

**ALTERED AUTONOMIC NERVOUS SYSTEM FUNCTION IN CHICKENS  
DIVERGENTLY SELECTED FOR BODY WEIGHT**

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**Keywords:** autonomic nervous system, blood pressure, chicken, heart rate, sympathetic nervous system, Parasympathetic nervous system, reserpine

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# **ALTERED AUTONOMIC NERVOUS SYSTEM FUNCTION IN CHICKENS DIVERGENTLY SELECTED FOR BODY WEIGHT**

by

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## **ABSTRACT**

Autonomic nervous system activity is related to body weight regulation. Based on the MONA LISA hypothesis it has been suggested that most obese subjects and animals have low sympathetic nervous system activity. The aim of this study was to investigate whether there are differences in autonomic nervous system activity between lines of chickens selected for either high (HWS) or low body weight (LWS). In Exp. 1, various pharmacological agents were injected intravenously, and the changes in blood pressure (BP) and heart rate (HR) of both HWS and LWS chickens were compared. The results showed that the HWS birds had a greater increase in BP and HR than the LWS following injection of atropine, a muscarinic receptor blocker, and LWS birds had a greater decrease in BP and HR to propranolol, a beta- adrenergic receptor blocker than the HWS birds. These results suggested that HWS chickens have higher parasympathetic tone, whereas LWS chickens have a higher sympathetic nervous system tone regulating the cardiovascular system. HWS and LWS chickens displayed a similar response in BP and HR following injection of the ganglion blocker tetraethylammonium chloride. These results suggest that there is no significant difference in the central autonomic nervous system in the cardiovascular regulation between HWS and LWS together. Since there does not appear to be any differences in the activity of the autonomic nervous system activity at the level of the central nervous system, these findings imply that the difference in response to atropine and propranolol could be caused by differences in adrenal activity. The ratio of heart rate and blood pressure after the injection of phenylephrine showed significant difference between these two lines of birds, but not when phenylephrine was injected following atropine. This result indicated that HWS are more dependent on the parasympathetic nervous system to regulate the baroreceptor reflex. The percentage of adrenal and sympathetic impact on the regulation of heart rate showed that LWS females required greater adrenal activity than the other birds. In Exp. 2, the body weight and food intake responses of HWS and LWS chickens to ip injections of reserpine were compared. Reserpine caused a transitory decrease in food intake and body weight in both lines of birds. However HWS chickens recovered more slowly from the depression caused by reserpine than the LWS chickens. This could be due to lower sympathetic nervous system activity. In conclusion, it appears that HWS may have lower sympathetic activity than LWS. Combining the results of both experiments, it appears that the HWS birds have lower sympathetic and higher parasympathetic activity. Furthermore central nervous system autonomic activity in BP and HR regulation is not different between HWS and LWS, but the activity of the adrenal gland may be different between these two lines of birds.

**Key words:** autonomic nervous system, blood pressure, chicken, heart rate, sympathetic nervous system, parasympathetic nervous system, reserpine

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## **Introduction**

Many mechanisms involved in controlling body weight have been described. Hypothalamic, genetic, dietary, and adrenoendocrine forms of experimental obesity are all associated with reduced activity of the sympathetic nervous system (Bray, 1987; Fukushima *et al.*, 1987; Sakaguchi *et al.*, 1988a; Sakaguchi *et al.*, 1988b). The MONA LISA hypothesis, an acronym for Most Obesities kNown Are Low In Sympathetic Activity, restates the autonomic hypothesis in memorable terms (Bray, 1991a). The MONA LISA hypothesis is associated with food intake regulation, synaptosomal uptake of norepinephrine, local concentration of monoamine neurotransmitters, ventromedial hypothalamus (VMH) lesion syndrome and adrenergic activation of the VMH, and adrenal dependence of hypothalamic obesity (Bray, 1991a).

### **1. *Autonomic Nervous System Activity with Obesity and Food Intake***

Obesity, a disproportional increase of the body fat mass, is often accompanied by alterations in both sympathetic and parasympathetic function (Bray and York, 1979; Levin *et al.*, 1980; Levin *et al.*, 1984; Jeanrenaud *et al.*, 1985; Martin *et al.*, 1986). The central nervous system has sites controlling both the parasympathetic and sympathetic nervous system. Therefore autonomic function is affected by the central nervous system, and damage to various hypothalamic areas can cause either an increase or decrease in body weight.

#### **1.1 *Reciprocal Relationship with Food Intake***

##### **1.1.1 *Ventromedial Hypothalamus with Autonomic Activity***

The ventromedial hypothalamic nucleus (VMH) has been suggested to act as a satiety center. Lesions of the VMH of the rat produced hypoactivity, hyperphagia and obesity (Brooks, 1946; Cox and Powley, 1981). Lesioning the VMH reduced postingestional thermogenesis, disrupted intracerebroventricular glucose regulation and induced obesity (Tokunaga *et al.*, 1989; Le Feuvre *et al.*, 1991; Thornton *et al.*, 1991; Monda *et al.*, 1997). These findings provide the basis for the MONA LISA hypothesis. Obesity following damage to the VMH is associated with hyperphagia and a variety of metabolic disturbances which can be largely attributed to alterations in the function of the autonomic nervous system and the resulting hyperinsulinemia (Hustvedt *et al.*, 1976; Bray and York, 1979). There is clear evidence for enhanced activity of the vagus nerve (Inoue *et al.*, 1977; Berthoud and Jeanrenaud, 1979; Cox and Powley, 1981), and reduced activity of the sympathetic nervous system following VMH lesions (Bray *et al.*, 1981).

There is a functional link between the VMH and interscapular brown adipose tissue (Nijima *et al.*, 1984). Brown adipose tissue has been recognized as a major site for thermogenesis during cold acclimation and spontaneous hyperphagia in rats. The role of the hypothalamus in the regulation of brown adipose tissue thermogenesis and its relationship to energy balance and development of obesity has been investigated (Hogan

*et al.*, 1982; Himms-Hagen, 1984; Saito *et al.*, 1985). Electrical stimulation of the VMH of rats caused a marked increase in lipogenesis in brown adipose tissue, but not in white adipose tissue or the liver (Shimazu and Takahashi, 1980; Takahashi and Shimazu, 1982). Also, it was demonstrated that stimulation of the VMH elicited a biphasic temperature change in brown adipose tissue similar to that produced by sympathetic nerve stimulation (Perkins *et al.*, 1981). Since thermogenesis in brown adipose tissue is believed to be under direct control of the sympathetic nervous system, it is conceivable that the stimulatory effects of the VMH on brown adipose tissue is mediated through increased activity of the sympathetic nerve to this tissue.

### **1.1.2 Lateral Hypothalamus with Autonomic Activity**

The activity of the sympathetic nervous system is also important in the hypophagia and weight loss associated with lateral hypothalamus (LH) lesioning (Yoshida *et al.*, 1983; Arase *et al.*, 1987). Lesioning the LH increased metabolic rate, increased norepinephrine turnover (Yoshida *et al.*, 1983; Garofalo *et al.*, 1996), and increased the firing rate of the sympathetic nerves to the brown adipose tissue (Freeman and Wellman, 1984; Cannon and Nedergaard, 1985). Bernardis and Bellinger (1996) reported that the LH is involved in catecholaminergic and serotonergic feeding systems, and glucose sensitive neurons have been identified in the LH. Furthermore, bilateral LH dopaminergic cell transplants, which presumably resulted in chronically and persistently enhanced dopaminergic activity in the LH, were associated with overeating and, consequently, weight gain (Yang *et al.*, 1996).

Robert *et al.* (1990) indicated that increased dopaminergic activity in the striatum could enhance food intake while increased dopamine in the lateral hypothalamus (LH) could decrease food intake. Decreased turnover of norepinephrine, dopamine, and serotonin in the LH stimulated activity of the sympathetic nervous system in brown adipose tissue. LH induced stimulation of sympathetic activity may have occurred by suppression of norepinephrine, dopamine and serotonin turnover. Yang and Meguid (1995) reported that the mean basal dopamine levels were significantly higher in obese Zucker rats than lean rats. Meguid *et al.* (1999) studied the role of LH dopamine and serotonin in the regulation of the feeding pattern during obesity by grafting the lean rat's embryonic dopaminergic and serotonergic neurons into the LH of the obese Zucker rats. The results showed that both serotonin-grafted and dopamine-grafted rats decreased the number of meals and feed intake. This indicated that dopamine and serotonin in the LH are involved in the regulation of feeding pattern during obesity.

### **1.1.3. Paraventricular Nucleus with Autonomic Activity**

Leibowitz *et al.* (1981) showed that electrolytic lesions of the paraventricular nucleus (PVN) in male or female rats induced hyperphagia and marked weight gain. Intracerebroventricular injection of norepinephrine and epinephrine into the PVN increased feed intake and body weight gain in rats (Leibowitz, 1978; Matthews *et al.*, 1978; Leibowitz and Brown, 1980). Evidence suggested that the PVN might play a role in the control of meal size, long-term feeding behavior, body weight regulation

(Leibowitz *et al.*, 1984; Lichtenstein *et al.*, 1985) and carbohydrate intake (Leibowitz *et al.*, 1985).

A feeding inhibitory pathway courses laterally from the PVN, and then turns caudally to take a longitudinal path in the perifornical region of the hypothalamus (Sclafani, 1982). The pathway is not limited to the hypothalamus, but appears to pass into the midbrain as evidenced by the findings that lesions in the midbrain tegmentum also produced hyperphagia and obesity (Sclafani, 1982; Kirchgessner and Sclafani, 1988).

There is a high density of insulin receptors in the PVN which appear to be involved in the central effects of insulin on energy balance (Baskin *et al.*, 1983; Corp *et al.*, 1986). Evidence suggests that insulin in the PVN acts as a signal to integrate a metabolic-behavioral response and regulate body fat (Menendez and Atrons, 1991). It was reported that glucocorticoids diminished basal and stress-induced catecholaminergic activity in the PVN; noradrenergic neurons may be a target site for stress-induced glucocorticoid to decrease the activity of corticotropin releasing factor (Pacak *et al.*, 1995a; Pacak *et al.*, 1995b).

It is known that the autonomic nervous system activity has a relationship to food intake and body weight. VMH lesions increased food intake and decreased sympathetic nervous system activity whereas lesions in the LH decreased food intake and body weight while increasing sympathetic activity (Bray, 1991a).

### **1.2. Local Concentrations of Monoamine Neurotransmitters**

Norepinephrine and serotonin, as well as several peptides, have reciprocal effects on food intake and sympathetic activity (Bray, 1992). Injections of norepinephrine into the VMH and PVN increased food intake and body weight gain (Leibowitz and Brown, 1980). Studies showed that corticotropin releasing factor (CRF) inhibited norepinephrine release in the lateral hypothalamus and decreased food intake (Osumi, 1990; Bray, 1991b). Intracerebroventricular injection of CRF increased sympathetic nervous activity and decreased food intake (Egawa *et al.*, 1990). Injection of CRF mimicked many components of the stress response including decreases in exploration, increases in sympathetic activity, decreases in parasympathetic outflow, and a decrease in appetite behavior (Spina *et al.*, 1996). Melanin concentrating hormone (MCH) injected directly into the PVN decreased food intake (Rossi *et al.*, 1999; Tritos and Maratos-Flier, 1999), and increased sympathetic activity while decreasing parasympathetic activity (van Kijk *et al.*, 1994). Neuropeptide Y, which coexists with norepinephrine in sympathetic nerves, increased food intake when injected into the PVN (Morris *et al.*, 1997; Morris *et al.*, 1998). Neuropeptide Y induced norepinephrine release and further reduced food intake and body weight (Matos *et al.*, 1996; Nishimura *et al.*, 1996). Increasing levels of neuropeptide Y in the PVN induced by exercise or fasting were followed by increased appetite and food intake, and increase parasympathetic activity but suppressed sympathetic activity (Kokot and Ficek, 1999).

### **1.2.1. Drugs Affect Neurotransmitter Activity**

Other drugs were also found to have a reciprocal relationship on food intake and sympathetic nervous system activity. Fenfluramine, an amphetamine derivative which is used as a weight-reducing agent in the treatment of obesity, decreased food intake and altered autonomic outflow resulting in increased metabolism (Appel *et al.*, 1991). It was suggested that fenfluramine blocks a feeding reward system which is stimulated by a high-fat diet (Fisler *et al.*, 1993). Mazindol, a monoamine reuptake blocker with effects on dopamine and serotonin, decreased food intake and increased sympathetic activity (Kleven and Woolverton, 1993; Mattei and Carini, 1995; Yoshida *et al.*, 1996). Conversely, 2-deoxy-glucose, an analog of glucose that blocks its intracellular metabolism, stimulated food intake and reduced sympathetic activity when injected into the third ventricle of lean rats (Tsujii and Bray, 1990).

### **1.3. Reciprocal relationship of synaptosomal uptake of norepinephrine**

Uptake of norepinephrine by hypothalamic synaptosomes is reciprocally related to sympathetic activity (Shimizu *et al.*, 1990). When sympathetic nervous system activity is low, food intake, body fat and norepinephrine turnover increased which enhanced norepinephrine reuptake (Cantu and Goodman, 1967; Fredholm, 1985; Youngstrom and Bartness, 1995).

### **1.4. Ventromedial Hypothalamus Lesion Syndrome and Adrenergic Activation of the VMH**

Lesioning the VMH or infusing norepinephrine or epinephrine into the VMH caused hyperphagia, hyperinsulinemia and increased body weight while reducing the activity of the sympathetic nervous system (Cransac *et al.*, 1996). However, a similar response was not noted in the PVN. Thus, adrenergic receptors in the VMH, but not in the PVN, mediate the VMH syndrome. Increased VMH norepinephrine activity is common among obese insulin-resistant animal models showing development of the VMH syndrome (Cincotta *et al.*, 2000).

### **1.5. Adrenal Dependence of Hypothalamic Obesity**

An important role for adrenal hormones in the short and long term regulation of food intake and body weight has been strongly suggested (Bray, 1984; Leibowitz, 1986). Adrenalectomy reverses hypothalamic obesity, and injection of corticosterone restores the hyperphagia and obesity in such animals (Tokunaga *et al.*, 1989). Adrenal glucocorticoids have an effect on the development of hypothalamic obesity and appear to be essential for the development of all forms of genetic obesity (Satio and Bray, 1984; Shimizu *et al.*, 1988; Bray *et al.*, 1989). Genetic and lesion-induced obesity are strongly related to the high level of corticosterone which greatly enhance fat intake (King and Smith, 1985; Kumar and Libowitz, 1988).

Adrenalectomy decreased the secretion of corticosteroids, increased the release of CRF, decrease food intake, and stimulated the activity of the sympathetic nervous system (Brown *et al.*, 1982). Adrenalectomy decreased food intake to normal levels in obese mice, increased energy expenditure, and restored catecholamine turnover to normal in brown adipose tissue, and returned sympathetic nervous system activity to normal (Satio and Bray, 1984; Vander Tuig *et al.*, 1984 York *et al.*, 1985).

### **1.6. Genetic Obesity Model**

Sympathetic nervous system activity is decreased in most forms of obesity, even in various genetic models. It has been reported that ob/ob mice have a leptin deficiency, and leptin regulates body weight and fat deposition through effects on metabolism and appetite (Pelleymounter *et al.*, 1995). Because leptin released from adipose tissue effects the autonomic nervous system acting within the hypothalamus, it can act as a feedback signal from adipose tissue to modulate neuronal and hormonal systems involved in the regulation of body weight and food intake (Blum, 1997; Mistry *et al.*, 1997). Harris *et al.* (1998) reported that giving ob/ob mice high concentrations of leptin caused increased sympathetic activity. The reason ob/ob mice have low sympathetic activity could be due to a smaller brain weight which can be reversed by leptin treatment during the development of the brain (Steppan and Swick, 1998). The Pima Indians appear similar to the ob/ob mouse in that they lack leptin and are prone to obesity and have low sympathetic nervous system activity (Ravussin and Gautier, 1999).

## **2. Obesity and Health Risks**

Obesity has a profound impact on the heart and vascular system. It is known that obesity is a factor that causes various cardiovascular disorders. The qualitative and quantitative assessment of sympathetic nervous system function is fundamentally important because heart rate variability indicates the relative predominance of sympathetic tone, and has been associated with a high cardiovascular risk (Kleiger *et al.*, 1987).

It has been reported that obesity either increases or decreases cardiovascular sympathetic nervous system activity. For example, Bunag *et al.*, (1990) reported that obese rats had borderline hypertension, and their baroreceptor reflex was less than that of control rats. This finding suggested that cardiovascular and baroreceptor reflex malfunction in obese rats may result from an autonomic imbalance involving diminished parasympathetic activity. It has also been reported in the literature that obese human parents tend to have higher norepinephrine concentrations in their plasma than non-obese parents (Piccirillo *et al.*, 1998). Obese human parents also demonstrated a decreased cardiovascular sympathetic response.

### **2.1. Evidence of Increased Autonomic Cardiovascular System Activity**

Hypertension is a common cardiovascular disorder. While the correlation between body weight and blood pressure has been recognized the mechanisms causing

the development of hypertension are not clear. Various obese animal models and obese humans showed elevated resting heart rate and pulmonary arterial pressures indicating decreased parasympathetic activity and reduced baroreceptor reflex sensitivity (Wehberg *et al.*, 1990; Hirsch *et al.*, 1991; Stoddard *et al.*, 1992). It is suggested that an autonomic imbalance characterized by decreased parasympathetic and increased sympathetic activity may be responsible for altering cardiovascular regulation in dietary-induced obesity (Bunag *et al.*, 1990).

Richter *et al.* (1996) reported that impaired cardiac autonomic nerve function was found in obese subjects with diminished insulin sensitivity, and parameters for the evaluation of autonomic nerve impairment correlated with the degree of insulin sensitivity. Hyperinsulinemia, which is characteristic of obesity, can contribute to the development of hypertension by inducing sodium retention and activating the sympathetic nervous system (Berchtold *et al.*, 1983). Overnutrition with increased consumption of sodium and lack of physical exercise contributes to the metabolic syndromes of obesity. The pressor effect of insulin in obesity may be further enhanced by the observation that its vasodilatory action can be blunted in obese subjects (Mikhail *et al.*, 1999).

## **2.2. Evidence of Decreased Autonomic Cardiovascular System Activity**

Evidence showing decreased sympathetic functions in obese subjects and the association between obesity and cardiovascular problems led to studies into the relationship between the sympathetic nervous system and hypertension. Plasma catecholamine concentration was shown to be correlated to blood pressure. It was also shown to have an effect on increased sensitivity of the arterial pressure to interruption of the sympathetic tone, which is consistent with an alteration of sympathetic function in obese subjects (Kotchen *et al.*, 1982; Hall *et al.*, 1993). Studies showed that reduced sympathetic cardiac responsiveness depended on adrenoreceptor desensitization and impaired post-receptor signal transduction (Hammond *et al.*, 1993; White *et al.*, 1994). While Young and Macdonald (1992) reported low plasma norepinephrine levels in obese subjects, Piccirillo *et al.* (1998) reported higher plasma noradrenaline levels but decreased sympathetic modulation of the heart. This led to the possibility that reduced sympathetic nervous system activity in the obese subject might only exist in the end organs.

The peptide hormone leptin not only has an affect on the sympathetic nervous system to regulate food intake and body weight but also on cardiovascular function. Based on knowing that obesity is associated with hypertension, and increased leptin concentration will decrease food intake, Dunbar *et al.* (1997) studied the blood pressure change after intracerebroventricular (ICV) injection of leptin in normal rats. The result showed that ICV injection of leptin increased blood pressure by decreasing arterial blood flow to the skeletal muscle. This increased peripheral resistance is the result of an increased activity of the sympathetic nerves. A similar study by Lu *et al.* (1998) compare the cardiovascular sympathetic activity after ICV injection of leptin in obese and control rats. ICV injections of leptin increased blood pressure of the control rats, but not in obese

rats. Suggesting that obesity was associated with a decreased central nervous system response to leptin leading to a decreased leptin effect on the cardiovascular system.

### **3. Heavy Body weights and Obesity**

#### **3.1. Identification of Heavy Weight and Obesity**

To define healthy weight and obesity, nutritionists often prefer to use the body mass index values, which is a standard derived from height and weight measures. The body mass index mathematically correlates heights and weights with risks to health. It is especially useful for evaluating health risks of obesity but fails to measure body fat distribution (Sizer and Whitney, 1997). The following equations are more often use in determine the value of body mass index.

$$\text{Body mass index} = \text{weight (kg)} / \text{height (m)}^2$$

Or

$$\text{Body mass index} = \text{weight (lb)} / \text{height (in)}^2$$

Most nutritionists consider that a normal body mass index should be between 19 to 26, 26 to 28 is heavy weight and over 30 is consider obese. While the body mass index values are most valuable for evaluating degrees of obesity, they do not provide information about the weight and location of the fat. For example, a body mass index value may not be the most practical method for applying in the sports performer, because they may have higher muscle mass and muscle weight, but not fat weight. Their body mass index could be higher than the normal condition (Pettersson, 2000); however, they are not obese or under any health risk.

#### **3.2. High and Low Body Weight Selected Chickens**

Foundation stock for high (HWS) and low body weight selected (LWS) chickens consisted of crosses of 7 inbred lines of White Plymouth Rocks (Siegel, 1962). For this segregating gene pool, 2 lines were selected on the basis of body weight at 8 weeks of age. Chickens with heavier body weights formed the parent stock for the HWS whereas those with the lighter body weights were used as parents for the LWS. Thereafter, individual phenotypic selection was practiced within each line for the single characteristic, body weight at 8 weeks of age.

After forty yeas selection for body weight, the differences of body weight between HWS and LWS chickens was 8 fold. The LWS female does not consume enough feed *ad libitum* to attain a minimum threshold of body weight, body composition, or both, which are essential to commence egg production (Zelenka *et al.*, 1987). Forced-feeding could improve the situation. LWS males still produced semen. In the HWS line, feed restriction program is used after 8 weeks of age. If the feed restriction is not applied, egg production is impaired. Because feed consumption exceeds skeletal and lean body weight needs, fat deposition increases in the HWS. The resulting obesity is accompanied by undesirable reproductive complications (Katanbaf *et al.*, 1989; Liu *et al.*, 1994). This indicates that the HWS natural puritanical result in obesity.

#### **4. Conclusion**

Current research indicates that autonomic nervous system activity plays an important role in regulating food intake and body weight. Obesity has also been shown to affect autonomic nervous system activity either increasing or decreasing input to the cardiovascular system affecting health. Research is needed to characterize autonomic nervous system activity at different body weights. HWS chickens have a propensity to become obese whereas LWS chickens do not consume enough feed which can impair egg production. These two lines of birds were chosen to be the experimental subjects in this project. The primary objective of the following experiments were to compare the autonomic nervous system activity in high and low body weight selected chickens. Furthermore, attempts were made to determine the site of altered autonomic nervous system function between high and low body weight selected chicken. Experiments were designed to investigate both the central and peripheral cardiovascular autonomic nervous system activity in both lines of birds. Finally, studies were conducted to determine if the lines differed in response to reserpine, a compound that depletes catecholamines and indoleamines. Further understanding of the relationship between body weight regulation and autonomic nervous system activity in both animals and humans, will help address obesity problems.

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## **Chapter 1**

### **The Change in Blood Pressure and Heart Rate of High and Low Body Weight Selected Chickens in Response to Various Pharmacological Agents**

#### **Abstract**

This study was conducted to investigate whether there are differences in the autonomic nervous system function of chickens from lines selected for high (HWS) or low body-weight (LWS). The cardiovascular response to various pharmacological agents was used as an indicator of autonomic nervous system response. Ten birds from each line and sex were used. Catheters were introduced into the left brachial artery and vein, and connected to a MP100-BIOPAC system to record blood pressure and heart rate. Birds were injected with phenylephrine, atropine, propranolol, and tetraethylammonium chloride (TEAC). There were no differences in the heart rate or blood pressure responses of HWS and LWS chickens to phenylephrine. The LWS birds exhibited a greater increase in blood pressure and less increase in heart rate than the HWS ones following atropine. The response to atropine showed a line and sex interaction, in which male birds had a greater increase in heart rate than females, and LWS females had a lower increase in heart rate than the HWS females. Phenylephrine injected following pretreatment with atropine caused a baroreceptor reflex in which males showed a greater decrease in heart rate than females. In response to the beta-adrenergic receptor blocker propranolol, females displayed a greater decrease in blood pressure than males, and LWS birds had a greater decrease in heart rate than HWS birds. In response to the autonomic ganglionic blocker TEAC, blood pressure and heart rate was decreased equally in both lines. The percentage of adrenal and sympathetic impact on regulation of heart rate showed these LWS females required greater adrenal activity than the other birds. The change in heart rate and blood pressure ratio in the phenylephrine was significant between lines, however it was not in the phenylephrine following after atropine. This suggested that HWS birds had greater parasympathetic nervous system activity in order to maintain cardiovascular function. These results suggest that a higher parasympathetic nervous system tone is present in the HWS than LWS birds, and a higher sympathetic nervous system tone is present in the LWS than HWS birds. It is suggested that differences between the lines could be at the level of the adrenal gland.

Key words: autonomic nervous system, blood pressure, chickens, heart rate, parasympathetic nervous system, sympathetic nervous system.

## Introduction

Many studies have investigated the role of the autonomic nervous system in the control of body weight. While some studies reported that sympathetic nervous system activity increased in obese subjects while parasympathetic nervous system activity decreased (Bunag *et al.*, 1990, Petretta *et al.*, 1995), most reported decreased sympathetic nervous system function associated with obesity (Young and Landsberg, 1983; Bray *et al.*, 1989; Young and Macdonald, 1992).

Several investigation have shown that obesity contributes to cardiovascular disorders (Peterson *et al.*, 1988; Landsberg, 1992; and Truett *et al.*, 1996). Since plasma catecholamine levels only provide presynaptic indexes of sympathetic function, but not postsynaptic responsiveness, it has been observed that heart rate variability provides a reliable, non-invasive method for quantitative assessment of cardiac autonomic control (Malpas and Maling, 1990; Guzzetti *et al.*, 1991). Spectral analysis has been used to determine quantitative cardiac autonomic control by translating blood pressure readings to electrocardiographic signals. Two spectral components can be measured using this technique. The one at high frequency reflects parasympathetic activity whereas the low and high frequency ratio reflects sympathetic function. It has been used to provide an overall index of vagal/sympathetic cardiovascular modulation and thus an assessment of end-organ autonomic activity (Kingwell *et al.*, 1994).

The baroreceptor reflex is another indicator of cardiac autonomic regulation. It is well documented that obesity reduces baroreceptor reflex control of heart rate thus contributing to the impairment of cardiac parasympathetic function (Bruce *et al.*, 1995) which may predispose an animal to the development of hypertension (Bunag and Barringer, 1996).

Although several animal models such as the ob/ob and db/db mouse have been used to study obesity (Leigh *et al.*, 1992; Truett *et al.*, 1996; Harris *et al.*, 1998), most obesities are not caused by a single gene or factor. The lines chickens used in the present study provide a unique genetic model for studying the regulation of body weight because they have under gone long term divergent selection for high or low juvenile body weight (Dunnington and Siegel, 1996). The aim of this study was to investigate the change in blood pressure and heart rate of both male and female, high and low body weight selected chickens in response to various pharmacological agents.

## Materials and Methods

White Rock chickens from the S<sub>41</sub> generation of two lines divergently selected for high and low body weight at eight weeks of age (HWS and LWS) were used. Siegel (1962; 1970) and Liu *et al.* (1994) described the selection procedures used in the development of these lines.

## **Experimental protocol.**

Chick from each line were hatched on the same day and raised separately in floor pens. The birds were provided a standard diet (20% crude protein; 2864 Kcal/kg metabolizable energy) and water for *ad libitum* consumption. After injecting lidocaine hydrochloride (VEDCO, Inc. St. Joseph, MO) as a local anesthetic, a polyethylene catheter (PE-60, Clay Adams) was introduced into the left brachial artery for measuring arterial blood pressure. Blood pressure was measured with a Harvard transducer (DA100B), and recorded using an MP100- BIOPAC system (Santa Barbara, CA). A second catheter (PE-60, Clay Adams) was introduced into the left brachial vein for intravenous injections.

Following cannulation, 0.5 mL of heparinized saline (100 units/mL) was introduced into each catheter to prevent clotting. A 20-min period was allowed for the animal to achieve a steady hemodynamic state before introducing drugs, and control measurements were obtained.

## **Drugs used.**

Heparin sodium, L- phenylephrine hydrochloride, atropine, propranolol hydrochloride and tetraethylammonium chloride were obtained from Sigma Chemical CO., (St. Louis, MO).

## **Test solutions.**

All drugs were dissolved in 0.85% avian physiological saline, and prepared fresh daily. Solutions were slowly injected in a volume of 0.5 ml over a 30-sec interval. At least 15 min was allowed for recovery between each drug tested. Drugs were injected intravenously in the following order: 1) Phenylephrine, an  $\alpha$ -adrenoceptor agonist (40  $\mu$ g/kg/ 0.2 ml); 2) Atropine, a muscarinic receptor blocker (0.5 mg/kg/ 0.2ml); 3) Propranolol, a  $\beta$ -adrenoceptor blocker (1.0 mg/kg/0.2 ml); 4) Tetraethylammonium chloride, a ganglionic blocker (10 mg/kg/0.2 ml). Doses were according to Szeto *et al.* (1977) and Barnes and Eltherington (1991).

## **Data analysis.**

Data were analyzed using ANOVA with line and sex as the main effects and initial blood pressure as a covariant (SAS, 1998). When the interaction between line and sex was significant, comparisons were made within each line and sex. All values are reported as means  $\pm$  SE. Significant differences imply  $P \leq .05$ .

## **Results and Discussion**

### **Response caused by Atropine**

The basal blood pressure and heart rate in the HWS males was 109.6 mmHg and 336 beats/min, HWS females was 119.6 mmHg and 315 beats/min, LWS males was

151.9 mmHg and 314 beats/min, and LWS females was 140.6 mmHg with 333 beats/min heart rate.

In response to the iv injection of atropine, the LWS birds displayed a greater increase in blood pressure and less increase in heart rate than the HWS (Table 2). There was a line by sex interaction for heart rate because in females, atropine caused a much greater increase in the HWS whereas the response in males was similar between lines (Table 3). Since atropine is a muscarinic receptor blocker, these results suggest that the HWS females may have greater parasympathetic input to the heart than the LWS.

### **Response caused by Propranolol**

The iv infusion of propranolol decreased blood pressure and heart rate. While there was no line effect for blood pressure, females displayed a greater decrease in blood pressure than males (Table 1 and 2). There was a line effect for heart rate with the LWS birds showing a greater decrease than the HWS birds. Sex had no effect on the heart rate response (Table 4).

Propranolol, a  $\beta$ -adrenergic blocker, decreases sympathetic input to the heart. The increased heart rate responsiveness of the LWS to propranolol suggests that the LWS birds have higher sympathetic nervous system activity to maintain heart rate and blood pressure. This result is consistent with a study showing that obese patients have significantly higher cardiac parasympathetic activity and lower cardiac sympathetic response (Piccirillo *et al.*, 1996). In addition, obese subjects had higher norepinephrine plasma concentrations, but this did not correspond with an enhanced cardiovascular sympathetic response (Piccirillo *et al.*, 1998). Therefore obese subjects may be more resistant to  $\beta$ -adrenergic stimulation (Christin *et al.*, 1993), and have lower  $\beta$ -adrenergic sensitivity (Tataranni *et al.*, 1998).

### **Response caused by TEAC**

The iv injection of TEAC decreased blood pressure and heart rate similarly in both lines and sexes (Table 1,2, and 5). TEAC blocks the autonomic ganglion thus leaving only the peripheral autonomic nervous system to control blood pressure and heart rate.

Because TEAC blocks the connection between the preganglionic neurons and the postganglionic neurons, these results suggest that the central nervous system input controlling the cardiovascular system was not different between HWS and LWS birds.

### **Percentage of sympathetic and adrenal control**

It is known that the amount of heart rate decreased by TEAC indicates the control of the parasympathetic, sympathetic, and adrenal input to the heart rate. Atropine is used to the degree of parasympathetic control to the heart rate; propranolol has an effect on  $\beta$ -receptors and indicates effects on both sympathetic and adrenal control of the heart rate.

The following equations were used to define the percentage of sympathetic and adrenal impact on the regulation of heart rate in each line and sex.

$$\begin{aligned} \text{Sympathetic input} &= \text{TEAC}_{\text{change heart rate}} - \text{Atropine}_{\text{change heart rate}} \\ \text{Propranolol}_{\text{change heart rate}} - \text{Sympathetic input} &= \text{Adrenal input} \\ \text{Sympathetic input} / \text{Propranolol}_{\text{change heart rate}} &= \text{percentage of sympathetic input} \\ \text{Adrenal input} / \text{Propranolol}_{\text{change heart rate}} &= \text{percentage of adrenal input} \end{aligned}$$

The LWS female has 43.4% sympathetic input and 56.6% of adrenal input, compare with the others, the sympathetic impact were between 68 to 71% and adrenal impact were 21 to 31% (Table 6). The result indicated that LWS females have greater adrenal effect on the heart rate regulation than the other birds. At the same time, the LWS birds depend less on sympathetic activity to regulate heart rate than the HWS. It is suggested that the adrenal activity between each line and sex may be different.

### **Response caused by Phenylephrine**

A comparison of the baroreceptor reflex in obese and lean Zucker rats showed that receptor sensitivity was attenuated in obese Zucker rats as evidenced by a consistently weaker response compared to lean rats (Bunag and Barringer, 1988). This made the Zucker obese rats more prone to hypertension. In the present study, there was no significant difference in the baroreceptor reflex between HWS and LWS chickens (Table 7 and 8). Therefore, selection for high body weight has not made the HWS birds more prone to hypertension.

### **Response caused by Atropine+Phenylephrine**

In order to further study the baroreceptor reflex, phenylephrine was injected following pretreatment with atropine, which caused an increase in blood pressure and a reflexive decrease in heart rate. Heart rate decreased less in females than males; there was no significant difference in the change in blood pressure (Table 7 and 8). These results suggest that females may have a lower baroreceptor reflex compared to males when the muscarinic receptor was blocked.

Studies indicated that the baroreceptor reflex sensitivity was different between male and female rats with females exhibiting substantially lower baroreceptor reflex sensitivity after injection of phenylephrine (Luzier *et al.*, 1998, Abdel-Rahman, 1999). Reports have shown that a higher percentage of women had depressed baroreceptor reflex sensitivity which was associated with a high risk of arrhythmias (La Rovere *et al.*, 1988; Farrel *et al.*, 1992). The mechanisms responsible for lower baroreceptor reflex sensitivity in women have not been clarified. Luzier *et al.* (1998) reported that women might have greater alpha-adrenergic receptor sensitivity than men, whereas beta 1-adrenergic receptor sensitivity is similar between genders. This results in greater baroreceptor reflex sensitivity in men than in women. Therefore, the sensitivity of the adrenergic receptor may be different between genders thus causing a different baroreceptor reflex response.

El-Mas and Abdel-Rahaman (1998a) reported that ovariectomized rats showed a decreased baroreceptor reflex response which could be normalized with estrogen replacement therapy. It was suggested that the sex hormones could play a role in the gender-related difference in baroreceptor reflex sensitivity (Huikuri *et al.*, 1996, El-Mas and Abdel-Rahaman, 1998a; El-Mas and Abdel-Rahaman, 1998b).

### **Response caused by Propranolol+phenylephrine**

In order to study the baroreceptor reflex, phenylephrine was injected following pretreatment with propranolol which caused an increase in blood pressure and a reflexive decrease in heart rate. The blood pressure and heart rate response was not affected by line or sex, suggesting that the baroreceptor reflex was not affected by line or sex (Table 7 and 8).

### **Response caused by TEAC+ phenylephrine**

Following the injection of TEAC, phenylephrine caused significant differences in blood pressure and heart rate responses between both lines and sexes (Table 7 and 8). This is in contrast to the results obtained following atropine in which there was a line and sex interaction, and a difference between the lines on blood pressure change. Therefore, the baroreceptor reflex was different between the sexes when the muscarinic receptors were blocked.

### **The ratio of change of heart rate and change of blood pressure**

The ratio of heart rate to blood pressure (beats per min/ mmHg) was calculated (Table 9). This ratio indicates the change in heart rate caused by a 1 mmHg change in blood pressure. The ratio was significantly different between HWS and LWS birds after the injection of phenylephrine. However, the ratio was not affected by phenylephrine injection following atropine in either line or sex. It is suggested that HWS birds have greater parasympathetic input than the LWS bird and also greater need of parasympathetic nervous system to maintain cardiovascular homeostasis.

### **General discussion**

These responses suggest that HWS birds have a higher parasympathetic and lower sympathetic nervous system tone, while the converse is true in the LWS. The LWS females showed less parasympathetic nervous system activity than HWS males or females, or the LWS males. Zucker and Wang (1991) studied the baroreceptor reflex before and after heart failure by using various agents. They indicated that depression of baroreceptor reflex activity indicated high risk of heart failure. The HWS and LWS birds showed no significant differences in the central nervous system control of the cardiovascular response. This suggests that the difference between HWS and LWS bird is in the peripheral cardiovascular autonomic nervous system.

The effects of epinephrine are predominantly on the cardiovascular system, but it also effects body weight homeostasis (Robertshaw, 1977; Russek *et al.*, 1980; Tordoff *et*

*al.*, 1982). Knowing that the peripheral cardiovascular autonomic nervous system activity between HWS and LWS birds is altered, and that epinephrine impacts the cardiovascular system activity and is primarily secreted by the adrenal medulla, this suggests that different adrenal gland activities or epinephrine concentrations might cause the different cardiovascular autonomic activity or different body weight between these two lines of chickens. Leigh *et al* (1992) reported that genetically obese (ob/ob) mice have higher urinary epinephrine output than the lean mice suggesting altered peripheral nervous system activity.

Leptin has an effect on the autonomic nervous system acting within the hypothalamus to regulate food intake and body weight gain (Blum, 1997). The paraventricular nucleus (PVN) is a central nervous system structure suggested as a site where leptin acts to exert its central effects, and is also involved in the control of gastrointestinal function. Smith *et al.* (1998) reported that microinjection of leptin into the PVN decreased body weight and food intake and increased energy expenditure. In addition, leptin increased sympathetic nervous system activity in the ob/ob mice (Harris *et al.*, 1998). Lesioning the PVN blocks the decrease in body weight and increase in sympathetic system activity in response to leptin (Bray, 1998).

The leptin receptor was identified on the epinephrine secreting cells in the adrenal medulla, but not in the adrenal cortex, suggesting that leptin may have a direct effect on epinephrine secretion (Cao *et al.*, 1997). Epinephrine also might play a role in mediating the effects of leptin to reduce body weight. The results from the present experiment suggested that the HWS and LWS birds might have differences in the peripheral autonomic nervous system activity. It is possible that the adrenal medulla epinephrine secreting activity is different between these two lines of birds. Based on the fact that leptin can regulate sympathetic nervous system activity, epinephrine has an effect on the cardiovascular system, and leptin can affect epinephrine secretion, these could be factors causing the differences between the HWS and LWS chickens in the autonomic cardiovascular system responses.

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**Table 1 . The change in heart rate (beats/min) of HWS and LWS, male and female chickens following the i.v. injection of various pharmacological agents<sup>1</sup>**

| Treatment   | Line       |               |
|-------------|------------|---------------|
|             | HWS        | LWS           |
| Atropine    |            |               |
| Propranolol | -106 ± 5.2 | * -123 ± 5.2  |
| TEAC        | -29 ± 4.4  | NS -25 ± 3.6  |
|             | Sex        |               |
|             | Male       | Female        |
| Atropine    |            |               |
| Propranolol | -111 ± 6.1 | NS -119 ± 4.6 |
| TEAC        | -20 ± 2.0  | NS -33 ± 4.7  |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect.

NS = No significant difference between lines or sexes.

= Line by sex interaction is significant, see Table 3 .

**Table 2. The change in blood pressure (mmHg) of HWS and LWS, male and female chickens following the i.v. injection of various pharmacological agents<sup>1</sup>**

| Treatment   | Line         |        |
|-------------|--------------|--------|
|             | HWS          | LWS    |
| Atropine    | 10.4 ± 0.95  | *      |
| Propranolol | -20.4 ± 1.27 | NS     |
| TEAC        | -31.7 ± 1.68 | NS     |
|             | Sex          |        |
|             | Male         | Female |
| Atropine    | 12.4 ± 1.34  | NS     |
| Propranolol | -19.9 ± 1.00 | *      |
| TEAC        | -29.6 ± 2.44 | NS     |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line or sex effect.

NS = No significant difference between lines or sexes.

**Table 3 . The change in heart rate (beats/min) and blood pressure (mmHg) of HWS and LWS, male and female chickens following the i.v. injection of atropine<sup>1</sup>**

| Treatment |        | Lines                 |    |             |
|-----------|--------|-----------------------|----|-------------|
|           |        | HWS                   |    | LWS         |
|           |        | Heart rate (beat/min) |    |             |
| Atropine  | Male   | 46 ±3.4               | NS | 55 ±7.4     |
|           |        | NS                    |    | *           |
|           | Female | 49 ±2.5               | *  | 25 ±4.4     |
|           |        | Blood pressure (mmHg) |    |             |
|           | Male   | 9.4 ± 0.91            | NS | 15.8 ± 2.18 |
|           |        | NS                    |    | NS          |
|           | Female | 11.3 1.65             | NS | 15.9 ± 1.74 |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line and sex effect.

NS = No significant difference between lines and sexes.

**Table 4 . The change in heart rate (beats/min) and blood pressure (mmHg) of HWS and LWS, male and female chickens following the i.v. injection of propranolol<sup>1</sup>**

| Treatment   |        | Lines                 |    |              |
|-------------|--------|-----------------------|----|--------------|
|             |        | HWS                   |    | LWS          |
|             |        | Heart rate (beat/min) |    |              |
| Propranolol | Male   | -103 ±8.1             | NS | -118 ±9.0    |
|             |        | NS                    |    | NS           |
|             | Female | -110 ± 6.7            | NS | -128 ±5.0    |
|             |        |                       |    |              |
|             |        | Blood pressure (mmHg) |    |              |
|             | Male   | -18.5 ± 1.07          | NS | -21.4 ±1.60  |
|             |        | NS                    |    | NS           |
|             | Female | -22.1 ± 2.13          | NS | -26.5 ± 2.74 |
|             |        |                       |    |              |

<sup>1</sup>Value represents Mean ± SE.

NS = No significant difference between lines and sexes.

**Table 5. The change in heart rate (beats/min) and blood pressure (mmHg) of HWS and LWS, male and female chickens following the i.v. injection of TEAC<sup>1</sup>**

| Treatment |        | Lines                 |    |              |
|-----------|--------|-----------------------|----|--------------|
|           |        | HWS                   |    | LWS          |
|           |        | Heart rate (beat/min) |    |              |
| TEAC      | Male   | -20 ± 2.5             | NS | -20 ± 3.4    |
|           |        | NS                    |    | NS           |
|           | Female | -36 ± 7.6             | NS | -29 ± 5.7    |
|           |        | Blood pressure (mmHg) |    |              |
|           | Male   | -27.8 ± 2.09          | NS | -31.6 ± 4.67 |
|           |        | NS                    |    | NS           |
|           | Female | -36.0 ± 1.86          | NS | -29.1 ± 4.77 |

<sup>1</sup>Value represents Mean ± SE.

NS = No significant difference between lines and sexes.

**Table 6. The percentage of sympathetic and adrenal impact in the regulation of heart rate<sup>1</sup>**

| Lines and sexes | %Sympathetic      | %Adrenal          |
|-----------------|-------------------|-------------------|
| HWS Male        | 71.9 <sup>a</sup> | 28.1 <sup>b</sup> |
| HWS Female      | 78.8 <sup>a</sup> | 21.2 <sup>b</sup> |
| LWS Male        | 68.8 <sup>a</sup> | 31.2 <sup>b</sup> |
| LWS Female      | 43.4 <sup>b</sup> | 56.6 <sup>a</sup> |

<sup>1</sup>Value represents Mean.

<sup>a,b</sup> Means within a column with different superscripts are significantly different.

**Table 7 . The change in heart rate (beats/min) of HWS and LWS, male and female chickens following the i.v. injection of phenylephrine and various pharmacological agents and following by phenylephrine<sup>1</sup>**

| Treatment                 | Line       |    |            | Function                                             |
|---------------------------|------------|----|------------|------------------------------------------------------|
|                           | HWS        | NS | LWS        |                                                      |
| Phenylephrine             | -82 ± 6.6  | NS | -69 ± 7.1  |                                                      |
| Atropine+Phenylephrine    | -57 ± 7.2  | NS | -46 ± 5.0  | Block parasympathetic nervous system                 |
| Propranolol+Phenylephrine | -121 ± 5.0 | NS | -134 ± 4.9 | Block sympathetic nervous system                     |
| TEAC+Phenylephrine        | -34 ± 4.6  | NS | -28 ± 3.8  | Block parasympathetic and sympathetic nervous system |
|                           | Sex        |    |            |                                                      |
|                           | Male       | NS | Female     |                                                      |
| Phenylephrine             | -79 ± 8.1  | NS | -72 ± 5.3  |                                                      |
| Atropine+Phenylephrine    | -58 ± 7.5  | *  | -46 ± 4.2  | Block parasympathetic nervous system                 |
| Propranolol+Phenylephrine | -122 ± 5.8 | NS | -133 ± 4.0 | Block sympathetic nervous system                     |
| TEAC+Phenylephrine        | - 24 ± 2.3 | NS | -37 ± 4.9  | Block parasympathetic and sympathetic nervous system |

<sup>1</sup>Value represents Mean ± SE.

\* = There is significant line or sex effect.

NS = No significant difference between lines or sexes.

**Table 8. The change in blood pressure (mmHg) of HWS and LWS, male and female chickens following the i.v. injection of phenylephrine and various pharmacological agents and following by phenylephrine<sup>1</sup>**

| Treatment                 | Line        |    |             | Function                                             |
|---------------------------|-------------|----|-------------|------------------------------------------------------|
|                           | HWS         |    | LWS         |                                                      |
| Phenylephrine             | 20.0 ± 0.96 | NS | 25.7 ± 1.62 |                                                      |
| Atropine+Phenylephrine    | 34.1 ± 1.62 | NS | 27.7 ± 1.93 | Block parasympathetic nervous system                 |
| Propranolol+Phenylephrine | 17.2 ± 1.60 | NS | 22.3 ± 1.63 | Block sympathetic nervous system                     |
| TEAC+Phenylephrine        | 26.5 ± 2.63 | NS | 37.7 ± 2.91 | Block parasympathetic and sympathetic nervous system |
|                           | Sex         |    |             |                                                      |
|                           | Male        |    | Female      |                                                      |
| Phenylephrine             | 22.8 ± 0.81 | NS | 23.0 ± 1.92 |                                                      |
| Atropine+Phenylephrine    | 32.2 ± 1.83 | NS | 29.5 ± 1.99 | Block parasympathetic nervous system                 |
| Propranolol+Phenylephrine | 19.1 ± 1.64 | NS | 20.3 ± 1.79 | Block sympathetic nervous system                     |
| TEAC+Phenylephrine        | 33.2 ± 3.66 | NS | 31.1 ± 2.47 | Block parasympathetic and sympathetic nervous system |

<sup>1</sup>Value represents Mean ± SE.

NS = No significant difference between lines or sexes.

**Table 9. The ratio of heart rate/Blood pressure (beat per min/mmHg) after the injection of phenylephrine and phenylephrine following after atropine<sup>1</sup>**

| Treatment              | Lines        |     |
|------------------------|--------------|-----|
|                        | HWS          | LWS |
| Phenylephrine          | -4.39 ± 0.42 | *   |
| Atropine+Phenylephrine | -1.89 ± 0.31 | NS  |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect.

NS = No significant difference between lines.

## **Chapter 2**

### **The effect of reserpine on body weight, feed intake, catecholamine and indoleamine levels in high and low body weight selected chickens**

#### **Abstract**

The effect of reserpine on body weight, food intake, and neurotransmitter levels in lines of chickens selected for high (HWS) and low (LWS) body weight was investigated. HWS or LWS chicks were randomly assigned to three treatments groups. Saline, 1.25mg/kg, and 2.5mg/kg reserpine were injected intraperitoneally at hatch, and at six weeks of age. Body weight and feed intake were measured weekly and feed efficiency determined. At eight weeks of age, ten females from each group were sacrificed to measure catecholamine and serotonin levels by high performance liquid chromatography. Weekly body weights, feed efficiency, and cumulative feed efficiency were different between the two lines throughout the study. Reserpine decreased body weight in both lines at both injection ages. However, the second reserpine injection at six weeks of age dose-dependently decreased body weight gain in the HWS whereas the effect in the LWS was not dose-dependent. The HWS birds were more sensitive than LWS birds ones to the effects of reserpine with body weight decreasing nearly 25 % and 11%, respectively, at 7 weeks of age. In addition, the HWS birds recovered more slowly from the decrease in body weight caused by reserpine. Reserpine significantly decreased feed consumption in both lines. The second reserpine injection decreased feed consumption in a dose-dependent manner at 7 weeks of age in the HWS, but not in the LWS birds. The second reserpine injection given at 6 weeks dose-dependently decreased cumulative feed efficiency at 7 weeks of age in the HWS birds, but not LWS birds. The results of this study showed that the HWS birds were more sensitive to the body weight-reducing effects of reserpine than LWS birds. It has been reported that obese subjects have lower beta-adrenergic sensitivity. Similarly, the HWS chickens may have lower sympathetic nervous system activity than those from LWS.

Key words: body weight, catecholamine, chickens, feed intake, reserpine.

## **Introduction**

The sympathetic nervous system has a role in the etiology of body weight gain through its impact on food intake. Two opposite hypotheses concerning these mechanisms have been proposed. The first is that obesity results from low sympathetic nervous system activity (Bray, 1991b; Ravussin and Gautier, 1999). The second suggests that sympathetic nervous system activation occur as a result of chronic overeating (Rumantir et al., 1999). Both hypotheses suggest that the sympathetic nervous system can alter food intake and body weight through the central and peripheral nervous system (Bray, 1991a, Ravussin, 1995).

It is known that various neurotransmitters including norepinephrine (NE), epinephrine (Epi), dopamine (DA), and serotonin (5HT) in the hypothalamus have an impact on the regulation of body weight and food intake in rats (Leibowitz and Brown, 1980; Robert et al., 1990). Early studies suggest that dopamine is involved in maintaining normal feeding (Heffner et al., 1980), and there is increased DA release in the lateral hypothalamus (LH) associated with feeding (Meguid et al., 1995, Meguid et al., 1997). Furthermore, Cincotta (1997) demonstrated that obese (ob/ob) mice treated with DA D1 and D2 receptor agonists reduce food intake, body weight and fat mass.

Injecting NE into the ventromedial hypothalamus (VMH) and paraventricular nucleus (PVN) enhanced food intake, and ultimately, body weight gain (Leibowitz and Brown, 1980; Leibowitz et al., 1984; Lichtenstein et al., 1984). 5HT also acts in the central nervous system to regulate food intake and body weight gain (Samanin and Garattini, 1988). Increasing 5HT in the medial hypothalamus reduced ingestion of carbohydrate while sparing protein intake (Leibowitz and Shor-Posner, 1986). Injection of 5HT or its agonists into the perifornical hypothalamus (Goldman et al., 1971), LH or medial PVN of rats decreased food intake (Gardier et al., 1989). Denbow et al. (1983) reported that intracerebroventricular injection of 5HT decreased food intake in fasted Leghorns. Many circulating hormones also impact serotonergic function and satiety (Leibowitz and Alexander, 1998).

Reserpine impairs the storage of biogenic amines, and results in depletion of NE, DA, and 5HT in both the central and peripheral nervous systems, and disturbs the digestive system and decreases food intake (Ahmed et al., 1997). The aim of this study was to compare the response of HWS and LWS of chickens to reserpine.

## **Materials and Methods**

Day-old male and female HWS and LWS broiler chicks were vaccinated for Marek's disease and placed in electric heated batteries with raised wire floors. Thirty-six pens were randomly assigned to each of three treatment groups. The groups were injected with 5 ml/ kg i.p. of either 1.25 mg/kg or 2.50 mg/kg reserpine, or 0.85% saline which served as the control (Ahmed et al., 1986). Birds were provided a starter (20% protein, 2684 kcal/ kg) diet and water available for ad libitum consumption. The birds

were exposed to continuous fluorescent lighting. At four weeks of age, the birds were transferred to grower batteries (75.12 cm W X 38.10 cm L X 38.10 cm H).

At eight weeks of age, ten females from each group were sacrificed for catecholamine and indoleamine assays. Blood was collected via cardiac puncture using a syringe coated with heparin. The blood was centrifuged at 300 x g for 20 min, and the plasma collected and stored at -70 °C until analysis. The brain and heart were removed, quickly frozen in liquid nitrogen, and stored at -70 °C until analysis.

Neurotransmitter levels were analyzed using high performance liquid chromatography with electrochemical detection (HPLC-EC). Brain and heart samples were homogenized in ice cold homogenization buffer which contained 150 mg of Na<sub>2</sub> EDTA and 4.3 ml of 70% perchloric acid in 850 ml of distilled water (APPENDIX C) (Arjona, 1991). The homogenized mixtures were centrifuged (4 °C) at 11,000 g for 15 min and the homogenate collected. The homogenate was then centrifuged (4 °C) at 1600 g for 5 min and the supernatant filtered using a 0.22 µm swinnex filter. Finally, the brain and heart homogenates, and plasma samples were centrifuged using the Amicon MPS-1 micropartition system (Amicon Co., Beverly MA) that filters out all protein above 3000 MW.

The HPLC system consisted of a Beckman 344 modular system (Beckman Instruments Inc., San Ramon, CA) using two solvent delivery modules (Model 110B) and a manual sample injector (Model 340) with a 100 µl loop and a 5µ C18 HC column (Phenomenex, Torrance, CA). The detection system consisted of an ESA model 5200 Coulochem II multi-electrode electrochemical detector (Environmental Sciences Association, Bedford, MA). Data were interfaced (Beckman 406 analogs) and processed by a analytical series system (Beckman DATA <sup>TM</sup>). One channel on the analog interface was connected to the dual channel coulometric electrochemical detector to monitor the dual electrode transducer at an applied voltage of 150 mV at the analysis electrode with an overall sensitivity of 100 nA and 50 nA, respectively (ESA HPLC guide).

Body weight and feed intake were measured at weekly intervals and feed efficiency determined. Data were analyzed using the General Linear Model of the Statistical Analysis System with line, treatment, and the interactions between them in the model (SAS Institute Inc., 1997). The line by treatment interaction was significant comparisons were made when within each line and treatment. Means were separated using Duncan's multiple range tests. Significant differences imply  $P \leq .05$ .

## **Result**

HWS chickens were heavier than LWS chickens throughout the experiment (Table 1). Injection of reserpine at day of hatch significantly decreased body weight (BW) in both the HWS and LWS chickens during the first three weeks (Table 1, Fig 1.), but BW recovered by the weeks 4 to 6 such that there were no differences between treatments. Following the second injection of reserpine at 6 weeks of age, BW was again significantly decreased with the response being dose-dependent in the HWS birds (Table

5). The response in the LWS birds was not dose-dependent. By 8 weeks, only the BW of the HWS birds treated with reserpine remained lower while in the LWS birds, there was no difference between saline and reserpine injected birds.

Weekly body weight gain per period (GAIN) was significantly different between the HWS and LWS birds throughout the experiment (Table 1). Reserpine significantly reduced gain in both lines during the first week, but there were no differences from 2 to 6 weeks of age. The second injection of reserpine dose-dependently decreased GAIN in the HWS birds (Table 6). While reserpine decreased GAIN in the LWS, the effect was not dose-dependent (Table 6, Fig 2). Cumulative body weight gain (CMGAIN) was generally similar to GAIN. It was significantly different between the lines throughout the experiment. The second reserpine injection dose-dependently decreased CMGAIN in HWS at 7 weeks of age. Although CMGAIN was reduced in LWS birds, it was not dose-dependent (Table 4, Table 9).

Weekly feed consumption (FDCNS) was different between the lines throughout the experiment (Table 2). Reserpine decreased FDCNS in both lines during the first two weeks. At 3 weeks of age, FDCNS remained depressed in the HWS, but not LWS (Table 7). From 4-6 weeks of age, FDCNS was not different among treatments. Following the second injection of reserpine, FDCNS was significantly reduced with the response being dose-dependent for HWS birds. The second injection of reserpine decreased FDCNS in the LWS birds, but effects were not dose-dependent (Table 7, Figure 3).

Cumulative feed consumption (CMFECNS) was different between the HWS and LWS birds throughout the experiment (Table 2). Reserpine significantly decreased CMFECNS in the lines at 2 and 3 weeks of age. The second injection of reserpine significantly decreased CMFECNS in HWS but not LWS birds at 7 weeks of age. By 8 weeks of age, CMFECNS was decreased in a dose-dependent manner only in the HWS birds (Table 7, Fig 4).

Weekly feed efficiency (FE) was different between the two lines through six weeks (Table 3, Fig 5). Reserpine decreased weekly FE at 1 and 7 weeks of age. At 8 weeks of age, the groups receiving 1.25 mg/kg reserpine had significantly higher FE than the other groups in both HWS and LWS chickens (Table 8).

Cumulative feed efficiency (CMFE) was higher in the HWS birds than the LWS birds following the first and second injections of reserpine (Table 3). However, there was no significant difference between all three-dosage groups during the first injection period. Following the second injection of reserpine, CMFE decreased with the effect being dose-dependent in the HWS birds. By the 7<sup>th</sup> weeks of age, reserpine CMFE remained decreased in the HWS birds (Table 8, Fig 6).

### **Catecholamine and indoleamine levels in the brain**

The concentrations of Epi, 5-hydroxyindoleacetic acid (5HIAA), and DA in the brain were significantly different between HWS and LWS birds. Reserpine significantly

decreased the concentration of NE and 5HT in the two lines. Reserpine decreased 5HIAA concentration in the HWS birds while significantly increasing the concentrations of Epi, 5HIAA and DA in LWS birds (Table 11).

### **Catecholamine and indoleamine levels in the heart**

The concentration of 5HIAA, DA and 5HT in the heart was significantly different between the two lines of birds. Reserpine significantly decreased the concentration of DA and 5HT in the HWS, but not LWS. Reserpine injections had no effect in the concentrations of NE and Epi (Table 10, Table 12).

### **Catecholamine and indoleamine levels in the plasma**

The concentration of NE, Epi, 5HIAA, and DA in the plasma was significantly different between the HWS and LWS birds. The injections of reserpine significantly increased the concentrations of DA and 5HT in the HWS birds. In the LWS birds, the injections of reserpine significantly decreased the level of 5HT (Table 10, Table 12).

## **Discussion**

In this experiment, the HWS birds were heavier, had greater BW, GAIN, FE, CMFE, FECNS, CMFE, CMFDCNS, and CMGAIN than the LWS birds. This is consistent with previous reports (Dunnington and Siegel, 1996).

After the second injection of reserpine the HWS birds had a greater response to reserpine than the LWS as indicated by their decreased body weight at 8 weeks of age. This conclusion is supported by the effect on body weight gain per period, feed efficiency, combined feed efficiency, and cumulative body weight gain per period which were similarly decreased. It has been suggested that low sympathetic nervous system activity ability to regulate energy balance and energy expenditure is limited (Saad et al., 1991; Chaloupka et al., 1998). The HWS birds had less sympathetic nervous system activity and higher parasympathetic nervous system activity; therefore, they would have less ability to regulate energy balance and expenditure and less ability recover from the depression caused by the reserpine. The opposite response was found in the LWS birds.

Changes in concentration of neurotransmitters at different sites of the central nervous system could have various effects on food intake and body weight regulation. Infusing NE or Epi into the VMH and PVN increased food intake, body weight gain and reduced sympathetic nervous system activity (Leibowitz and Brown, 1980; Cransac et al., 1996). Robert et al. (1990) indicated that increased dopaminergic activity in the striatum enhanced food intake while increased dopamine in the LH decreased food intake. Meguid et al (1999) reported that transplanting embryonic dopaminergic and serotonergic neurons of lean rats into the LH of obese Zucker rats decreased food intake, indicating involvement of LH DA and 5HT in the regulation of feeding during obesity. Since reserpine caused an overall depletion of catecholamines and 5HT in both the central and peripheral nervous system, and body weight and food intake are controlled by multiple

mechanisms, it is difficult to identify the actual site of depletion that caused the differences between HWS and LWS chickens.

The concentration of DA, NE, and Epi was greater in the heart tissue of LWS than to HWS birds. Studies suggest that low NE concentration in the heart tissue and high plasma NE levels in the obese subjects could be due to less stimulation of the cardiovascular sympathetic nervous system activity (Levin et al, 1983; Piccirillo et al., 1996). It was suggested (Chapter1) that LWS birds have higher sympathetic nervous system activity which could lead to the greater levels of catecholamines in the heart tissue.

The results from the present study suggest differences in autonomic nervous system activity HWS and LWS chickens. Studies have indicated that obese Zucker rats have a higher  $\alpha_2$  receptor activity and less  $\beta_3$  activity which cause increases in food intake and BW (Jhanwar-Uniyal et al., 1991; Tsujii and Bray, 1992; Tsujii, and Bray, 1998). Christin et al. (1993) compared the sympathetic nervous system activity in the Pima Indians who have a higher prevalence of obesity than white Caucasians. The results indicated that Pima Indians and white Caucasians have similar plasma norepinephrine appearance rates, but Pima Indians tend to be more resistant to beta-adrenergic stimulation. Tataranni et al. (1998) compared the sympathetic nervous system beta-adrenergic receptor's sensitivity in Pima Indian and Caucasians. The result showed that Pima Indian males had lower beta-adrenergic sensitivity than Caucasians. Therefore, increased body weight may be associated with lower beta-adrenergic sensitivity which can cause low sympathetic nervous system activity (Tataranni et al., 1998).

Denbow et al. (1984) reported that intracerebroventricular injection of serotonin decreased food intake, and the HWS birds were more sensitive to the intracerebroventricular injection of serotonin as evidence by its longer action than in LWS birds. Moreover, the ventromedial nucleus serotonergic activity was reduced in obese Zucker rats (Routh et al., 1994). These results suggest that animals differing in body weight may have differing numbers of receptors, and rate of metabolism of serotonin.

In conclusion, reserpine depressed overall sympathetic nervous system activity, which decreased body weight and feed intake in both HWS and LWS chickens. However, the LWS birds recovered from the depression faster than HWS birds confirming earlier results (Chapter 1) showing that LWS birds have greater sympathetic nervous system activity to maintain cardiovascular homeostasis. HWS chickens are heavier and have greater feed intake than LWS birds, which may be caused by less stimulation of  $\beta$  receptors. Another suggestion is that HWS birds may be more sensitive to 5HT than the LWS.

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**Table 1. Effect of reserpine on body weight (g) and body weight gain per period (g) of high weight and low weight selected lines of chickens<sup>1</sup>**

| wks | Line                            |   |              | Treatment                  |                           |                           |
|-----|---------------------------------|---|--------------|----------------------------|---------------------------|---------------------------|
|     | HWS                             |   | LWS          | Saline                     | 1.25mg/kg                 | 2.50mg/kg                 |
|     | Body Weight (g)                 |   |              |                            |                           |                           |
| 1   | ⑤                               |   |              |                            |                           |                           |
| 2   | 154.2 ± 2.72                    | * | 46.6 ± 0.87  | 106.7 ± 17.50 <sup>a</sup> | 98.0 ± 15.46 <sup>b</sup> | 96.5 ± 16.05 <sup>b</sup> |
| 3   | ⑤                               |   |              |                            |                           |                           |
| 4   | ⑤                               |   |              |                            |                           |                           |
| 5   | 635.6 ± 11.99                   | * | 108.8 ± 2.48 | 381.9 ± 84.52              | 364.0 ± 76.49             | 370.6 ± 79.12             |
| 6   | 861.9 ± 12.33                   | * | 135.9 ± 3.43 | 509.7 ± 115.13             | 489.8 ± 106.49            | 497.3 ± 108.18            |
| 7   | ⑤                               |   |              |                            |                           |                           |
| 8   | ⑤                               |   |              |                            |                           |                           |
|     | Body weight gain per period (g) |   |              |                            |                           |                           |
| 1   |                                 |   |              |                            |                           |                           |
| 2   | 80.2 ± 1.72                     | * | 15.2 ± 0.57  | 49.8 ± 10.38               | 47.1 ± 9.43               | 46.1 ± 9.90               |
| 3   | ⑥                               |   |              |                            |                           |                           |
| 4   | 162.4 ± 2.34                    | * | 20.1 ± 1.08  | 90.9 ± 22.10               | 91.7 ± 21.05              | 91.2 ± 21.60              |
| 5   | 197.6 ± 6.90                    | * | 23.8 ± 1.31  | 111.4 ± 28.24              | 107.6 ± 26.00             | 113.1 ± 26.41             |
| 6   | 226.3 ± 4.77                    | * | 27.2 ± 1.48  | 127.8 ± 31.42              | 125.8 ± 30.34             | 126.6 ± 29.23             |
| 7   | ⑥                               |   |              |                            |                           |                           |
| 8   | ⑥                               |   |              |                            |                           |                           |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

⑤⑥ = Indicated line by treatment interaction is significant and in shown Table 5 and 6, respectively.

**Table 2. Effect of reserpine on feed consumption (g) and cumulative feed consumption (g) of high weight and low weight selected chickens<sup>1</sup>**

| wks | Line                            |               | Treatment                  |                            |                             |  |
|-----|---------------------------------|---------------|----------------------------|----------------------------|-----------------------------|--|
|     | HWS                             | LWS           | Saline                     | 1.25mg/kg                  | 2.50mg/kg                   |  |
|     | Feed Consumption (g)            |               |                            |                            |                             |  |
| 1   | 71.5 ± 2.17 *                   | 46.7 ± 2.23   | 66.1 ± 4.23 <sup>a</sup>   | 55.6 ± 4.81 <sup>b</sup>   | 55.8 ± 4.10 <sup>b</sup>    |  |
| 2   | 146.3 ± 3.53 *                  | 78.2 ± 4.25   | 121.6 ± 12.67 <sup>a</sup> | 104.6 ± 10.30 <sup>b</sup> | 110.5 ± 10.34 <sup>ab</sup> |  |
| 3   | ⑦                               |               |                            |                            |                             |  |
| 4   | 345.8 ± 4.78 *                  | 142.9 ± 7.23  | 239.9 ± 34.01              | 250.0 ± 28.20              | 243.2 ± 32.17               |  |
| 5   | 465.0 ± 10.00 *                 | 165.5 ± 4.80  | 316.9 ± 48.85              | 313.4 ± 44.03              | 315.5 ± 45.52               |  |
| 6   | 564.6 ± 7.32 *                  | 175.7 ± 5.64  | 378.1 ± 60.94              | 363.8 ± 59.14              | 368.5 ± 57.34               |  |
| 7   | ⑦                               |               |                            |                            |                             |  |
| 8   | ⑦                               |               |                            |                            |                             |  |
|     | Cumulative Feed Consumption (g) |               |                            |                            |                             |  |
| 2   | 217.9 ± 5.31 *                  | 124.9 ± 5.00  | 187.7 ± 16.10 <sup>a</sup> | 160.2 ± 14.59 <sup>b</sup> | 166.3 ± 14.22 <sup>b</sup>  |  |
| 3   | 466.6 ± 9.30 *                  | 195.4 ± 5.53  | 353.2 ± 45.21 <sup>a</sup> | 317.2 ± 39.13 <sup>b</sup> | 322.6 ± 40.52 <sup>b</sup>  |  |
| 4   | 812.4 ± 13.12 *                 | 338.3 ± 10.17 | 593.0 ± 78.85              | 567.3 ± 66.81              | 565.8 ± 72.38               |  |
| 5   | 1277.4 ± 21.22 *                | 503.9 ± 13.95 | 909.9 ± 127.21             | 880.7 ± 110.46             | 881.3 ± 117.70              |  |
| 6   | 1842.0 ± 26.80 *                | 679.6 ± 17.82 | 1288.0 ± 187.80            | 1244.5 ± 169.12            | 1249.8 ± 174.80             |  |
| 7   | ⑦                               |               |                            |                            |                             |  |
| 8   | ⑦                               |               |                            |                            |                             |  |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

⑦ = Indicated line by treatment interaction is significant and in shown Table 7, respectively.

**Table 3. Effect of reserpine on feed efficiency and cumulative feed efficiency of high weight and low weight selected chickens<sup>1</sup>**

| wks | Line                                             |     | Treatment   |                         |                          |                         |
|-----|--------------------------------------------------|-----|-------------|-------------------------|--------------------------|-------------------------|
|     | HWS                                              | LWS | Saline      | 1.25mg/kg               | 2.50mg/kg                |                         |
|     | Feed Efficiency (g body weight/ g feed consumed) |     |             |                         |                          |                         |
| 1   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.4 ± 0.05 <sup>a</sup> | 0.3 ± 0.05 <sup>b</sup>  | 0.3 ± 0.05 <sup>b</sup> |
| 2   | 0.6 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.4 ± 0.05              | 0.4 ± 0.05               | 0.4 ± 0.06              |
| 3   | 0.5 ± 0.01                                       | *   | 0.3 ± 0.01  | 0.4 ± 0.04              | 0.4 ± 0.03               | 0.4 ± 0.03              |
| 4   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.02  | 0.3 ± 0.05              | 0.3 ± 0.05               | 0.3 ± 0.05              |
| 5   | 0.4 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.3 ± 0.04              | 0.3 ± 0.04               | 0.3 ± 0.04              |
| 6   | 0.4 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.3 ± 0.04 <sup>a</sup> | 0.3 ± 0.04 <sup>ab</sup> | 0.3 ± 0.04 <sup>b</sup> |
| 7   | -0.3 ± 0.23                                      |     | -0.6 ± 0.34 | 0.3 ± 0.04              | -0.7 ± 0.43              | -1.0 ± 0.37             |
| 8   | ⑧                                                |     |             |                         |                          |                         |
|     | Cumulative Feed Efficiency                       |     |             |                         |                          |                         |
| 2   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.4 ± 0.05              | 0.4 ± 0.05               | 0.4 ± 0.06              |
| 3   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.4 ± 0.05              | 0.4 ± 0.04               | 0.4 ± 0.05              |
| 4   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.3 ± 0.05              | 0.3 ± 0.05               | 0.3 ± 0.05              |
| 5   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.3 ± 0.05              | 0.3 ± 0.04               | 0.3 ± 0.05              |
| 6   | 0.5 ± 0.01                                       | *   | 0.2 ± 0.01  | 0.3 ± 0.04              | 0.3 ± 0.04               | 0.1 ± 0.04              |
| 7   | ⑧                                                |     |             |                         |                          |                         |
| 8   | 0.3 ± 0.01                                       |     | 0.1 ± 0.01  | 0.3 ± 0.03 <sup>a</sup> | 0.3 ± 0.03 <sup>a</sup>  | 0.2 ± 0.03 <sup>b</sup> |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

⑧ = Indicated line by treatment interaction is significant and in shown Table 8, respectively.

**Table 4. Effect of reserpine on combined body weight gain per period (g) of high body weight and low body weight selected chickens<sup>1</sup>**

| wks | Line          |                | Treatment      |                |                |
|-----|---------------|----------------|----------------|----------------|----------------|
|     | HWS           | LWS            | Saline         | 1.25mg/kg      | 2.50mg/kg      |
| 2   | ⑨             |                |                |                |                |
| 3   | ⑨             |                |                |                |                |
| 4   | ⑨             |                |                |                |                |
| 5   | 597.2 ± 11.94 | * 85.1 ± 2.48  | 350.9 ± 82.31  | 332.9 ± 74.27  | 339.6 ± 76.90  |
| 6   | 823.5 ± 12.23 | * 112.2 ± 3.41 | 478.7 ± 112.91 | 458.7 ± 107.25 | 466.2 ± 105.97 |
| 7   | ⑨             |                |                |                |                |
| 8   | ⑨             |                |                |                |                |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect ( $P \leq .05$ ).

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different ( $P \leq .05$ ).

⑨ = Indicated line by treatment interaction is significant and in shown Table 9, respectively.

**Table 5. Effect of reserpine on body weight (g) of HWS and LWS chickens where line by treatment interaction was significant<sup>1</sup>**

| wks | Treatment | Line                        |    |                           |
|-----|-----------|-----------------------------|----|---------------------------|
|     |           | HWS                         |    | LWS                       |
| 1wk | Saline    | 80.3 ± 1.67 <sup>a</sup>    | *  | 33.4 ± 0.70 <sup>a</sup>  |
|     | 1.25mg/kg | 71.1 ± 1.98 <sup>b</sup>    | *  | 30.8 ± 0.89 <sup>b</sup>  |
|     | 2.50mg/kg | 70.7 ± 1.18 <sup>b</sup>    | *  | 23.0 ± 0.43 <sup>b</sup>  |
| 3wk | Saline    | 294.3 ± 7.04 <sup>a</sup>   | *  | 64.9 ± 1.55               |
|     | 1.25mg/kg | 262.5 ± 7.80 <sup>b</sup>   | *  | 66.9 ± 2.62               |
|     | 2.50mg/kg | 270.1 ± 5.50 <sup>b</sup>   | *  | 62.8 ± 1.07               |
| 4wk | Saline    | 458.2 ± 10.19               | *  | 82.8 ± 1.78               |
|     | 1.25mg/kg | 423.3 ± 12.74               | *  | 89.6 ± 4.59               |
|     | 2.50mg/kg | 432.6 ± 6.98                | *  | 82.6 ± 1.87               |
| 7wk | Saline    | 1123.7 ± 28.12 <sup>a</sup> | *  | 153.0 ± 3.80 <sup>a</sup> |
|     | 1.25mg/kg | 816.6 ± 33.76 <sup>b</sup>  | *  | 117.9 ± 6.87 <sup>b</sup> |
|     | 2.50mg/kg | 733.6 ± 16.16 <sup>c</sup>  | *  | 123.3 ± 8.68 <sup>b</sup> |
| 8wk | Saline    | 1344.0 ± 37.52 <sup>a</sup> | *  | 184.6 ± 5.85              |
|     | 1.25mg/kg | 1012.8 ± 23.22 <sup>b</sup> | NS | 164.1 ± 9.69              |
|     | 2.50mg/kg | 720.4 ± 43.46 <sup>c</sup>  | NS | 156.4 ± 7.96              |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect ( $P \leq .05$ ).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different ( $P \leq .05$ ).

**Table 6. Effect of reserpine on body weight gain per period (g) of HWS and LWS chickens where line by treatment interaction was significant<sup>1</sup>**

| wks | Treatment | Line                        |    |                           |
|-----|-----------|-----------------------------|----|---------------------------|
|     |           | HWS                         |    | LWS                       |
| 1wk | Saline    | 42.0 ± 1.56 <sup>a</sup>    | *  | 9.8 ± 0.53 <sup>a</sup>   |
|     | 1.25mg/kg | 32.4 ± 1.42 <sup>b</sup>    | *  | 7.1 ± 0.53 <sup>b</sup>   |
|     | 2.50mg/kg | 32.3 ± 1.23 <sup>b</sup>    | *  | 6.2 ± 0.41 <sup>b</sup>   |
| 3wk | Saline    | 129.8 ± 4.47 <sup>a</sup>   | *  | 15.9 ± 1.10               |
|     | 1.25mg/kg | 113.8 ± 5.85 <sup>b</sup>   | *  | 19.7 ± 1.46               |
|     | 2.50mg/kg | 120.8 ± 2.20 <sup>ab</sup>  | *  | 19.2 ± 1.37               |
| 7wk | Saline    | 233.9 ± 8.28 <sup>a</sup>   | *  | 23.4 ± 2.38 <sup>a</sup>  |
|     | 1.25mg/kg | -23.9 ± 29.47 <sup>b</sup>  | *  | -21.3 ± 5.67 <sup>b</sup> |
|     | 2.50mg/kg | -121.9 ± 23.66 <sup>c</sup> | *  | -15.8 ± 6.69 <sup>b</sup> |
| 8wk | Saline    | 220.3 ± 11.89 <sup>a</sup>  | NS | 31.7 ± 3.21               |
|     | 1.25mg/kg | 196.2 ± 30.24 <sup>b</sup>  | NS | 46.3 ± 4.49               |
|     | 2.50mg/kg | -13.2 ± 31.94 <sup>b</sup>  | NS | 33.1 ± 5.65               |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

**Table 7. Effect of reserpine on feed consumption (g) and cumulative feed consumption (g) of HWS and LWS chickens where line by treatment interaction was significant<sup>1</sup>**

| wks                                       | Treatment | Line     |                     |                     |          |                     |
|-------------------------------------------|-----------|----------|---------------------|---------------------|----------|---------------------|
|                                           |           | HWS      |                     | Feed Consumption(g) | LWS      |                     |
|                                           |           |          |                     |                     |          |                     |
| 3wk                                       | Saline    | 262.8 ±  | 6.79 <sup>a</sup>   | *                   | 68.2 ±   | 3.71                |
|                                           | 1.25mg/kg | 238.4 ±  | 7.71 <sup>b</sup>   | *                   | 75.7 ±   | 6.68                |
|                                           | 2.50mg/kg | 245.0 ±  | 4.05 <sup>ab</sup>  | *                   | 67.6 ±   | 3.06                |
| 7wk                                       | Saline    | 610.2 ±  | 16.36 <sup>a</sup>  | *                   | 172.3 ±  | 20.79 <sup>a</sup>  |
|                                           | 1.25mg/kg | 264.1 ±  | 20.17 <sup>b</sup>  | *                   | 51.0 ±   | 12.79 <sup>b</sup>  |
|                                           | 2.50mg/kg | 145.0 ±  | 25.01 <sup>c</sup>  | P=0.0583            | 79.8 ±   | 17.44               |
| 8wk                                       | Saline    | 1176.7 ± | 11.89 <sup>a</sup>  | *                   | 340.9 ±  | 28.18 <sup>a</sup>  |
|                                           | 1.25mg/kg | 868.5 ±  | 30.24 <sup>a</sup>  | *                   | 185.0 ±  | 30.30 <sup>b</sup>  |
|                                           | 2.50mg/kg | 329.6 ±  | 31.94 <sup>b</sup>  | NS                  | 241.1 ±  | 38.02 <sup>ab</sup> |
| ———— Cumulative Feed Consumption (g) ———— |           |          |                     |                     |          |                     |
| 7wk                                       | Saline    | 2516.9 ± | 57.25 <sup>a</sup>  | *                   | 841.7 ±  | 21.72               |
|                                           | 1.25mg/kg | 2061.4 ± | 52.14 <sup>b</sup>  | *                   | 742.7 ±  | 60.39               |
|                                           | 2.50mg/kg | 1966.9 ± | 21.18 <sup>b</sup>  | *                   | 757.5 ±  | 60.08               |
| 8wk                                       | Saline    | 3693.5 ± | 85.75 <sup>a</sup>  | *                   | 1182.6 ± | 45.19 <sup>a</sup>  |
|                                           | 1.25mg/kg | 2929.9 ± | 195.28 <sup>b</sup> | NS                  | 927.6 ±  | 54.17 <sup>b</sup>  |
|                                           | 2.50mg/kg | 2296.5 ± | 68.79 <sup>c</sup>  | NS                  | 998.6 ±  | 90.33 <sup>ab</sup> |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

**Table 8. Effect of reserpine on feed efficiency (g/g) and cumulative feed efficiency (g/g) of HWS and LWS chickens where line by treatment interaction was significant<sup>1</sup>**

|                                    |           | Line                     |    |                          |
|------------------------------------|-----------|--------------------------|----|--------------------------|
| Wks                                | Treatment | HWS                      |    | LWS                      |
| —Feed Efficiency (g/ g )—          |           |                          |    |                          |
| 8wk                                | Saline    | 0.2 ± 0.02 <sup>a</sup>  | *  | 0.1 ± 0.01 <sup>b</sup>  |
|                                    | 1.25mg/kg | 0.3 ± 0.05 <sup>a</sup>  | NS | 0.3 ± 0.07 <sup>a</sup>  |
|                                    | 2.50mg/kg | -0.3 ± 0.26 <sup>b</sup> | NS | 0.2 ± 0.06 <sup>ab</sup> |
| —Cumulative Feed Efficiency (g/g)— |           |                          |    |                          |
| 7wk                                | Saline    | 0.43 ± 0.01 <sup>a</sup> | *  | 0.15 ± 0.01              |
|                                    | 1.25mg/kg | 0.37 ± 0.01 <sup>b</sup> | *  | 0.13 ± 0.01              |
|                                    | 2.50mg/kg | 0.35 ± 0.01 <sup>c</sup> | *  | 0.13 ± 0.01              |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

**Table 9. Effect of reserpine on combined body weight gain per period (g) of HWS and LWS chickens where line by treatment interaction was significant<sup>1</sup>**

| Wks | Treatment | Line                        |    |                           |
|-----|-----------|-----------------------------|----|---------------------------|
|     |           | HWS                         |    | LWS                       |
| 2wk | Saline    | 0.5 ± 0.01 <sup>a</sup>     | *  | 0.2 ± 0.01 <sup>a</sup>   |
|     | 1.25mg/kg | 0.5 ± 0.02 <sup>b</sup>     | *  | 0.2 ± 0.02 <sup>a</sup>   |
|     | 2.50mg/kg | 0.5 ± 0.01 <sup>b</sup>     | *  | 0.2 ± 0.02 <sup>b</sup>   |
| 3wk | Saline    | 255.9 ± 6.92 <sup>a</sup>   | *  | 41.3 ± 1.69               |
|     | 1.25mg/kg | 224.0 ± 7.41 <sup>b</sup>   | *  | 43.2 ± 2.42               |
|     | 2.50mg/kg | 231.7 ± 5.48 <sup>b</sup>   | *  | 39.0 ± 0.98               |
| 4wk | Saline    | 419.9 ± 10.11               | *  | 59.2 ± 1.84               |
|     | 1.25mg/kg | 384.7 ± 12.43               | *  | 65.9 ± 4.46               |
|     | 2.50mg/kg | 394.3 ± 6.88                | *  | 58.8 ± 1.84               |
| 7wk | Saline    | 1085.3 ± 27.98 <sup>a</sup> | *  | 129.4 ± 3.83 <sup>a</sup> |
|     | 1.25mg/kg | 778.0 ± 33.34 <sup>b</sup>  | *  | 94.2 ± 6.74 <sup>b</sup>  |
|     | 2.50mg/kg | 695.3 ± 16.68 <sup>c</sup>  | *  | 99.5 ± 8.75 <sup>b</sup>  |
| 8wk | Saline    | 1305.6 ± 37.38 <sup>a</sup> | *  | 161.0 ± 6.01              |
|     | 1.25mg/kg | 974.2 ± 22.73 <sup>b</sup>  | NS | 140.5 ± 9.60              |
|     | 2.50mg/kg | 682.1 ± 44.01 <sup>c</sup>  | NS | 132.7 ± 8.01              |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

**Table 10. Effect of reserpine on NE, Epi, 5HIAA, DA and 5HT levels of HWS and LWS chicken's heart tissue and plasma**

| Neurotransmitter | Line          |    |               | Treatment                 |                            |                            |
|------------------|---------------|----|---------------|---------------------------|----------------------------|----------------------------|
|                  | HWS           |    | LWS           | Saline                    | 1.25mg/kg                  | 2.50mg/kg                  |
| Heart            |               |    |               |                           |                            |                            |
| NE               | 84.1 ± 15.84  | *  | 395.9 ± 53.76 | 190.4 ± 51.55             | 213.9 ± 48.20              | 300.3 ± 73.28              |
| Epi              | 6.0 ± 1.57    | *  | 36.2 ± 7.06   | 14.7 ± 7.13               | 21.9 ± 7.03                | 27.2 ± 7.74                |
| 5HIAA            | 32.0 ± 5.55   | *  | 229.7 ± 50.40 | 63.9 ± 13.91 <sup>b</sup> | 107.7 ± 35.13 <sup>b</sup> | 201.5 ± 66.75 <sup>a</sup> |
| DA               |               |    |               |                           |                            |                            |
| 5HT              |               |    |               |                           |                            |                            |
| Plasma           |               |    |               |                           |                            |                            |
| NE               | 125.1 ± 29.04 | NS | 70.8 ± 24.18  | 80.6 ± 21.74              | 68.6 ± 22.94               | 145.2 ± 46.53              |
| Epi              | 146.3 ± 23.72 | NS | 98.2 ± 16.52  | 127.8 ± 23.41             | 95.2 ± 18.83               | 143.7 ± 32.21              |
| 5HIAA            |               |    |               |                           |                            |                            |
| DA               |               |    |               |                           |                            |                            |
| 5HT              |               |    |               |                           |                            |                            |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect ( $P \leq .05$ ).

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different ( $P \leq .05$ ).

□ = Indicated line by treatment interaction is significant and in shown Table 11 and 12, respectively.

**Table 11. Effect of reserpine on NE, Epi, 5HIAA, DA and 5HT levels of HWS and LWS chicken's brain tissue where line by treatment interaction was significant**

| Neurotransmitter | Treatment | Line                          |    |  |                               |  |
|------------------|-----------|-------------------------------|----|--|-------------------------------|--|
|                  |           | HWS                           |    |  | LWS                           |  |
| NE               | Saline    | 4463.6 ± 1145.75 <sup>a</sup> | *  |  | 9375.8 ± 1862.31 <sup>a</sup> |  |
|                  | 1.25mg/kg | 3252.9 ± 736.68 <sup>ab</sup> |    |  | 2411.5 ± 784.08 <sup>b</sup>  |  |
|                  | 2.50mg/kg | 1021.9 ± 264.97 <sup>b</sup>  | *  |  | 208.7 ± 77.19 <sup>c</sup>    |  |
| Epi              | Saline    | 112.8 ± 34.64                 | NS |  | 95.7 ± 32.19 <sup>b</sup>     |  |
|                  | 1.25mg/kg | 55.3 ± 14.73                  | NS |  | 37.4 ± 9.91 <sup>c</sup>      |  |
|                  | 2.50mg/kg | 63.4 ± 19.82                  | *  |  | 300.1 ± 18.50 <sup>a</sup>    |  |
| 5HIAA            | Saline    | 643.8 ± 66.99 <sup>a</sup>    | NS |  | 739.8 ± 96.85 <sup>ab</sup>   |  |
|                  | 1.25mg/kg | 229.1 ± 31.99 <sup>b</sup>    | NS |  | 428.3 ± 69.10 <sup>b</sup>    |  |
|                  | 2.50mg/kg | 178.5 ± 49.22 <sup>b</sup>    | *  |  | 982.3 ± 175.83 <sup>a</sup>   |  |
| DA               | Saline    | 121.9 ± 17.20                 | *  |  | 1120.4 ± 468.05 <sup>a</sup>  |  |
|                  | 1.25mg/kg | 124.7 ± 24.29                 | NS |  | 193.9 ± 40.04 <sup>b</sup>    |  |
|                  | 2.50mg/kg | 78.4 ± 24.02                  | *  |  | 785.8 ± 155.02 <sup>a</sup>   |  |
| 5HT              | Saline    | 1541.6 ± 454.32 <sup>a</sup>  | NS |  | 1148.1 ± 378.41 <sup>a</sup>  |  |
|                  | 1.25mg/kg | 205.8 ± 43.23 <sup>b</sup>    | *  |  | 401.0 ± 73.17 <sup>b</sup>    |  |
|                  | 2.50mg/kg | 100.4 ± 13.19 <sup>b</sup>    | *  |  | 418.8 ± 48.64 <sup>b</sup>    |  |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

**Table 12. Effect of reserpine on NE, Epi, 5HIAA, DA and 5HT levels of HWS and LWS chicken's heart tissue and plasma where line by treatment interaction was significant**

| Neurotransmitter | Treatment | Line     |                      |       |          |                     |
|------------------|-----------|----------|----------------------|-------|----------|---------------------|
|                  |           | HWS      |                      | Heart | LWS      |                     |
|                  |           |          |                      |       |          |                     |
| DA               | Saline    | 20.9 ±   | 5.24 <sup>b</sup>    | *     | 103.8 ±  | 16.59               |
|                  | 1.25mg/kg | 21.8 ±   | 5.75 <sup>b</sup>    | *     | 111.2 ±  | 13.77               |
|                  | 2.50mg/kg | 39.8 ±   | 6.06 <sup>a</sup>    | *     | 92.2 ±   | 9.46                |
| 5HT              | Saline    | 0.2 ±    | 0.01 <sup>b</sup>    | *     | 1305.0 ± | 472.49 <sup>a</sup> |
|                  | 1.25mg/kg | 40.1 ±   | 26.46 <sup>b</sup>   | NS    | 31.7 ±   | 30.41 <sup>b</sup>  |
|                  | 2.50mg/kg | 304.0 ±  | 81.94 <sup>a</sup>   | *     | 1.1 ±    | 0.10 <sup>b</sup>   |
| 5HIAA            | Saline    | 971.7 ±  | 224.32               | *     | 183.4 ±  | 41.67               |
|                  | 1.25mg/kg | 428.8 ±  | 185.72               | NS    | 229.8 ±  | 65.21               |
|                  | 2.50mg/kg | 453.0 ±  | 171.82               | NS    | 641.1 ±  | 231.02              |
| DA               | Saline    | 2740.3 ± | 440.76 <sup>a</sup>  | NS    | 2463.2 ± | 380.65              |
|                  | 1.25mg/kg | 1903.2 ± | 456.27 <sup>ab</sup> | NS    | 1499.0 ± | 276.30              |
|                  | 2.50mg/kg | 1060.8±  | 242.84 <sup>b</sup>  | *     | 3216.1 ± | 659.79              |
| 5HT              | Saline    | 3963.0 ± | 1064.30 <sup>a</sup> | *     | 1315.1 ± | 150.99              |
|                  | 1.25mg/kg | 1236.6 ± | 232.15 <sup>b</sup>  | NS    | 896.4 ±  | 267.07              |
|                  | 2.50mg/kg | 652.6 ±  | 131.57 <sup>b</sup>  | NS    | 1038.8 ± | 27.92               |

<sup>1</sup>Value represents Mean ± SE.

\* = Significant line effect (P ≤ .05).

NS = Means where line was not significant.

<sup>a,b,c</sup> Means within a treatment with different superscripts are significantly different (P ≤ .05).

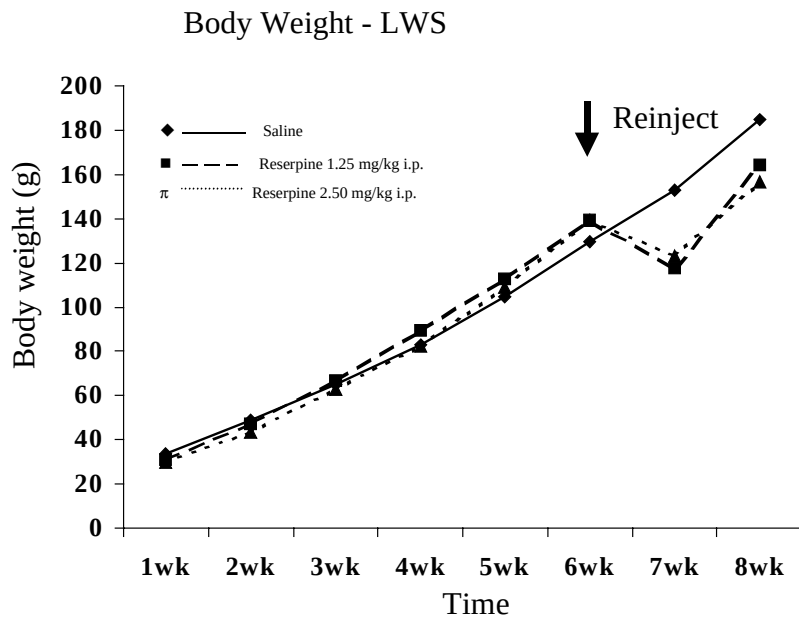
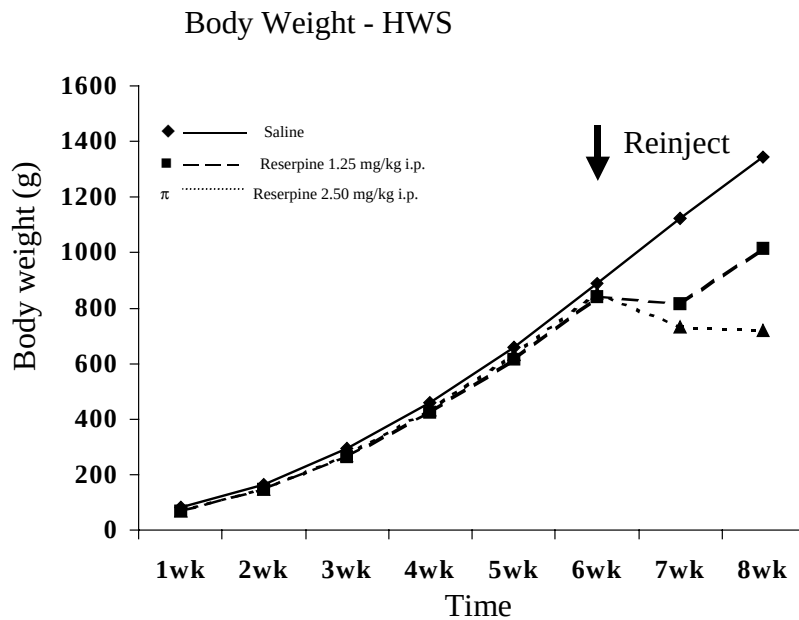


**Figure 1.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on average body weight (BW) of high (HWS) and low (LWS) body weight select chicken.

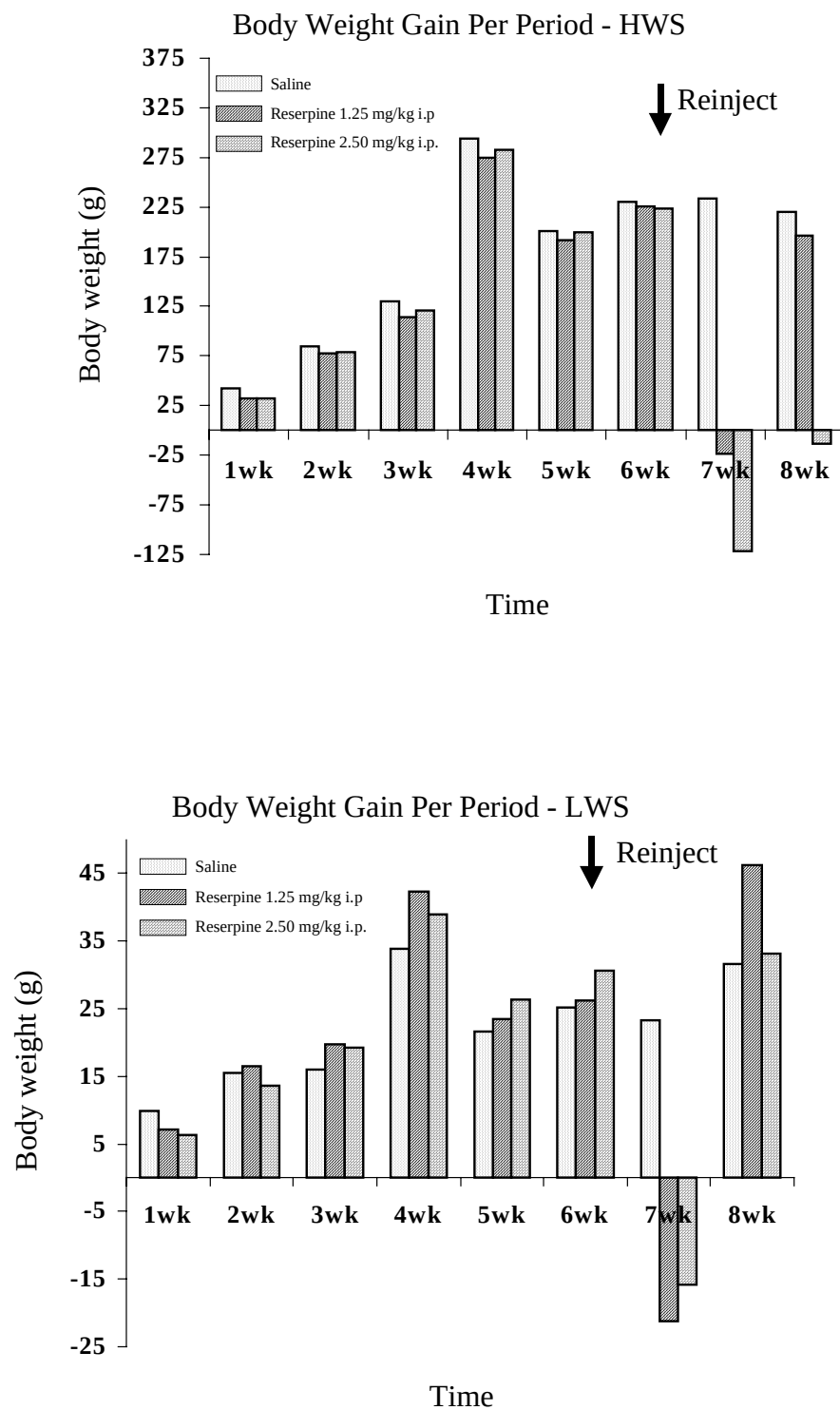


**Figure 2.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on body weight gain per day (GAINPD) of high (HWS) and low (LWS) body weight select chicken.

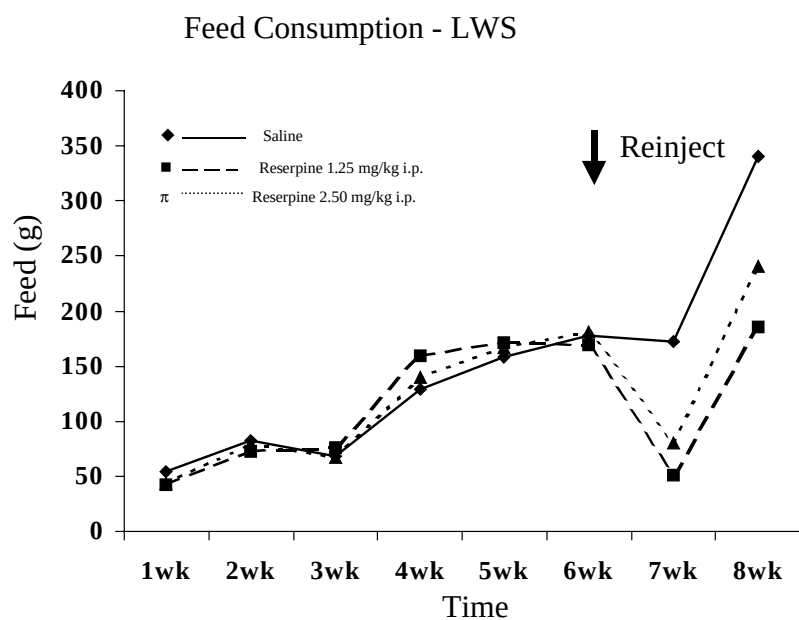
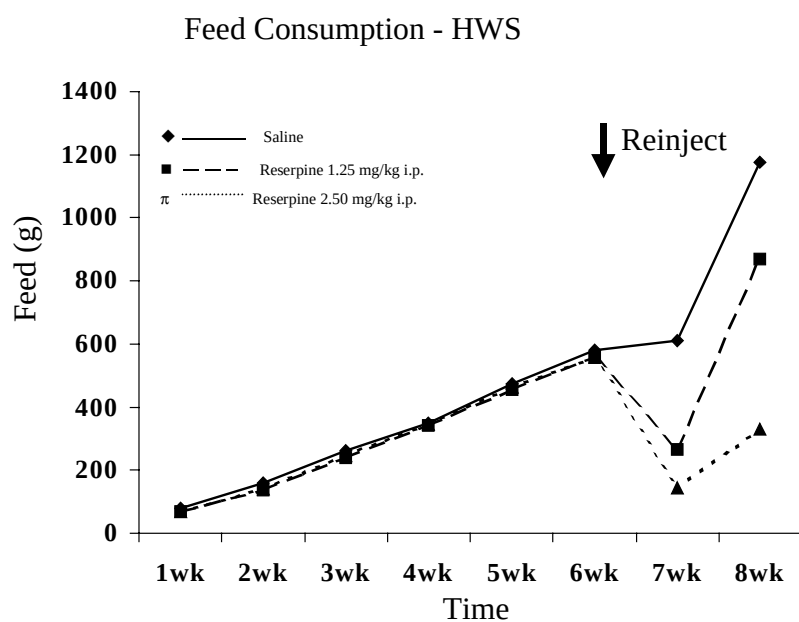


**Figure 3.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on average feed consumed (FDCNS) of high (HWS) and low (LWS) body weight select chicken.

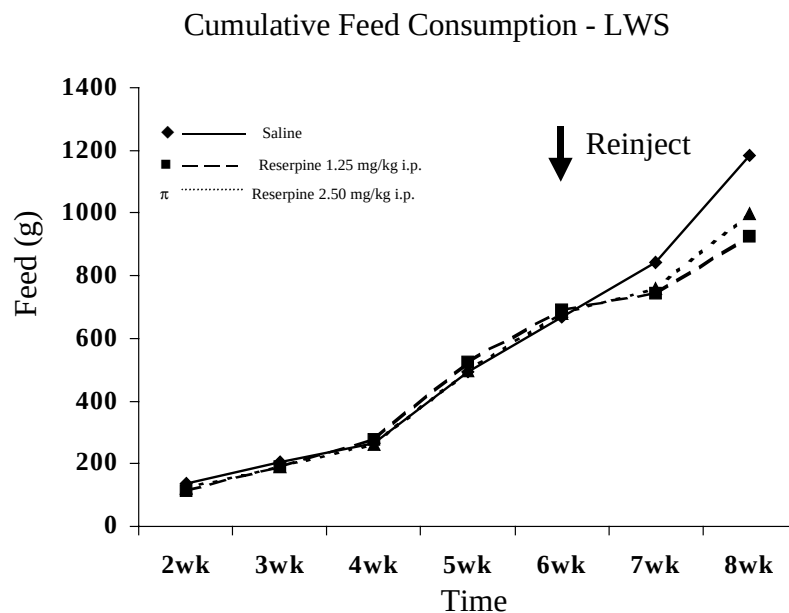
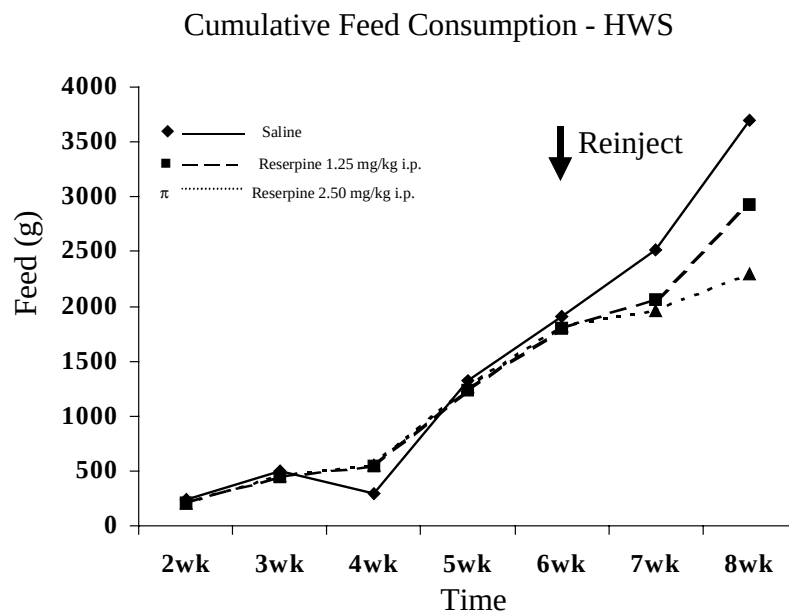


**Figure 4.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on cumulative feed consumption (CMFDCNS) of high (HWS) and low (LWS) body weight select chicken

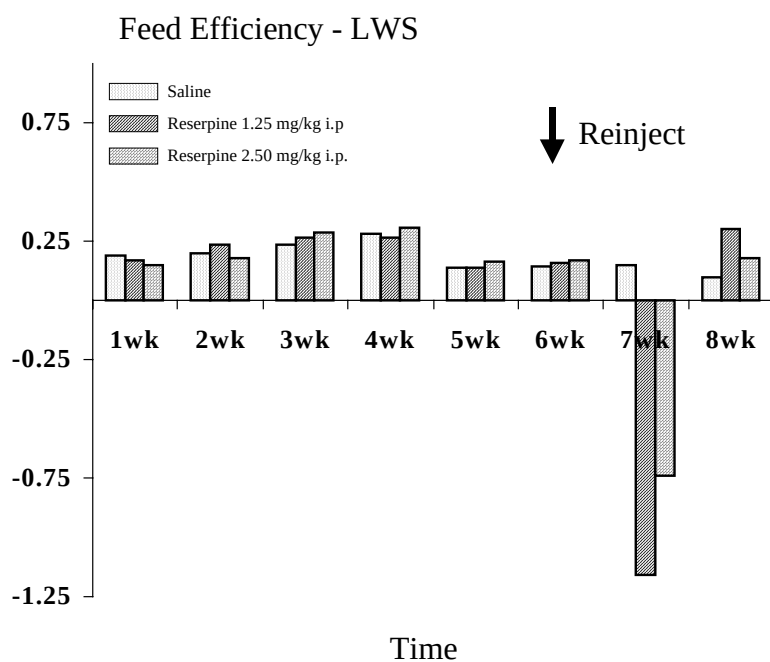
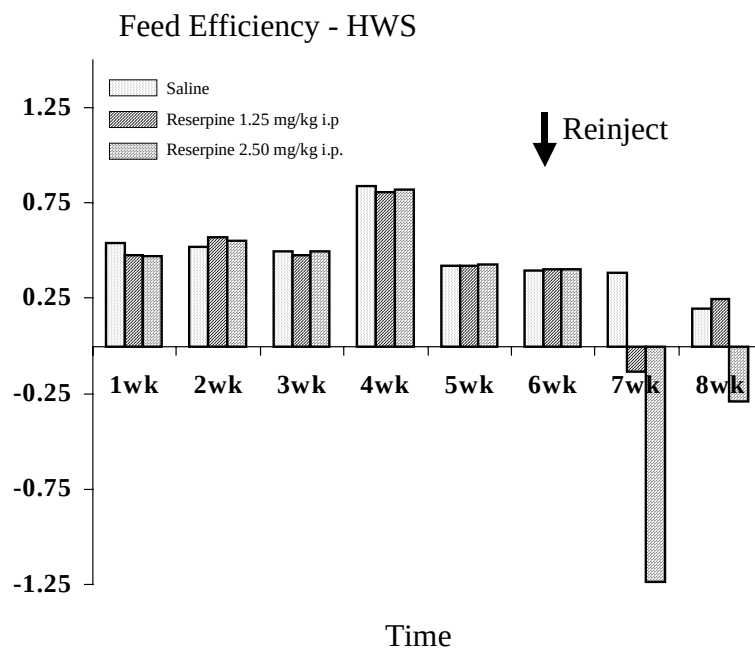


**Figure 5.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on average feed efficiency (FE) of high (HWS) and low (LWS) body weight select chicken

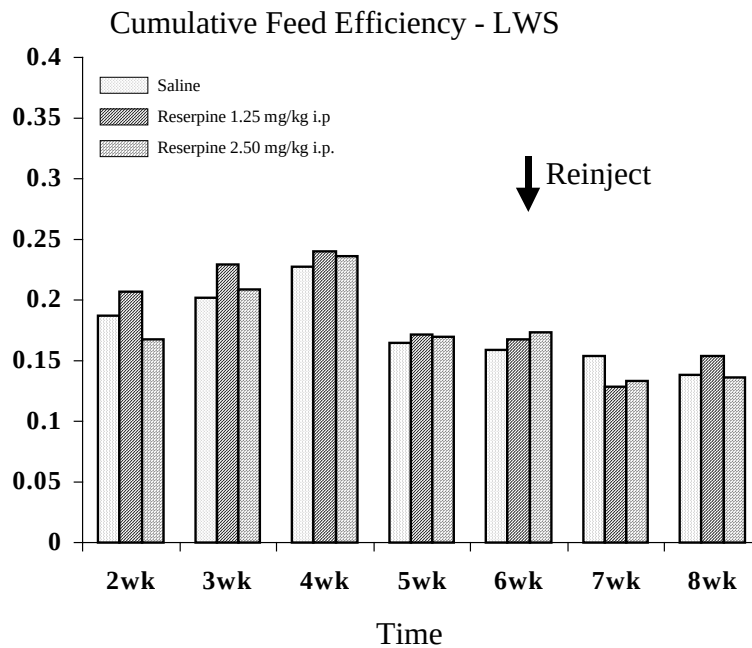
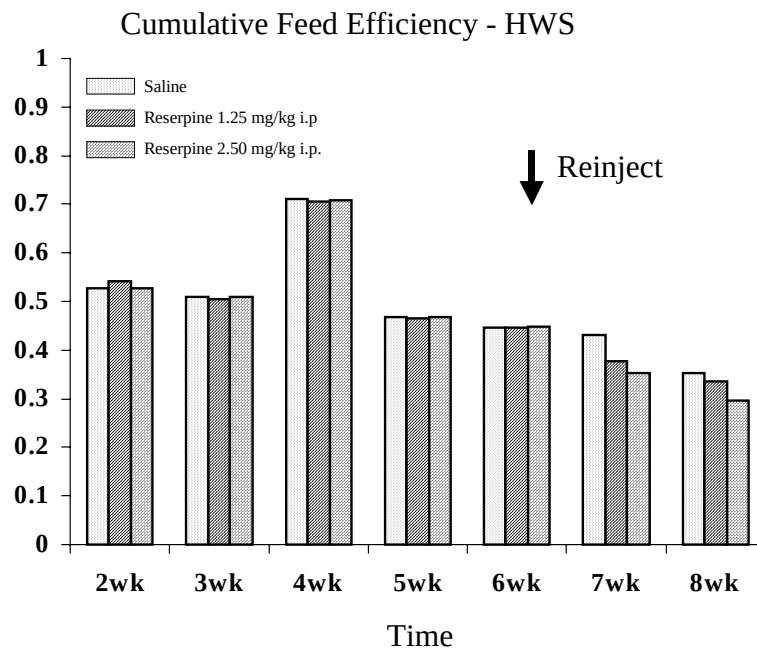


**Figure 6.** Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on cumulative feed efficiency (CMFE) of high (HWS) and low (LWS) body weight select chicken

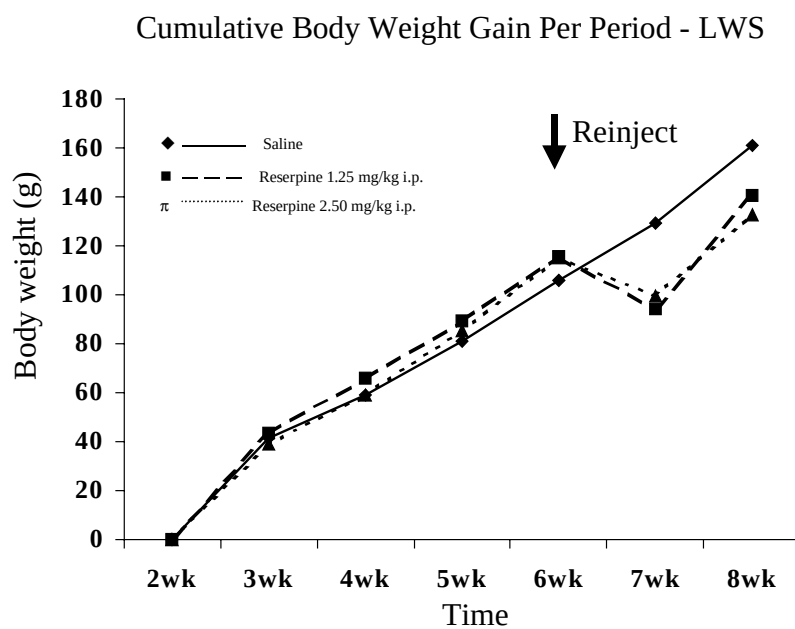
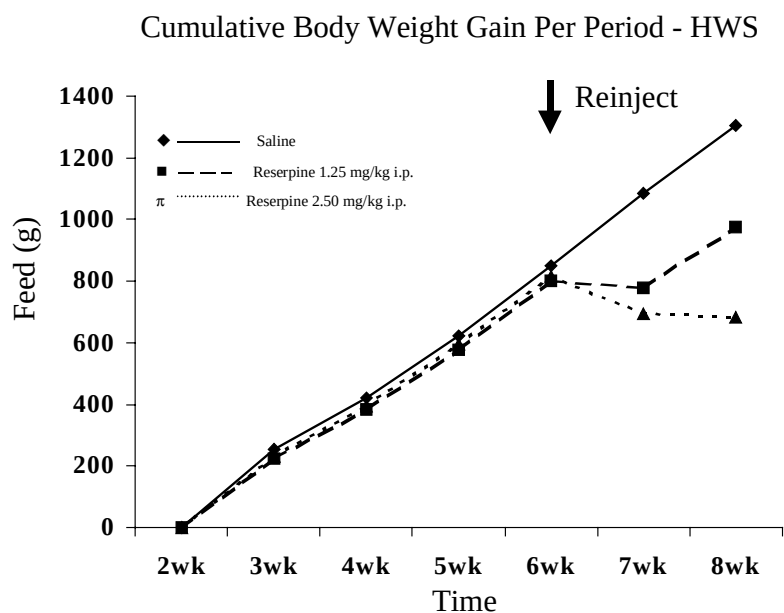


**Figure 7.** . Effect of intraperitoneal injection of saline, 1.25mg/kg of reserpine, and 2.50 mg/kg reserpine on cumulative body weight gain per day (CMGAIN) of high (HWS) and low (LWS) body weight select chicken.

## SUMMARY

The results of studies examining the regulation of selection for body weight reduced sympathetic nervous system activity. In the peripheral autonomic nervous system cardiovascular activity suggest that HWS birds have lower sympathetic nervous system activity and higher parasympathetic input to maintain homeostasis. The result also suggested that LWS females have greater dependence to the adrenal function than HWS male, female and LWS male. Furthermore, this altered activity appears to results from sites outside the central nervous system. The results imply that the peripheral autonomic cardiovascular response could be due to different adrenal activity.

It is known that leptin has an effect on central and peripheral feeding-related neuronal networks in animals. Studies show that leptin receptors are present on the epinephrine-secreting cells in the adrenal medulla. These leads to the possibility that varying leptin activity may causes these two lines of birds to have different sympathetic activity. The effect of leptin might not be directly cause the body weight difference between these two lines of birds, however, there is a possibility that leptin could affect the body weight in certain level. Further studies are necessary to investigate the adrenal activity by using agents that may alter adrenal gland activity. The levels of leptin in various tissues between HWS and LWS bird should also be compare. Concentration and sensitivity of leptin receptors in the adrenal medulla also could be the reason for difference between HWS and LWS chicken in the cardiovascular regulation.

The second study showed that HWS birds slower recovery from reserpine. The results suggest that HWS have lower sympathetic activity than the LWS. Differing endogenous could be the reason for these varying results. The result from the catecholamines and indoalamine assay was not conclusive in determining whether such differences exist.

It is known catecholamines and serotonin have different effects at different locations in the brain and peripheral nervous system. In the future, the levels of catecholamines and serotonin between lines, sexes and at different locations in the brain and heart should be investigated.

It is know several leptin receptors have been identified. Also leptin from human and various animals has been colon include chicken leptin. However, the effect of chicken leptin on the feed intake and body weight regulation in the avian has not been documented. The difference between avian leptin and other animal's leptin also has not been compare. It is possible that the leptin gene between each species could has control body weight and food intake regulation by follow different mechanism.

In conclusion, high body weight could lead the lower sympathetic activity by several mechanisms. Since body weight gain is not control by only one factor, and without knowing actual site that caused the different responses of HWS and LWS chickens, more studies are necessary to elucidate the mechanism by which selection has altered body weight.

## **APPENDIX A**

### **CANNULATION OF THE BRACHIAL ARTERY AND VEIN IN BIRDS**

#### **SOLUTION:**

1. Sodium Heparin – 1g = 156,8000 units

|                            |        |
|----------------------------|--------|
| Compound                   | Amount |
| Sodium Heparin             | 60 mg  |
| Avian Physiological Saline | 10 ml  |

  - Will produce a solution containing 1000 units/ml

#### **PROCEDURES:**

##### **NOTES:**

- Avoid noise environment
  - Stable room temperature
  - Use drop to cover bird's head to avoid stress cause environment
1. Secure the bird on the V shape surgical station. Cover with drop cloth with only left wing exposed.
  2. Remove the feathers from the ventral side of the wing.
  3. Scrub the area with 70% ethanol. Make a 3 cm longitudinal incision where of the exposed area.
  4. After carefully incising the brachial artery from underneath the brachial muscle.
  5. Carefully incise the brachial artery with a pair of scissors, insert the cannula (PE 60). Secure the cannulae to prevent blood backflow.
  6. Arterial pressure is measured, using a pressure transducer (TSD 104 A) attached to MP100 Biopac system.
  7. Drugs are infused into the brachial vein. All infusions are done at the rate of 0.5 ml/30-sec interval using a infusion pump.
  8. Flush the cannulae with heparinized saline (1000 units/ml) in order to assure patency.

## **APENDIX B**

### **SOLUTION PREPARATION**

#### **AVIAN PHYSIOLOGICAL SALINE**

8.5 g sodium chloride in 1000 mL distilled water.

#### **SODIUM HEPARIN 100 U/mL (FOR USE IN SURGERY)**

Use .137g heparin sodium salt (146 unit/mg) in 200 mL avian saline

#### **PHENYLEPHRINE SOLUTION**

*Phenylephrine powder mixed with avian saline at dosage 40 µg/ .2 ml.*

#### **ATROPINE SOLUTION**

Atropine powder mixed with avian saline at dosage .5 mg/ .2ml

#### **PROPRANOLOL SOLUTION**

Propranolol powder mixed with avian saline at dosage 1.0 mg/ .2 ml

#### **TETRAETHYLAMMONIUM CHLORIDE SOLUTION**

Tetraethylammonium chloride powder mixed with avian saline at dosage 10 mg/ .2 ml

#### **RESERPINE SOLUTION**

Reserpine powder mixed with avian saline at dosage 1.25 and 2.50 mg/ 5ml.

## APPENDIX C

### BRAIN, HEART, AND PLASMA CATECHOLAMINE ASSAY FOR HPLC SOLUTIONS

a. Mobile Phase:

- 19.2 g of Citric Acid
- 350 mg of NaOH
- 0.150 g of Na<sub>2</sub>EDTA
- 200 mg of Sodium Octyl Sulfate-Variable
- 85 ml of Acetonitrile
- 1.8 ml of Triethylamine
- Add the chemicals listed above to approximately 900 ml of HPLC grade water and stir. Bring to a total volume of 1000ml using water.
- Adjust pH to 2.6 using NaOH and/or Citric Acid. Filter and degas the solution by vacuum, helium injection, or by passing the solution through a degasser before it is utilized in the HPLC machine.

b. Buffer for Homogenization:

- 150 mg of Na<sub>2</sub> EDTA
  - 4.3 ml of 70% Perchloric Acid
  - Add above to approximately 850 ml of HPLC Grade water and stir. Bring up to 1000 ml with water and refrigerate.
- NOTE:** All water used for this procedure (reagent prep., rinsing, etc) must be HPLC grade.

### STANDARDS

C. Preparation of Stock Solutions – 50 mg/100ml

- Can be stored refrigerated for 1 month
- 0.1 M HClO<sub>4</sub> – 8.6 ml of 70% HClO<sub>4</sub>/L
- NE – 94.36 mg of NE per 100 ml of 0.1 M HClO<sub>4</sub>
- Epi – 90.97 mg of Epi per 100 ml of 0.1 M HClO<sub>4</sub>
- DOPA – 50.0 mg of DOPA per 100 ml of 0.1 M HClO<sub>4</sub>
- DA – 61.91 mg of DA per 100 ml of 0.1 M HClO<sub>4</sub>
- 5HT – 110.56 mg of 5HT per 100 ml of 0.1 M HClO<sub>4</sub>
- 5HIAA – 50.0 mg of 5HIAA per 100 ml of 0.1 M HClO<sub>4</sub>
- META – 59.24 mg of META per 100 ml of 0.1 M HClO<sub>4</sub>
- NORMO – 59.24 mg of NORMO per 100 ml of 0.1 M HClO<sub>4</sub>
- MHPG – 65.4 mg of MHPG per 100 ml of 0.1 M HClO<sub>4</sub>
- DOPEG – 50.0 mg of DOPEG per 100 ml of 0.1 M HClO<sub>4</sub>
- DHBA – 80.0 mg of DHBA per 100 ml of 0.1 M HClO<sub>4</sub>

d. Preparation of Working Stock Solutions – 10 µg/ml

Can store refrigerated for 2 weeks

- For all the above standards add 200  $\mu$ l of the 50 mg/100 ml Stock solution to a 10 ml volumetric flask.
- Dilute to 10 ml using 0.1 M HClO<sub>4</sub>
- e. Preparation of Mixed Standard for Analysis (Conc. is 2 ng/100  $\mu$ l, except DHBA which is 5 ng/100  $\mu$ l)
  - For all the standards, except DHBA, add 50  $\mu$ l of 10  $\mu$ g/ml working solution to a 25 ml volumetric flask. Also add 100  $\mu$ l of the 10  $\mu$ g/ml DHBA solution to the flask.
  - Dilute to 10 ml using the Homogenization Buffer. Make the Mixed Standard fresh daily. Refrigerate between uses.

## PROCEDURE

Plan how many samples will be run per day. Each sample takes between 20-30 min. to run on the HPLC depending on the conditions. In addition to samples, standard must be run after every 4 samples. Keep brains frozen until ready to weigh. Keep brains, hearts and all solutions on ice while performing the following steps. Homogenize and run similar brain and heart parts at the same time.

- a. Weigh the brain sections into 10 ml centrifuge tubes and record the weight.
- b. Add 3.0 ml of brain buffer and 15  $\mu$ l of the 10  $\mu$ g/ml solution of DHBA for the diencephalon, brain stem, and cerebellum. Add 6.0 ml of brain buffer and 30  $\mu$ l of the 10  $\mu$ g/ml solution of DHBA for the telencephalon.
- c. Homogenize using the Brinkman Polytron.
- d. Place in the refrigerated centrifuge at 4°C and centrifuge at 11,000g for 15 min.
- e. Pour supernatant into BAS microfilters and centrifuge, using the refrigerated centrifuge, at 1600g for 5 min.
- f. All samples are centrifuged by using Amicon MPS-1 micropartition system that filters out all protein above 300 MW before putting in column.
- g. Put filtered extract into appropriate size tubes and freeze immediately by placing in the ultralow freezer. Keep frozen until ready to inject.
- h. Calibrate the HPLC by injecting 100  $\mu$ l of the mixed standard. Refer to HPLC set up instructions for the more information.
- i. Inject 100  $\mu$ l of the extract into the HPLC.

## HPLC CONDITIONS

- a. Detector Conditions:

- Model 5011 high sensitivity cell
  - Conditioning cell : + .35 volts
  - Electrode1 : .15 volts
  - Electrode2 : -.24 volts Time Const. – 20 Sec.
- b. Column:
- Beckman Ultrasphere XL
- c. Anspec HPLC Pump
- Pressure Limit – MAX 25.0; MIN 0.2
  - Flow – 1.5 ml/ min DC
  - Drain Valve - Closed
  - Compressibility – Auto

## CALCULATIONS

SAMPLE CONCENTRATION IN UG/G

$$\frac{\text{Pk ht of x/pk ht of IS unknown}}{\text{Pk ht of y/pk ht of IS unknown}} \text{---(STD. CONC)(DILUT. FACTOR)}$$


---

### BRAIN WEIGHT

Pk ht = Peak height

x = Catecholamine or indoleamine of interest from sample run  
 IS unknown = The peak height of IS from the calibration run

STD. CONC. = The concentration of the standard used for calibration

DILUT. FACTOR = The dilution factor takes into account the amount injected into the HPLC versus the amount of extract. For example, if 100 µl are injected and there is 3 ml of extract, the dilution factor would be 3000 µl/ 100 µl or 30.

Note: The integrator and the computer can be programmed to calculate the numerator of the above equation.

## APPENDIX D

Chapter 1. Analysis of variance of blood pressure of high and low body weight selected male and female chickens in response to various pharmacological agents.

| Source of variation              | df | Mean of squares | P Value |
|----------------------------------|----|-----------------|---------|
| <b>Phenylephrine</b>             |    |                 |         |
| LINE                             | 1  | 47.4105         | 0.2557  |
| SEX                              | 1  | 1.2780          | 0.8506  |
| LINE*SEX                         | 1  | 81.5193         | 0.1388  |
| PHENYLEPHRINE BASELINE           | 1  | 51.0080         | 0.2388  |
| <b>Atropine</b>                  |    |                 |         |
| LINE                             | 1  | 142.7379        | 0.0289  |
| SEX                              | 1  | 10.4233         | 0.5416  |
| LINE*SEX                         | 1  | 8.1289          | 0.5897  |
| ATROPINE BASELINE                | 1  | 0.0431          | 0.9686  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| LINE                             | 1  | 129.3250        | 0.1617  |
| SEX                              | 1  | 55.8199         | 0.3537  |
| LINE*SEX                         | 1  | 4.3404          | 0.7947  |
| ATROPINE BASELINE                | 1  | 4.0265          | 0.8021  |
| <b>Propranolol</b>               |    |                 |         |
| LINE                             | 1  | 115.9940        | 0.0995  |
| SEX                              | 1  | 199.1728        | 0.0333  |
| LINE*SEX                         | 1  | 1.6809          | 0.8396  |
| PROPRANOLOL BASELINE             | 1  | 19.4715         | 0.4922  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| LINE                             | 1  | 80.8697         | 0.2285  |
| SEX                              | 1  | 16.3859         | 0.5845  |
| LINE*SEX                         | 1  | 35.6750         | 0.4210  |
| PROPRANOLOL BASELINE             | 1  | 2.8001          | 0.8209  |
| <b>TEAC</b>                      |    |                 |         |
| LINE                             | 1  | 35.0620         | 0.6052  |
| SEX                              | 1  | 66.6816         | 0.4768  |
| LINE*SEX                         | 1  | 171.2692        | 0.2570  |
| TEAC BASELINE                    | 1  | 12.7266         | 0.7552  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| LINE                             | 1  | 429.1072        | 0.1034  |
| SEX                              | 1  | 50.3857         | 0.5699  |
| LINE*SEX                         | 1  | 52.3544         | 0.5624  |
| TEAC BASELINE                    | 1  | 47.1954         | 0.5823  |

Chapter 1. Analysis of variance of blood pressure of high and low body weight selected chickens in response to various pharmacological agents.

| Source of variation              | df | Mean of squares | P Value |
|----------------------------------|----|-----------------|---------|
| <b>HWS</b>                       |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| SEX                              | 1  | 17.6246         | 0.3270  |
| <b>Atropine</b>                  |    |                 |         |
| SEX                              | 1  | 19.1414         | 0.3131  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| SEX                              | 1  | 17.4621         | 0.5595  |
| <b>Propranolol</b>               |    |                 |         |
| SEX                              | 1  | 62.5453         | 0.1597  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| SEX                              | 1  | 0.9693          | 0.8949  |
| <b>TEAC</b>                      |    |                 |         |
| SEX                              | 1  | 318.0074        | 0.0099  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| SEX                              | 1  | 14.0160         | 0.7539  |
| <b>LWS</b>                       |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| SEX                              | 1  | 32.8756         | 0.4433  |
| <b>Atropine</b>                  |    |                 |         |
| SEX                              | 1  | 0.0625          | 0.9673  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| SEX                              | 1  | 43.2929         | 0.4507  |
| <b>Propranolol</b>               |    |                 |         |
| SEX                              | 1  | 124.6828        | 0.1348  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| SEX                              | 1  | 50.0900         | 0.3332  |
| <b>TEAC</b>                      |    |                 |         |
| SEX                              | 1  | 30.0840         | 0.7116  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| SEX                              | 1  | 158.9002        | 0.3352  |

Chapter 1. Analysis of variance of blood pressure of male and female chickens in response to various pharmacological agents.

| Source of variation                      | df | Mean of squares | P Value |
|------------------------------------------|----|-----------------|---------|
| <b>Male</b>                              |    |                 |         |
| <b>Phenylephrine</b><br>LINE             | 1  | 56.6479         | 0.0280  |
| <b>Atropine</b><br>LINE                  | 1  | 196.2465        | 0.0115  |
| <b>Atropine+Phenylephrine</b><br>LINE    | 1  | 146.6328        | 0.1224  |
| <b>Propranolol</b><br>LINE               | 1  | 38.0657         | 0.1509  |
| <b>Propranolol+Phenylephrine</b><br>LINE | 1  | 55.5045         | 0.3235  |
| <b>TEAC</b><br>LINE                      | 1  | 68.2932         | 0.4521  |
| <b>TEAC+Phenylephrine</b><br>LINE        | 1  | 1030.2774       | 0.0339  |
| <b>Female</b>                            |    |                 |         |
| <b>Phenylephrine</b><br>LINE             | 1  | 316.1159        | 0.0347  |
| <b>Atropine</b><br>LINE                  | 1  | 105.5655        | 0.0710  |
| <b>Atropine+Phenylephrine</b><br>LINE    | 1  | 216.5662        | 0.0900  |
| <b>Propranolol</b><br>LINE               | 1  | 97.0289         | 0.2205  |
| <b>Propranolol+Phenylephrine</b><br>LINE | 1  | 233.6966        | 0.0465  |
| <b>TEAC</b><br>LINE                      | 1  | 226.6133        | 0.2130  |
| <b>TEAC+Phenylephrine</b><br>LINE        | 1  | 290.2534        | 0.1259  |

Chapter 1. Analysis of variance of heart rate of high and low body weight selected male and female chickens in response to various pharmacological agents.

| Source of variation              | df | Mean of squares | P Value |
|----------------------------------|----|-----------------|---------|
| <b>Phenylephrine</b>             |    |                 |         |
| LINE                             | 1  | 1670.1086       | 0.1952  |
| SEX                              | 1  | 456.0596        | 0.4944  |
| LINE*SEX                         | 1  | 330.2538        | 0.5605  |
| PHENYLEPHRINE BASELINE           | 1  | 119.6601        | 0.7256  |
| <b>Atropine</b>                  |    |                 |         |
| LINE                             | 1  | 1222.2199       | 0.0067  |
| SEX                              | 1  | 1619.7709       | 0.0022  |
| LINE*SEX                         | 1  | 1023.3844       | 0.0124  |
| ATROPINE BASELINE                | 1  | 3191.4308       | 0.0001  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| LINE                             | 1  | 15.6877         | 0.8433  |
| SEX                              | 1  | 1736.3595       | 0.0436  |
| LINE*SEX                         | 1  | 1046.6464       | 0.1129  |
| ATROPINE BASELINE                | 1  | 12491.2367      | 0.0001  |
| <b>Propranolol</b>               |    |                 |         |
| LINE                             | 1  | 1636.7974       | 0.0219  |
| SEX                              | 1  | 69.0720         | 0.6251  |
| LINE*SEX                         | 1  | 42.4466         | 0.7015  |
| PROPRANOLOL BASELINE             | 1  | 9591.6365       | 0.0001  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| LINE                             | 1  | 775.9032        | 0.1116  |
| SEX                              | 1  | 280.0265        | 0.3335  |
| LINE*SEX                         | 1  | 16.1758         | 0.8151  |
| PROPRANOLOL BASELINE             | 1  | 7364.5683       | 0.0001  |
| <b>TEAC</b>                      |    |                 |         |
| LINE                             | 1  | 8.6459          | 0.8224  |
| SEX                              | 1  | 687.3036        | 0.0521  |
| LINE*SEX                         | 1  | 6.9631          | 0.8404  |
| TEAC BASELINE                    | 1  | 3700.8874       | 0.0001  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| LINE                             | 1  | 8.4842          | 0.8391  |
| SEX                              | 1  | 810.6285        | 0.0540  |
| LINE*SEX                         | 1  | 11.8923         | 0.8101  |
| TEAC BASELINE                    | 1  | 3529.7587       | 0.0002  |

Chapter 1. Analysis of variance of heart rate of high and low body weight selected chickens  
in response to various pharmacological agents.

| Source of variation              | df | Mean of squares | P Value |
|----------------------------------|----|-----------------|---------|
| <b>HWS</b>                       |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| SEX                              | 1  | 647.1953        | 0.3922  |
| <b>Atropine</b>                  |    |                 |         |
| SEX                              | 1  | 33.6111         | 0.5418  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| SEX                              | 1  | 544.5760        | 0.4735  |
| <b>Propranolol</b>               |    |                 |         |
| SEX                              | 1  | 217.8000        | 0.5378  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| SEX                              | 1  | 708.0500        | 0.2471  |
| <b>TEAC</b>                      |    |                 |         |
| SEX                              | 1  | 1162.6316       | 0.0773  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| SEX                              | 1  | 1207.5842       | 0.0841  |
| <b>LWS</b>                       |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| SEX                              | 1  | 13.6895         | 0.9092  |
| <b>Atropine</b>                  |    |                 |         |
| SEX                              | 1  | 4410.4500       | 0.0028  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| SEX                              | 1  | 832.0500        | 0.2061  |
| <b>Propranolol</b>               |    |                 |         |
| SEX                              | 1  | 520.2000        | 0.3365  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| SEX                              | 1  | 396.0500        | 0.3760  |
| <b>TEAC</b>                      |    |                 |         |
| SEX                              | 1  | 342.2250        | 0.2300  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| SEX                              | 1  | 429.0250        | 0.2041  |

Chapter 1. Analysis of variance of heart rate of male and female chickens in response to various pharmacological agents.

| Source of variation              | df | Mean of squares | P Value |
|----------------------------------|----|-----------------|---------|
| Male                             |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| LINE                             | 1  | 1638.0500       | 0.2765  |
| <b>Atropine</b>                  |    |                 |         |
| LINE                             | 1  | 396.0500        | 0.2866  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| LINE                             | 1  | 470.4500        | 0.5304  |
| <b>Propranolol</b>               |    |                 |         |
| LINE                             | 1  | 1125.0000       | 0.2314  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| LINE                             | 1  | 952.2000        | 0.2478  |
| <b>TEAC</b>                      |    |                 |         |
| LINE                             | 1  | 0.1838          | 0.9602  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| LINE                             | 1  | 25.5956         | 0.6095  |
| Female                           |    |                 |         |
| <b>Phenylephrine</b>             |    |                 |         |
| LINE                             | 1  | 296.0556        | 0.4592  |
| <b>Atropine</b>                  |    |                 |         |
| LINE                             | 1  | 2464.9000       | 0.0005  |
| <b>Atropine+Phenylephrine</b>    |    |                 |         |
| LINE                             | 1  | 668.2813        | 0.1592  |
| <b>Propranolol</b>               |    |                 |         |
| LINE                             | 1  | 1729.8000       | 0.0398  |
| <b>Propranolol+Phenylephrine</b> |    |                 |         |
| LINE                             | 1  | 583.2000        | 0.1857  |
| <b>TEAC</b>                      |    |                 |         |
| LINE                             | 1  | 252.0500        | 0.4621  |
| <b>TEAC+Phenylephrine</b>        |    |                 |         |
| LINE                             | 1  | 369.8000        | 0.3906  |

## APPENDIX E

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, feed efficiency at 1 week.

| Source of variation                | df | Mean Square | P value |
|------------------------------------|----|-------------|---------|
| <b>Body weight</b>                 |    |             |         |
| LINE                               | 1  | 16338.4200  | 0.0001  |
| TRT                                | 2  | 158.4247    | 0.0001  |
| LINE*TRT                           | 2  | 42.0243     | 0.0001  |
| <b>Body weight gain per period</b> |    |             |         |
| LINE                               | 1  | 6989.8230   | 0.0001  |
| TRT                                | 2  | 163.6135    | 0.0001  |
| LINE*TRT                           | 2  | 41.9022     | 0.0053  |
| <b>Feed consumption</b>            |    |             |         |
| LINE                               | 1  | 5541.9540   | 0.0001  |
| TRT                                | 2  | 431.4919    | 0.0056  |
| LINE*TRT                           | 2  | 2.5919      | 0.9635  |
| <b>Feed efficiency</b>             |    |             |         |
| LINE                               | 1  | 0.9812      | 0.0001  |
| TRT                                | 2  | 0.0091      | 0.0061  |
| LINE*TRT                           | 2  | 0.0010      | 0.5213  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 2 week.

| Source of variation                           | df | Mean Square | P value |
|-----------------------------------------------|----|-------------|---------|
| <b>Body weight</b>                            |    |             |         |
| LINE                                          | 1  | 104164.6168 | 0.0001  |
| TRT                                           | 2  | 366.1687    | 0.0022  |
| LINE*TRT                                      | 2  | 153.5085    | 0.0565  |
| <b>Body weight gain per period</b>            |    |             |         |
| LINE                                          | 1  | 37995.2142  | 0.0001  |
| TRT                                           | 2  | 43.8140     | 0.2252  |
| LINE*TRT                                      | 2  | 39.2663     | 0.2612  |
| <b>Feed consumption</b>                       |    |             |         |
| LINE                                          | 1  | 41763.3477  | 0.0001  |
| TRT                                           | 2  | 894.8958    | 0.0342  |
| LINE*TRT                                      | 2  | 224.9530    | 0.3976  |
| <b>Cumulative feed consumption</b>            |    |             |         |
| LINE                                          | 1  | 77732.2986  | 0.0001  |
| TRT                                           | 2  | 2502.6561   | 0.0034  |
| LINE*TRT                                      | 2  | 185.3903    | 0.6052  |
| <b>Feed efficiency</b>                        |    |             |         |
| LINE                                          | 1  | 1.0757      | 0.0001  |
| TRT                                           | 2  | 0.0063      | 0.1300  |
| LINE*TRT                                      | 2  | 0.0018      | 0.5328  |
| <b>Cumulative feed efficiency</b>             |    |             |         |
| LINE                                          | 1  | 1.0736      | 0.0001  |
| TRT                                           | 2  | 0.0002      | 0.1843  |
| LINE*TRT                                      | 2  | 0.0005      | 0.6573  |
| <b>Cumulative body weight gain per period</b> |    |             |         |
| LINE                                          | 1  | 77578.2756  | 0.0001  |
| TRT                                           | 2  | 373.5746    | 0.0014  |
| LINE*TRT                                      | 2  | 154.8739    | 0.0457  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 3 week.

| Source of variation                           | df | Mean Square | P value |
|-----------------------------------------------|----|-------------|---------|
| <b>Body weight</b>                            |    |             |         |
| LINE                                          | 1  | 399760.6360 | 0.0001  |
| TRT                                           | 2  | 792.6666    | 0.0111  |
| LINE*TRT                                      | 2  | 884.0596    | 0.0071  |
| <b>Body weight gain per period</b>            |    |             |         |
| LINE                                          | 1  | 95803.1463  | 0.0001  |
| TRT                                           | 2  | 113.1788    | 0.1907  |
| LINE*TRT                                      | 2  | 302.0441    | 0.0171  |
| <b>Feed consumption</b>                       |    |             |         |
| LINE                                          | 1  | 285912.0730 | 0.0001  |
| TRT                                           | 2  | 313.1096    | 0.2088  |
| LINE*TRT                                      | 2  | 763.7336    | 0.0282  |
| <b>Cumulative feed consumption</b>            |    |             |         |
| LINE                                          | 1  | 661802.7365 | 0.0001  |
| TRT                                           | 2  | 4513.7111   | 0.0078  |
| LINE*TRT                                      | 2  | 1578.0757   | 0.1525  |
| <b>Feed efficiency</b>                        |    |             |         |
| LINE                                          | 1  | 0.4558      | 0.0001  |
| TRT                                           | 2  | 0.0017      | 0.3599  |
| LINE*TRT                                      | 2  | 0.0023      | 0.2566  |
| <b>Cumulative feed efficiency</b>             |    |             |         |
| LINE                                          | 1  | 0.7838      | 0.0001  |
| TRT                                           | 2  | 0.0004      | 0.4981  |
| LINE*TRT                                      | 2  | 0.0008      | 0.2688  |
| <b>Cumulative body weight gain per period</b> |    |             |         |
| LINE                                          | 1  | 345802.3358 | 0.0001  |
| TRT                                           | 2  | 805.8342    | 0.0083  |
| LINE*TRT                                      | 2  | 888.2041    | 0.0055  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 4 week.

| Source of variation                           | df | Mean Square   | P value |
|-----------------------------------------------|----|---------------|---------|
| <b>Body weight</b>                            |    |               |         |
| LINE                                          | 1  | 1121967.6890  | 0.0001  |
| TRT                                           | 2  | 732.0210      | 0.1356  |
| LINE*TRT                                      | 2  | 1330.2170     | 0.0316  |
| <b>Body weight gain per period</b>            |    |               |         |
| LINE                                          | 1  | 182297.8236   | 0.0001  |
| TRT                                           | 2  | 1.9113        | 0.9707  |
| LINE*TRT                                      | 2  | 48.3359       | 0.4799  |
| <b>Feed consumption</b>                       |    |               |         |
| LINE                                          | 1  | 370522.6180   | 0.0001  |
| TRT                                           | 2  | 321.7086      | 0.6173  |
| LINE*TRT                                      | 2  | 1324.5068     | 0.1505  |
| <b>Cumulative feed consumption</b>            |    |               |         |
| LINE                                          | 1  | 2022704.8420  | 0.0001  |
| TRT                                           | 2  | 2814.2730     | 0.3010  |
| LINE*TRT                                      | 2  | 5592.8770     | 0.1004  |
| <b>Feed efficiency</b>                        |    |               |         |
| LINE                                          | 1  | 0.9198        | 0.0001  |
| TRT                                           | 2  | 0.0002        | 0.9115  |
| LINE*TRT                                      | 2  | 0.0003        | 0.8693  |
| <b>Cumulative feed efficiency</b>             |    |               |         |
| LINE                                          | 1  | 0.8549        | 0.0001  |
| TRT                                           | 2  | 0.0001        | 0.8986  |
| LINE*TRT                                      | 2  | 0.0001        | 0.8459  |
| <b>Cumulative body weight gain per period</b> |    |               |         |
| LINE                                          | 1  | 11030251.5800 | 0.0001  |
| TRT                                           | 2  | 744.4400      | 0.1226  |
| LINE*TRT                                      | 2  | 1336.344      | 0.0279  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 5 week.

| Source of variation                           | df | Mean Square  | P value |
|-----------------------------------------------|----|--------------|---------|
| <b>Body weight</b>                            |    |              |         |
| LINE                                          | 1  | 2497774.5380 | 0.0001  |
| TRT                                           | 2  | 974.2200     | 0.4874  |
| LINE*TRT                                      | 2  | 2129.6460    | 0.2168  |
| <b>Body weight gain per period</b>            |    |              |         |
| LINE                                          | 1  | 271654.8175  | 0.0001  |
| TRT                                           | 2  | 93.9006      | 0.8267  |
| LINE*TRT                                      | 2  | 93.8584      | 0.8267  |
| <b>Feed consumption</b>                       |    |              |         |
| LINE                                          | 1  | 806971.9116  | 0.0001  |
| TRT                                           | 2  | 36.8940      | 0.9701  |
| LINE*TRT                                      | 2  | 840.5818     | 0.5017  |
| <b>Cumulative feed consumption</b>            |    |              |         |
| LINE                                          | 1  | 5384880.0670 | 0.0001  |
| TRT                                           | 2  | 3339.3450    | 0.5594  |
| LINE*TRT                                      | 2  | 10768.9430   | 0.1657  |
| <b>Feed efficiency</b>                        |    |              |         |
| LINE                                          | 1  | 0.6908       | 0.0001  |
| TRT                                           | 2  | 0.0011       | 0.4420  |
| LINE*TRT                                      | 2  | 0.0002       | 0.8543  |
| <b>Cumulative feed efficiency</b>             |    |              |         |
| LINE                                          | 1  | 0.7891       | 0.0001  |
| TRT                                           | 2  | 0.0002       | 0.7754  |
| LINE*TRT                                      | 2  | 0.0001       | 0.8602  |
| <b>Cumulative body weight gain per period</b> |    |              |         |
| LINE                                          | 1  | 2359966.5240 | 0.0001  |
| TRT                                           | 2  | 990.6440     | 0.4776  |
| LINE*TRT                                      | 2  | 2137.6780    | 0.2120  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 6 week.

| Source of variation                           | df | Mean Square   | P value |
|-----------------------------------------------|----|---------------|---------|
| <b>Body weight</b>                            |    |               |         |
| LINE                                          | 1  | 4743403.9760  | 0.0001  |
| TRT                                           | 2  | 1210.5730     | 0.4322  |
| LINE*TRT                                      | 2  | 2808.6900     | 0.1527  |
| <b>Body weight gain per period</b>            |    |               |         |
| LINE                                          | 1  | 357007.1984   | 0.0001  |
| TRT                                           | 2  | 12.8484       | 0.9491  |
| LINE*TRT                                      | 2  | 109.4082      | 0.6448  |
| <b>Feed consumption</b>                       |    |               |         |
| LINE                                          | 1  | 1361091.6670  | 0.0001  |
| TRT                                           | 2  | 640.5980      | 0.4551  |
| LINE*TRT                                      | 2  | 535.3920      | 0.5164  |
| <b>Cumulative feed consumption</b>            |    |               |         |
| LINE                                          | 1  | 12160513.4100 | 0.0001  |
| TRT                                           | 2  | 6770.1800     | 0.4875  |
| LINE*TRT                                      | 2  | 13727.5700    | 0.2411  |
| <b>Feed efficiency</b>                        |    |               |         |
| LINE                                          | 1  | 0.5429        | 0.0001  |
| TRT                                           | 2  | 0.0007        | 0.5914  |
| LINE*TRT                                      | 2  | 0.0004        | 0.7486  |
| <b>Cumulative feed efficiency</b>             |    |               |         |
| LINE                                          | 1  | 0.7065        | 0.0001  |
| TRT                                           | 2  | 0.0002        | 0.5755  |
| LINE*TRT                                      | 2  | 0.0001        | 0.7169  |
| <b>Cumulative body weight gain per period</b> |    |               |         |
| LINE                                          | 1  | 4552756.9740  | 0.0001  |
| TRT                                           | 2  | 1228.8980     | 0.4195  |
| LINE*TRT                                      | 2  | 283.1010      | 0.1467  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 7 week.

| Source of variation                           | df | Mean Square   | P value |
|-----------------------------------------------|----|---------------|---------|
| <b>Body weight</b>                            |    |               |         |
| LINE                                          | 1  | 519743.9500   | 0.0001  |
| TRT                                           | 2  | 149657.3430   | 0.0001  |
| LINE*TRT                                      | 2  | 105848.7520   | 0.0001  |
| <b>Body weight gain per period</b>            |    |               |         |
| LINE                                          | 1  | 10373.8267    | 0.0156  |
| TRT                                           | 2  | 127990.2769   | 0.0001  |
| LINE*TRT                                      | 2  | 78194.4903    | 0.0001  |
| <b>Feed consumption</b>                       |    |               |         |
| LINE                                          | 1  | 512782.3990   | 0.0001  |
| TRT                                           | 2  | 268806.5566   | 0.0001  |
| LINE*TRT                                      | 2  | 105682.5930   | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |               |         |
| LINE                                          | 1  | 17667571.4300 | 0.0001  |
| TRT                                           | 2  | 357941.1600   | 0.0001  |
| LINE*TRT                                      | 2  | 177968.3900   | 0.0001  |
| <b>Feed efficiency</b>                        |    |               |         |
| LINE                                          | 1  | 0.5843        | 0.5026  |
| TRT                                           | 2  | 5.0432        | 0.0294  |
| LINE*TRT                                      | 2  | 1.7471        | 0.2678  |
| <b>Cumulative feed efficiency</b>             |    |               |         |
| LINE                                          | 1  | 0.5577        | 0.0001  |
| TRT                                           | 2  | 0.0083        | 0.0001  |
| LINE*TRT                                      | 2  | 0.0024        | 0.0151  |
| <b>Cumulative body weight gain per period</b> |    |               |         |
| LINE                                          | 1  | 497777.8380   | 0.0001  |
| TRT                                           | 2  | 149800.3960   | 0.0001  |
| LINE*TRT                                      | 2  | 105784.6180   | 0.0001  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period at 8 week.

| Source of variation                           | df | Mean Square   | P value |
|-----------------------------------------------|----|---------------|---------|
| <b>Body weight</b>                            |    |               |         |
| LINE                                          | 1  | 6614981.9160  | 0.0001  |
| TRT                                           | 2  | 319282.0560   | 0.0001  |
| LINE*TRT                                      | 2  | 266013.7140   | 0.0001  |
| <b>Body weight gain per period</b>            |    |               |         |
| LINE                                          | 1  | 85365.0712    | 0.0001  |
| TRT                                           | 2  | 51728.6194    | 0.0001  |
| LINE*TRT                                      | 2  | 47617.4060    | 0.0001  |
| <b>Feed consumption</b>                       |    |               |         |
| LINE                                          | 1  | 2585275.4660  | 0.0001  |
| TRT                                           | 2  | 672632.6780   | 0.0001  |
| LINE*TRT                                      | 2  | 46784.9910    | 0.0002  |
| <b>Cumulative feed consumption</b>            |    |               |         |
| LINE                                          | 1  | 33769583.0300 | 0.0001  |
| TRT                                           | 2  | 1926793.9300  | 0.0001  |
| LINE*TRT                                      | 2  | 1113164.1600  | 0.0001  |
| <b>Feed efficiency</b>                        |    |               |         |
| LINE                                          | 1  | 0.1808        | 0.1344  |
| TRT                                           | 2  | 0.3314        | 0.0221  |
| LINE*TRT                                      | 2  | 0.2584        | 0.0474  |
| <b>Cumulative feed efficiency</b>             |    |               |         |
| LINE                                          | 1  | 0.3131        | 0.0001  |
| TRT                                           | 2  | 0.0037        | 0.0196  |
| LINE*TRT                                      | 2  | 0.0024        | 0.0674  |
| <b>Cumulative body weight gain per period</b> |    |               |         |
| LINE                                          | 1  | 6389489.9700  | 0.0001  |
| TRT                                           | 2  | 319413.9970   | 0.0001  |
| LINE*TRT                                      | 2  | 265842.7660   | 0.0001  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, feed efficiency, of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 1 and 2.

| Source of variation                           | Df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 1</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 181.1047       | 0.0110  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 184.4588       | 0.0002  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 186.8390       | 0.1059  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0075         | 0.0237  |
| <b>Week 2</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 475.5349       | 0.0166  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 70.7795        | 0.2801  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 974.1152       | 0.0046  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 1991.0454      | 0.0096  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0037         | 0.2612  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0004         | 0.7490  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 481.7219       | 0.0135  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 3 and 4.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 3</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 1650.9016      | 0.0131  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 390.0091       | 0.0656  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 955.1754       | 0.0428  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 5685.3195      | 0.0148  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0006         | 0.3888  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0000         | 0.8008  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 1667.2736      | 0.0105  |
| <b>Week 4</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 1966.1696      | 0.0735  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 15.5798        | 0.8685  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 171.2249       | 0.6859  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 7659.7172      | 0.0760  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0000         | 0.8267  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 2.0608         | 0.9773  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 1984.8117      | 0.0665  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 5 and 6.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 5</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 2991.4633      | 0.3343  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 152.0485       | 0.8535  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 609.0947       | 0.7360  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 12478.9836     | 0.2236  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0002         | 0.8560  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0000         | 0.9126  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 3018.3162      | 0.3272  |
| <b>Week 6</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 3837.9493      | 0.2586  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 85.8510        | 0.8344  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 949.0308       | 0.3974  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 19739.5960     | 0.2265  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0000         | 0.9602  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0000         | 0.8135  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 3865.3505      | 0.2495  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 7 and 8.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 7</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 253365.1484    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 202627.3914    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 350403.3950    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 5188798.7590   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 4.1142         | 0.0048  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0095         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 253429.1064    | 0.0001  |
| <b>Week 8</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 584020.7990    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 98958.0768     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 1103049.8930   | 0.0003  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 2936063.4740   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.5268         | 0.0453  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0054         | 0.0156  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 583967.9990    | 0.0001  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, feed efficiency, of low weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 1 and 2.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 1</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 19.3442        | 0.0087  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 21.0570        | 0.0003  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 247.2448       | 0.0516  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0026         | 0.2020  |
| <b>Week 2</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 44.1637        | 0.0284  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 12.3008        | 0.1136  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 145.7336       | 0.6664  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 697.0010       | 0.2206  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0044         | 0.2840  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0022         | 0.1677  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 46.72654398    | 0.0112  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of low weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 3 and 4.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 3</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 25.8246        | 0.3186  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 25.2138        | 0.1237  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 121.6679       | 0.4283  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 406.4674       | 0.5056  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0034         | 0.3032  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0011         | 0.3421  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 26.7647        | 0.2810  |
| <b>Week 4</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 96.0695        | 0.2105  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 34.6674        | 0.1951  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 1474.9905      | 0.2168  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 747.4331       | 0.6959  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0005         | 0.8935  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0002         | 0.8643  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 95.9721        | 0.1989  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 5 and 6.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 5</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 112.4023       | 0.3859  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 35.7105        | 0.3366  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 267.6765       | 0.5536  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 1629.3043      | 0.6556  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0012         | 0.4862  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0002         | 0.7977  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 110.0063       | 0.3929  |
| <b>Week 6</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 181.3131       | 0.4518  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 39.4056        | 0.3908  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 226.9592       | 0.6989  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 758.1508       | 0.8888  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0010         | 0.4790  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0004         | 0.6374  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 176.6481       | 0.4564  |

Chapter 2. Analysis of variance of body weight gain, body weight gain per period, feed consumption, cumulative feed consumption, feed efficiency, cumulative feed efficiency. cumulative body weight gain per period of high weight selected chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine at age week 7 and 8.

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 7</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 2140.9464      | 0.0047  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 3557.3758      | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 24085.7545     | 0.0005  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 17110.7979     | 0.2144  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 2.6762         | 0.2934  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0012         | 0.1556  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 2155.9070      | 0.0046  |
| <b>Week 8</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| TRT                                           | 2  | 1274.9720      | 0.0636  |
| <b>Body weight gain per period</b>            |    |                |         |
| TRT                                           | 2  | 387.9486       | 0.0743  |
| <b>Feed consumption</b>                       |    |                |         |
| TRT                                           | 2  | 37424.7761     | 0.0127  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| TRT                                           | 2  | 103888.6208    | 0.0417  |
| <b>Feed efficiency</b>                        |    |                |         |
| TRT                                           | 2  | 0.0631         | 0.0373  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| TRT                                           | 2  | 0.0006         | 0.4059  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| TRT                                           | 2  | 1288.7641      | 0.0627  |

Chapter 2. Analysis of variance lines to treatment saline outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 1</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 6605.1965      | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 3403.3022      | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 1707.0797      | 0.0005  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3673         | 0.0001  |
| <b>Week 2</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 40021.4375     | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 14108.9797     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 18302.6422     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 31189.0007     | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3169         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.3494         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 30446.2425     | 0.0001  |

Chapter 2. Analysis of variance lines to treatment saline outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 3</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 157871.4414    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 38918.1385     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 113616.2926    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 263861.2235    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1985         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2846         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 138209.4557    | 0.0001  |
| <b>Week 4</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 422969.4589    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 64024.4991     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 147896.9320    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 806849.3623    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3061         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2961         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 390369.9817    | 0.0001  |

Chapter 2. Analysis of variance lines to treatment outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 5</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 924152.9659    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 967000.9280    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 301248.3262    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 2094124.0980   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.2381         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2743         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 87653.6653     | 0.0001  |
| <b>Week 6</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 1733753.7500   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 126303.5320    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 484274.2349    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 4592479.1110   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1953         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2484         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 1667083.2030   | 0.0001  |

Chapter 2. Analysis of lines to treatment saline outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 7</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 2826992.7260   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 132966.3369    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 5752269.3490   | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 8418540.6510   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1643         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2298         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 2741677.8030   | 0.0001  |
| <b>Week 8</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 4032183.4160   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 106708.9819    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 2095701.9000   | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 18914897.6800  | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.0283         | 0.0050  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.1404         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 3930166.1480   | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 1.25 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 1</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 4859.2292      | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 1928.0244      | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 1982.9811      | 0.0016  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.2955         | 0.0001  |
| <b>Week 2</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 30960.9070     | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 11288.8384     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 12109.7760     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 23893.4624     | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3446         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.3370         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 22547.4911     | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 1.25 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 3</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 114780.8743    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 26515.6101     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 79445.0922     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 190475.6493    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1309         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2303         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 97965.4726     | 0.0001  |
| <b>Week 4</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 334068.6590    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 57213.5148     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 97647.4499     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 560882.7840    | 0.0000  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3271         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2748         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 304911.4022    | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 1.25 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 5</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 756246.2848    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 85052.4264     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 241134.4846    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 1537539.3450   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.2389         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2582         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 712041.1769    | 0.0001  |
| <b>Week 6</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 1475401.6200   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 119050.4573    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 455780.1481    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 3667571.4570   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1845         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2328         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 1413393.3690   | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 1.25 mg/kg outcomes

| Source of variation                           | df | Mean of square  | P value |
|-----------------------------------------------|----|-----------------|---------|
| <b>Week 7</b>                                 |    |                 |         |
| <b>Body Weight</b>                            |    |                 |         |
| LINE                                          | 1  | 1464614.9410    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                 |         |
| LINE                                          | 1  | 19.7845         | 0.9335  |
| <b>Feed consumption</b>                       |    |                 |         |
| LINE                                          | 1  | 136137.7437     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                 |         |
| LINE                                          | 1  | 5216937.6340    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                 |         |
| LINE                                          | 1  | 3017097471.0000 | 0.2464  |
| <b>Cumulative feed efficiency</b>             |    |                 |         |
| LINE                                          | 1  | 0.1867          | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                 |         |
| LINE                                          | 1  | 1402836.2150    | 0.0001  |
| <b>Week 8</b>                                 |    |                 |         |
| <b>Body Weight</b>                            |    |                 |         |
| LINE                                          | 1  | 2160634.6120    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                 |         |
| LINE                                          | 1  | 67442.5123      | 0.0006  |
| <b>Feed consumption</b>                       |    |                 |         |
| LINE                                          | 1  | 1401750.8620    | 0.0019  |
| <b>Cumulative feed consumption</b>            |    |                 |         |
| LINE                                          | 1  | 12027143.6700   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                 |         |
| LINE                                          | 1  | 0.0084          | 0.5273  |
| <b>Cumulative feed efficiency</b>             |    |                 |         |
| LINE                                          | 1  | 0.1007          | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                 |         |
| LINE                                          | 1  | 2085456.0960    | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 2.50 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 1</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 4958.0418      | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 2042.3004      | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 1857.0767      | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.3210         | 0.0001  |
| <b>Week 2</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 33489.2893     | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 12648.9286     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 11800.8355     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 23020.6162     | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.4179         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.3884         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 24894.2895     | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 2.50 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 3</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 128876.4394    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 30973.4860     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 94378.1554     | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 210622.0152    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1312         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2705         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 111403.8158    | 0.0001  |
| <b>Week 4</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 367590.0055    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 61156.4815     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 127627.2497    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 666158.4506    | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.2872         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2843         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 337642.8875    | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 2.50 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 5</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 821634.5794    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 90089.1800     | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 266270.2644    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 1774754.5110   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.2142         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2568         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 776547.0378    | 0.0001  |
| <b>Week 6</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 1539865.9850   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 111872.0254    | 0.0001  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 422108.0679    | 0.0001  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 3927917.9770   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.1639         | 0.0001  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.2255         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 1477906.6030   | 0.0001  |

Chapter 2. Analysis of lines to treatment reserpine 2.50 mg/kg outcomes

| Source of variation                           | df | Mean of square | P value |
|-----------------------------------------------|----|----------------|---------|
| <b>Week 7</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 1117521.7870   | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 33776.6826     | 0.0015  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 12738.4922     | 0.0583  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 4388029.9270   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.7432         | 0.5249  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.1458         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 1064833.0550   | 0.0001  |
| <b>Week 8</b>                                 |    |                |         |
| <b>Body Weight</b>                            |    |                |         |
| LINE                                          | 1  | 954191.3167    | 0.0001  |
| <b>Body weight gain per period</b>            |    |                |         |
| LINE                                          | 1  | 6448.3890      | 0.1833  |
| <b>Feed consumption</b>                       |    |                |         |
| LINE                                          | 1  | 23506.6875     | 0.2965  |
| <b>Cumulative feed consumption</b>            |    |                |         |
| LINE                                          | 1  | 5053870.0110   | 0.0001  |
| <b>Feed efficiency</b>                        |    |                |         |
| LINE                                          | 1  | 0.6610         | 0.1045  |
| <b>Cumulative feed efficiency</b>             |    |                |         |
| LINE                                          | 1  | 0.0768         | 0.0001  |
| <b>Cumulative body weight gain per period</b> |    |                |         |
| LINE                                          | 1  | 905553.2594    | 0.0001  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| NE                  |    |                |         |
| LINE                | 1  | 16466250.3000  | 0.1795  |
| TRT                 | 2  | 188011719.7000 | 0.0001  |
| LINE*TRT            | 2  | 50250954.1000  | 0.0061  |
| Epi                 |    |                |         |
| LINE                | 1  | 64151.6682     | 0.0007  |
| TRT                 | 2  | 90016.3837     | 0.0001  |
| LINE*TRT            | 2  | 104820.4845    | 0.0001  |
| 5HIAA               |    |                |         |
| LINE                | 1  | 1896777.8770   | 0.0001  |
| TRT                 | 2  | 618691.6320    | 0.0019  |
| LINE*TRT            | 2  | 686360.7110    | 0.0011  |
| DA                  |    |                |         |
| LINE                | 1  | 4807065.2840   | 0.0007  |
| TRT                 | 2  | 970922.0390    | 0.0836  |
| LINE*TRT            | 2  | 1018366.9100   | 0.0739  |
| 5HT                 |    |                |         |
| LINE                | 1  | 21562.5100     | 0.8422  |
| TRT                 | 2  | 6801758.5600   | 0.0001  |
| LINE*TRT            | 2  | 657511.5500    | 0.3039  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine after square root.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| rtNE                |    |                |         |
| LINE                | 1  | 23.7168        | 0.8391  |
| TRT                 | 2  | 14372.5113     | 0.0001  |
| LINE*TRT            | 2  | 3334.1020      | 0.0052  |
| rtEpi               |    |                |         |
| LINE                | 1  | 102.3902       | 0.0111  |
| TRT                 | 2  | 170.2486       | 0.0001  |
| LINE*TRT            | 2  | 223.0167       | 0.0001  |
| rt5HIAA             |    |                |         |
| LINE                | 1  | 938.4396       | 0.0001  |
| TRT                 | 2  | 325.6188       | 0.0019  |
| LINE*TRT            | 2  | 375.7446       | 0.0008  |
| rtDA                |    |                |         |
| LINE                | 1  | 2184.2383      | 0.0001  |
| TRT                 | 2  | 250.7274       | 0.0732  |
| LINE*TRT            | 2  | 352.9736       | 0.0272  |
| rt5HT               |    |                |         |
| LINE                | 1  | 154.6826       | 0.2077  |
| TRT                 | 2  | 1861.2897      | 0.0001  |
| LINE*TRT            | 2  | 307.3257       | 0.0479  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations and concentrations square root of high weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 27432469.0800  | 0.0183  |
| Epi<br>TRT          | 2  | 8814.8387      | 0.2211  |
| 5HIAA<br>TRT        | 2  | 576720.7290    | 0.0001  |
| DA<br>TRT           | 2  | 6054.8668      | 0.2709  |
| 5HT<br>TRT          | 2  | 5857834.7500   | 0.0010  |
| rtNE<br>TRT         | 2  | 2233.1112      | 0.0341  |
| rtEpi<br>TRT        | 2  | 28.6438        | 0.2143  |
| rt5HIAA<br>TRT      | 2  | 446.0818       | 0.0001  |
| rtDA<br>TRT         | 2  | 23.3710        | 0.1331  |
| rt5HT<br>TRT        | 2  | 1838.7872      | 0.0001  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations and concentrations square root of low weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 214026041.5000 | 0.0001  |
| Epi<br>TRT          | 2  | 189081.8758    | 0.0001  |
| 5HIAA<br>TRT        | 2  | 770890.6600    | 0.0133  |
| DA<br>TRT           | 2  | 1984861.2240   | 0.0833  |
| 5HT<br>TRT          | 2  | 1635492.2540   | 0.0425  |
| rtNE<br>TRT         | 2  | 15747.2626     | 0.0001  |
| rtEpi<br>TRT        | 2  | 371.0471       | 0.0001  |
| rt5HIAA<br>TRT      | 2  | 280.0073       | 0.0245  |
| rtDA<br>TRT         | 2  | 583.7981       | 0.0465  |
| rt5HT<br>TRT        | 2  | 349.9000       | 0.0410  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received saline

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 108582363.5000 | 0.0391  |
| Epi<br>LINE         | 1  | 1321.4941      | 0.7218  |
| 5HIAA<br>LINE       | 1  | 46714.6258     | 0.3778  |
| DA<br>LINE          | 1  | 4486989.4180   | 0.0489  |
| 5HT<br>LINE         | 1  | 696908.1800    | 0.5152  |
| rtNE<br>LINE        | 1  | 4623.5987      | 0.0400  |
| rtEpi<br>LINE       | 1  | 7.2552         | 0.5831  |
| rt5HIAA<br>LINE     | 1  | 10.8945        | 0.5503  |
| rtDA<br>LINE        | 1  | 1258.1086      | 0.0251  |
| rt5HT<br>LINE       | 1  | 142.1439       | 0.4652  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 1.25 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 3353703.4940   | 0.4478  |
| Epi<br>LINE         | 1  | 1526.7326      | 0.3173  |
| 5HIAA<br>LINE       | 1  | 187820.6738    | 0.0221  |
| DA<br>LINE          | 1  | 21556.4927     | 0.1588  |
| 5HT<br>LINE         | 1  | 161463.0212    | 0.0420  |
| rtNE<br>LINE        | 1  | 450.1990       | 0.4299  |
| rtEpi<br>LINE       | 1  | 6.5908         | 0.4305  |
| rt5HIAA<br>LINE     | 1  | 105.3983       | 0.0896  |
| rtDA<br>LINE        | 1  | 28.2721        | 0.1954  |
| rt5HT<br>LINE       | 1  | 123.7029       | 0.0399  |

Chapter 2. Analysis of variance of brain catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 2.50 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 3132428.4240   | 0.0067  |
| Epi<br>LINE         | 1  | 280032.6782    | 0.0001  |
| 5HIAA<br>LINE       | 1  | 3230785.6900   | 0.0003  |
| DA<br>LINE          | 1  | 2370547.2930   | 0.0005  |
| 5HT<br>LINE         | 1  | 480295.6784    | 0.0001  |
| rtNE<br>LINE        | 1  | 1602.9507      | 0.0017  |
| rtEpi<br>LINE       | 1  | 551.4690       | 0.0001  |
| rt5HIAA<br>LINE     | 1  | 1678.8716      | 0.0001  |
| rtDA<br>LINE        | 1  | 1641.0761      | 0.0001  |
| rt5HT<br>LINE       | 1  | 511.5383       | 0.0001  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| NE                  |    |                |         |
| LINE                | 1  | 1389780.6510   | 0.0001  |
| TRT                 | 2  | 55828.7720     | 0.2910  |
| LINE*TRT            | 2  | 11927.8570     | 0.7643  |
| Epi                 |    |                |         |
| LINE                | 1  | 12624.5269     | 0.0002  |
| TRT                 | 2  | 793.4985       | 0.3814  |
| LINE*TRT            | 2  | 118.0648       | 0.8643  |
| 5HIAA               |    |                |         |
| LINE                | 1  | 49983.7387     | 0.0003  |
| TRT                 | 2  | 88840.2200     | 0.0695  |
| LINE*TRT            | 2  | 41558.6029     | 0.2772  |
| DA                  |    |                |         |
| LINE                | 1  | 75438.8584     | 0.0001  |
| TRT                 | 2  | 89.7627        | 0.9052  |
| LINE*TRT            | 2  | 1807.8826      | 0.1451  |
| 5HT                 |    |                |         |
| LINE                | 1  | 1499360.7730   | 0.0418  |
| TRT                 | 2  | 1889446.0570   | 0.0070  |
| LINE*TRT            | 2  | 3224398.9550   | 0.0004  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine after square root.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| rtNE                |    |                |         |
| LINE                | 1  | 1718.9524      | 0.0001  |
| TRT                 | 2  | 59.9193        | 0.2456  |
| LINE*TRT            | 2  | 102.7933       | 0.0941  |
| rtEpi               |    |                |         |
| LINE                | 1  | 143.2303       | 0.0001  |
| TRT                 | 2  | 13.6012        | 0.0904  |
| LINE*TRT            | 2  | 0.5303         | 0.9065  |
| rt5HIAA             |    |                |         |
| LINE                | 1  | 927.8499       | 0.0001  |
| TRT                 | 2  | 101.2901       | 0.0221  |
| LINE*TRT            | 2  | 12.5836        | 0.6021  |
| rtDA                |    |                |         |
| LINE                | 1  | 341.0614       | 0.0001  |
| TRT                 | 2  | 2.2534         | 0.4989  |
| LINE*TRT            | 2  | 10.2853        | 0.0487  |
| rt5HT               |    |                |         |
| LINE                | 1  | 202.4461       | 0.1809  |
| TRT                 | 2  | 529.3323       | 0.0123  |
| LINE*TRT            | 2  | 2169.9778      | 0.0001  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations and concentrations square root of high weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 42513.9820     | 0.0009  |
| Epi<br>TRT          | 2  | 210.0012       | 0.0360  |
| 5HIAA<br>TRT        | 2  | 5481.4548      | 0.0004  |
| DA<br>TRT           | 2  | 1100.2308      | 0.0426  |
| 5HT<br>TRT          | 2  | 258512.9020    | 0.0008  |
| rtNE<br>TRT         | 2  | 161.1479       | 0.0011  |
| rtEpi<br>TRT        | 2  | 6.8743         | 0.0164  |
| rt5HIAA<br>TRT      | 2  | 40.8101        | 0.0002  |
| rtDA<br>TRT         | 2  | 11.1529        | 0.0329  |
| rt5HT<br>TRT        | 2  | 627.5194       | 0.0001  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations and concentrations square root of low weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 26459.5228     | 0.7361  |
| Epi<br>TRT          | 2  | 724.5792       | 0.6227  |
| 5HIAA<br>TRT        | 2  | 124872.7487    | 0.1635  |
| DA<br>TRT           | 2  | 823.3193       | 0.5937  |
| 5HT<br>TRT          | 2  | 4983829.5200   | 0.0031  |
| rtNE<br>TRT         | 2  | 6.1982         | 0.9105  |
| rtEpi<br>TRT        | 2  | 7.3729         | 0.4546  |
| rt5HIAA<br>TRT      | 2  | 73.5814        | 0.2278  |
| rtDA<br>TRT         | 2  | 1.8251         | 0.6069  |
| rt5HT<br>TRT        | 2  | 2101.6421      | 0.0005  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received saline

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 579880.5432    | 0.0001  |
| Epi<br>LINE         | 1  | 2486.9672      | 0.0896  |
| 5HIAA<br>LINE       | 1  | 34306.4484     | 0.0001  |
| DA<br>LINE          | 1  | 29065.3399     | 0.0002  |
| 5HT<br>LINE         | 1  | 7211351.7740   | 0.0203  |
| rtNE<br>LINE        | 1  | 1094.3597      | 0.0001  |
| rtEpi<br>LINE       | 1  | 38.9029        | 0.0161  |
| rt5HIAA<br>LINE     | 1  | 161.6997       | 0.0001  |
| rtDA<br>LINE        | 1  | 135.3905       | 0.0001  |
| rt5HT<br>LINE       | 1  | 3310.4531      | 0.0047  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 1.25 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 363431.5841    | 0.0023  |
| Epi<br>LINE         | 1  | 5436.4562      | 0.0114  |
| 5HIAA<br>LINE       | 1  | 173487.9846    | 0.0031  |
| DA<br>LINE          | 1  | 36028.2272     | 0.0001  |
| 5HT<br>LINE         | 1  | 338.5783       | 0.8356  |
| rtNE<br>LINE        | 1  | 536.6313       | 0.0008  |
| rtEpi<br>LINE       | 1  | 61.2612        | 0.0024  |
| rt5HIAA<br>LINE     | 1  | 445.9064       | 0.0001  |
| rtDA<br>LINE        | 1  | 168.5549       | 0.0001  |
| rt5HT<br>LINE       | 1  | 8.5645         | 0.5724  |

Chapter 2. Analysis of variance of heart catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 2.50 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 451849.8973    | 0.0363  |
| Epi<br>LINE         | 1  | 5210.9604      | 0.0329  |
| 5HIAA<br>LINE       | 1  | 408251.0251    | 0.0279  |
| DA<br>LINE          | 1  | 13010.1481     | 0.0002  |
| 5HT<br>LINE         | 1  | 434583.0650    | 0.0028  |
| rtNE<br>LINE        | 1  | 219.9773       | 0.1075  |
| rtEpi<br>LINE       | 1  | 45.0359        | 0.0134  |
| rt5HIAA<br>LINE     | 1  | 379.0361       | 0.0124  |
| rtDA<br>LINE        | 1  | 52.7215        | 0.0001  |
| rt5HT<br>LINE       | 1  | 1075.5487      | 0.0001  |

Chapter 2. Analysis of variance of plasma catechoamines and indoleamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| NE                  |    |                |         |
| LINE                | 1  | 46445.5715     | 0.1420  |
| TRT                 | 2  | 34934.8339     | 0.1978  |
| LINE*TRT            | 2  | 15872.3366     | 0.4731  |
| Epi                 |    |                |         |
| LINE                | 1  | 34667.0075     | 0.0983  |
| TRT                 | 2  | 12222.3339     | 0.3755  |
| LINE*TRT            | 2  | 20463.8609     | 0.1978  |
| 5HIAA               |    |                |         |
| LINE                | 1  | 1191152.6820   | 0.0494  |
| TRT                 | 2  | 283825.6910    | 0.3879  |
| LINE*TRT            | 2  | 1161226.1620   | 0.0253  |
| DA                  |    |                |         |
| LINE                | 1  | 3491399.0400   | 0.1798  |
| TRT                 | 2  | 3949963.2800   | 0.1337  |
| LINE*TRT            | 2  | 9931478.8100   | 0.0083  |
| 5HT                 |    |                |         |
| LINE                | 1  | 11283106.3900  | 0.0311  |
| TRT                 | 2  | 19125733.9600  | 0.0007  |
| LINE*TRT            | 2  | 12548979.4800  | 0.0070  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations of high and low body weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine after square root.

| Source of variation | df | Mean of Square | P value |
|---------------------|----|----------------|---------|
| rtNE                |    |                |         |
| LINE                | 1  | 126.7403       | 0.0786  |
| TRT                 | 2  | 32.9439        | 0.4389  |
| LINE*TRT            | 2  | 61.5539        | 0.2191  |
| rtEpi               |    |                |         |
| LINE                | 1  | 53.6811        | 0.0969  |
| TRT                 | 2  | 17.1875        | 0.4072  |
| LINE*TRT            | 2  | 36.0665        | 0.1569  |
| rt5HIAA             |    |                |         |
| LINE                | 1  | 511.5969       | 0.0496  |
| TRT                 | 2  | 102.6003       | 0.4503  |
| LINE*TRT            | 2  | 430.4499       | 0.0409  |
| rtDA                |    |                |         |
| LINE                | 1  | 422.3876       | 0.1615  |
| TRT                 | 2  | 570.4781       | 0.0750  |
| LINE*TRT            | 2  | 1035.4883      | 0.0108  |
| rt5HT               |    |                |         |
| LINE                | 1  | 917.1883       | 0.0419  |
| TRT                 | 2  | 2306.4528      | 0.0001  |
| LINE*TRT            | 2  | 942.1673       | 0.0161  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations and concentrations square root of high weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 17304.5918     | 0.5209  |
| Epi<br>TRT          | 2  | 19358.3264     | 0.3289  |
| 5HIAA<br>TRT        | 2  | 848247.155     | 0.1274  |
| DA<br>TRT           | 2  | 6685699.43     | 0.0244  |
| 5HT<br>TRT          | 2  | 31221475.84    | 0.0021  |
| rtNE<br>TRT         | 2  | 32.7684        | 0.5032  |
| rtEpi<br>TRT        | 2  | 33.5685        | 0.2602  |
| rt5HIAA<br>TRT      | 2  | 369.6074       | 0.1073  |
| rtDA<br>TRT         | 2  | 928.0052       | 0.0149  |
| rt5HT<br>TRT        | 2  | 3049.3467      | 0.0004  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations and concentrations square root of low weight selected female chickens that received saline and 1.25 mg/kg and 2.50 mg/kg reserpine.

| Source of variation | df | Mean of square | P Value |
|---------------------|----|----------------|---------|
| NE<br>TRT           | 2  | 32857.8270     | 0.1441  |
| Epi<br>TRT          | 2  | 13327.8684     | 0.2003  |
| 5HIAA<br>TRT        | 2  | 619981.3100    | 0.0654  |
| DA<br>TRT           | 2  | 6995024.8600   | 0.0595  |
| 5HT<br>TRT          | 2  | 453237.6063    | 0.4738  |
| rtNE<br>TRT         | 2  | 61.0018        | 0.1688  |
| rtEpi<br>TRT        | 2  | 19.6856        | 0.2604  |
| rt5HIAA<br>TRT      | 2  | 174.3687       | 0.1949  |
| rtDA<br>TRT         | 2  | 645.8982       | 0.0802  |
| rt5HT<br>TRT        | 2  | 199.2733       | 0.2498  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received saline

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 70849.3839     | 0.0020  |
| Epi<br>LINE         | 1  | 74353.3541     | 0.0054  |
| 5HIAA<br>LINE       | 1  | 2943252.6500   | 0.0044  |
| DA<br>LINE          | 1  | 383920.6091    | 0.6399  |
| 5HT<br>LINE         | 1  | 35056468.5100  | 0.0241  |
| rtNE<br>LINE        | 1  | 234.9367       | 0.0013  |
| rtEpi<br>LINE       | 1  | 122.9583       | 0.0034  |
| rt5HIAA<br>LINE     | 1  | 1195.5007      | 0.0023  |
| rtDA<br>LINE        | 1  | 31.4387        | 0.6657  |
| rt5HT<br>LINE       | 1  | 2488.3760      | 0.0153  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 1.25 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 70849.3839     | 0.0020  |
| Epi<br>LINE         | 1  | 74353.3541     | 0.0054  |
| 5HIAA<br>LINE       | 1  | 2943252.6500   | 0.0044  |
| DA<br>LINE          | 1  | 383920.6091    | 0.6399  |
| 5HT<br>LINE         | 1  | 35056468.5100  | 0.0241  |
| rtNE<br>LINE        | 1  | 234.9367       | 0.0013  |
| rtEpi<br>LINE       | 1  | 122.9583       | 0.0034  |
| rt5HIAA<br>LINE     | 1  | 1195.5007      | 0.0023  |
| rtDA<br>LINE        | 1  | 31.4387        | 0.6657  |
| rt5HT<br>LINE       | 1  | 2488.3760      | 0.0153  |

Chapter 2. Analysis of variance of plasma catechoamines and indoalamine concentrations and concentrations square root of high and low weight selected female chickens that received 2.50 mg/kg reserpine

| Source of variation | df | Mean of square | P value |
|---------------------|----|----------------|---------|
| NE<br>LINE          | 1  | 3471.5914      | 0.7856  |
| Epi<br>LINE         | 1  | 591.8807       | 0.8712  |
| 5HIAA<br>LINE       | 1  | 176856.8905    | 0.5219  |
| DA<br>LINE          | 1  | 22002854.5100  | 0.0093  |
| 5HT<br>LINE         | 1  | 745818.9369    | 0.2382  |
| rtNE<br>LINE        | 1  | 1.5828         | 0.8836  |
| rtEpi<br>LINE       | 1  | 0.0074         | 0.9879  |
| rt5HIAA<br>LINE     | 1  | 39.9674        | 0.6531  |
| rtDA<br>LINE        | 1  | 2375.9548      | 0.0084  |
| rt5HT<br>LINE       | 1  | 124.5638       | 0.3415  |

## VITA

Alice Yi-Wen Kuo was born on September 18, 1971 in Taipei, Taiwan. In 1993, she entered the Department of Biology at the University of Hawaii at Hilo and received a Bachelor's of Art Degree in 1997. In the spring of 1998, she embarked on her graduate study and research in neurophysiology and neurochemical regulation under the supervision of Dr. D. Michael Denbow of the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University.

The candidate is an active member of the American Poultry Science Association, and the Hawaii State Department of Agriculture's Pesticide Control Association.

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