

CENTRAL CANNABINOID REGULATION OF FOOD INTAKE IN CHICKENS

JIN ZHANG

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APPROVED:

D. Michael Denbow, Chairman

Paul B. Siegel

Dongmin Liu

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Key Words: Chickens, Cannabinoid, Endogenous cannabinoid, Food intake.

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by

Jin Zhang

D.M. Denbow, Chairman

Department of Animal and Poultry Sciences

ABSTRACT

Marijuana has been used for medicinal and recreational purposes for thousands of years. Many people think of marijuana in the context of an illegal drug. Because of the antimarijuana attitude, research with cannabinoids was neglected for a long time. Although this substance is related to social problems, scientists are interested in its action and possible medicinal properties. Since the identification of the structure of Δ^9 -tetrahydrocannabinol, the main psychoactive ingredient of marijuana, there has been increased interest in this compound. Following the discovery of two cannabinoid receptors, CB1 and CB2 receptors, it was determined that CB1 receptors are in high density in the central nervous system while CB2 receptors are found primarily in the immune system. The endogenous cannabinoid ligands, anandamide and 2-arachidonoylglycerol, were observed in the central nervous system and peripheral tissues. Endocannabinoids differ from other "classical" neurotransmitters because they do not appear to be stored in synaptic vesicles, and they act as retrograde messengers within the brain. The endogenous cannabinoid signaling system includes cannabinoid receptors, their endogenous ligands called endocannabinoids, and the proteins for their synthesis and inactivation. The cannabinoid system appears to act as a neuromodulatory system. During the past ten years, the endogenous cannabinoid system has been implicated in a variety of physiological functions including pain reduction, motor regulation, learning, memory, and reward.

Because obesity and eating disorders are prevalent, scientists are working at the molecular level to study the mechanisms controlling body weight and regulation of food intake. Several of the neuropeptides present in hypothalamic nuclei contribute to energy

balance and food intake regulation. Endogenous cannabinoid and cannabinoid receptors are found in the hypothalamus and are associated with the regulation of food intake. Although the mechanisms whereby cannabinoids influence food intake remain unclear, results suggest that the cannabinoid system will be an important target in future studies in obesity.

Most research on cannabinoids has focused on their role in food intake regulation in mammalian species. It is important to determine the role of endocannabinoids in other species. The effect of intracerebroventricular injection of agonists and antagonists of both CB1 and CB2 receptors in 8 to 11 week-old male Single Comb White Leghorn and 3 to 6 weeks old male broilers was investigated. It was found that agonists of both the CB1 and CB2 receptor increased food intake significantly; however, the CB2 receptor agonist had a stronger and longer lasting effect. Antagonists of both receptors decreased food intake significantly. The CB1 receptor antagonist appeared to block both cannabinoid receptors in birds, whereas the CB2 receptor antagonist did not block both receptors. Previous studies have indicated that the CB2 receptor is found only outside the brain and spinal cord, and is involved with the immune system. From the present results, it appears that both cannabinoid receptors are present in the chicken brain. Furthermore, the CB2 receptor may also be localize in the chicken brain. There are also differences in cannabinoid system between Leghorn and broilers.

Key words: Chickens, Cannabinoid, Endogenous cannabinoid, Food intake.

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CHAPTER I

LITERATURE REVIEW

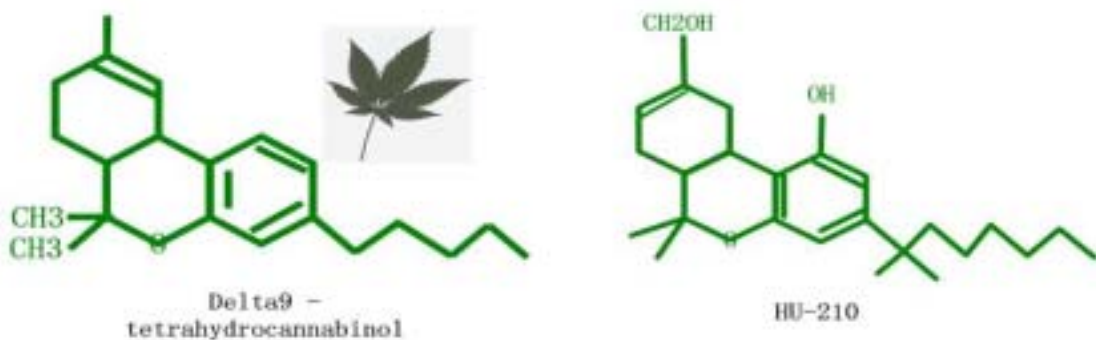
Marijuana has been cultivated and used medicinally and recreationally for thousands of years, but our knowledge of the chemistry and physiology of cannabinoids is quite recent. Studies began from the discovery of the structure of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the major chemical constituent of marijuana. Following the discovery of two cannabinoid receptors, scientists found that the endogenous cannabinoid ligands, anandamide and 2-arachidonoylglycerol, were located in the central nervous system and peripheral tissues. These compounds were called endocannabinoids. During the past ten years, endocannabinoids have been implicated in a variety of physiological functions including pain reduction, motor regulation, learning, memory, and reward. Because endocannabinoid and cannabinoid receptors are found in the hypothalamus, and several neuropeptides present in hypothalamic nuclei contribute to the energy balance and food intake regulation, most research has focused on their role in appetite stimulation and food intake regulation. Although the mechanisms whereby cannabinoids influence food intake remain unclear, data suggest the cannabinoid system will be an important target in future studies in obesity.

Cannabinoid and its receptors

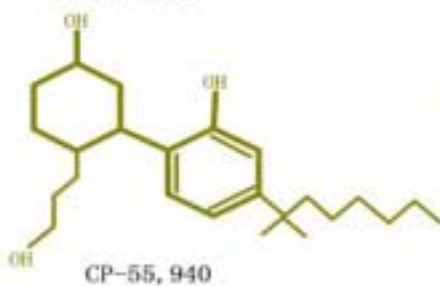
Cannabinoids were identified by Di Marzo et al. (2004) as natural lipophilic products from the flower of *Cannabis sativa*. Most have a typical bi-cyclic or tri-cyclic structure and a common biogenetic origin from olivetol. Δ^9 -tetrahydrocannabinol (Δ^9 -THC) was the first cannabinoid isolated, and was identified as the major psychoactive component of cannabis in 1964 (Gaoni and Mechoulam, 1964). Cannabinoids have been characterized according to their chemical structures and are divided into four major classes (Fig. 1-1) (Cota et al., 2003). The first group is the so called “classical” cannabinoids, and is composed of substances containing a 3-ring structure. This group contains 2 types of cannabinoids. The plant-derived cannabinoids contain more than 60 different compounds, including Δ^9 -THC, Δ^8 -THC and cannabidiol (CBD). Other cannabinoids in this group include compounds synthesized at Hebrew University in Israel,

such as HU-210 which induces effects typical for cannabinoids (Mechoulam et al., 1988; Howlett et al., 1990). A second group includes nonclassical cannabinoids, and contains bi- and tricyclic analogs of Δ^9 -THC such as CP-55,940 (Howlett et al., 1990). A third group of compounds are named aminoalkylindols, typified by WIN-55,212, which is a CB1 agonist (Howlett, 1998), which a fourth group is represented by endocannabinoids, which are the endogenous agonists of the cannabinoid receptors in animal organisms (Cota et al., 2003; Di Marzo et al., 2004).

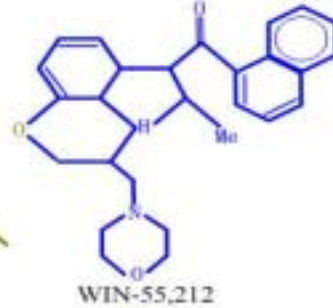
First group: Classical cannabinoid



Second group: non-classical cannabinoid



Third group: aminoalkylindolos



Fourth group: Endocannabinoid



Figure 1-1. Classification of cannabinoid compounds according to the chemical structures. (Cota et al., 2003- Copyright permission granted 6/10/2005)

After the isolation of Δ^9 -THC in 1964, the first cannabinoid receptor, CB1, was reported in 1988 (Devane et al., 1988), according to the binding of radioactive synthetic

cannabinoid CP 55,940 to rat brain homogenates. Two years later, a complementary DNA that encoded rat brain cannabinoid receptor was cloned (Matsuda et al., 1990). Three years later, the second cannabinoid receptor, CB2, was cloned from macrophages in the marginal zone of the spleen (Munro et al., 1993). CB1 has wide distribution in the central nervous system with the highest density in the basal ganglia, cerebellum, hippocampus, hypothalamus and cortex. It is also present in the peripheral nervous system and several peripheral organs including the pituitary gland, immune cells, reproductive tissues, gastrointestinal tissues, heart, lung, urinary bladder and adrenal gland (Pertwee, 1997). In the central nervous system, cannabinoids modulate neurotransmitter release via specific presynaptic CB1, including γ -aminobutyric acid (GABA), dopamine, noradrenaline, glutamate and serotonin (Schlicker and Kathmann, 2001). CB2, by contrast, is present mainly in immune tissues and including B-cells and natural killer cells (Galegue et al., 1995). Although the pharmacological and physiological importance of CB2 is not well understood, it may involve suppression of proinflammatory cytokine release and enhancement of anti-inflammatory cytokine release from immune cells (Berdyshev, 2000).

Both CB1 and CB2 receptors belong to the G-protein-coupled receptor family, where they appear to activate $G_{i/o}$ proteins and inhibit adenylate cyclases, stimulate mitogen-activated protein kinases, block voltage-dependent Ca^{2+} channels, and activate voltage-dependent K^+ channels (Fig. 1-2) (McAllister and Glass, 2002; Schlicker and Kathmann, 2001).

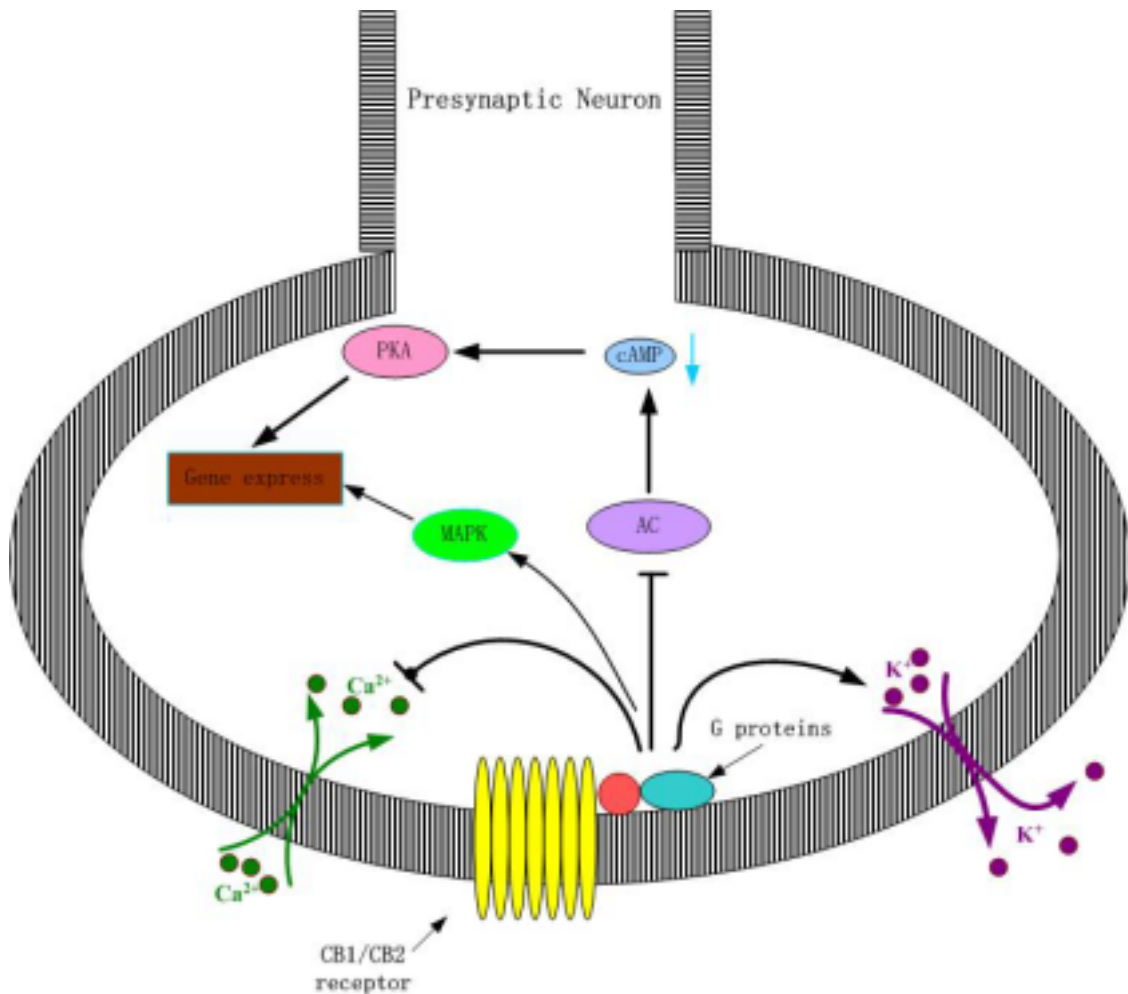


Figure 1-2. Major signaling pathways associated with cannabinoid receptor activation by agonists. Cannabinoid receptor agonists binds to and activate each of the cannabinoid receptors, and either stimulate $G_{i/o}$ heterotrimeric proteins which inhibit adenylate cyclase(AC) the inactivating the protein kinase A (PKA) phosphorylation pathway, or stimulate mitogen-activated protein kinase (MAPK). These intracellular events lead to the regulation of expression of several genes. The CB1, but not CB2 receptor via G_s proteins stimulate AC via $G_{i/o}$ proteins coupled to inhibition of voltage-activated Ca^{2+} channels and stimulate K^+ channels in neurons, thus inhibiting neurotransmitter release (Di Marzo et al., 2004- Copyright permission granted 6/9/2005).

Endocannabinoids

The identification and cloning of cannabinoid receptors indicated the presence of endogenous ligands (endocannabinoid). In 1992, Devane et al., (1992) discovered a small fatty acid produced in the brain that binds to CB1 and mimics all the activity of THC. Called anandamide (N-arachidonylethanolamine), it is the most widely investigated representative of the class of endocannabinoid polyunsaturated fatty acid ethanol amides. This substance, which binds to CB1 and CB2 receptors, has been found in human and rat hippocampus, striatum, and cerebellum; brain areas known to express high levels of CB1 receptors (Felder et al., 1996). Subsequently, another lipid called 2-arachidonoylglycerol (2-AG), was found to be an endogenous ligand present in canine gut and brain. It displays lower affinity for the CB1 receptor, but is selective for the CB2 receptor, and is more abundant in certain brain regions than anandamide (Mechoulam et al., 1995; Sugiura et al., 1995; Stella et al., 1997). Together, these two compounds are considered the major endocannabinoids.

Most of the pathways and enzymes involved in the biosynthesis and degradation of endocannabinoids have been identified (Fig 1-3). The hydrolysis of precursors derived from phospholipids produce both anandamide and 2-AG. The biosynthetic enzymes of anandamide, N-acyltransferase (NAT), N-acylphosphatidyl-ethanolamine-specific phospholipase D (NAPE-PLD), and the inactivating enzyme fatty acid amide hydrolase (FAAH) are all located on intracellular membranes. FAAH was found abundant on postsynaptic neurons of CB1 receptors, indicating that anandamide mainly acts on CB1 receptors, but it is unknown whether NAT and NAPE-PLD are located in the presynaptic or postsynaptic neurons (Schmid et al., 1983; Cravatt et al., 1996; Egertova et al., 2003; Okamoto et al., 2004). The biosynthetic enzyme of 2-AG, phospholipase C (PLC) and sn-1-selective diacylglycerol lipases (DAGL), are mostly localized on the plasma membrane. DAGL is found on postsynaptic neurons in the adult nervous system, and the inactivating enzyme monoacylglycerol lipase (MAGL) is localized in the presynaptic neurons, which supports a possible role as a retrograde messenger at presynaptic CB1 receptors for 2-AG (Bisogno et al., 1997; Bisogno et al., 2003; Dinh et al., 2001; Chevaleyre et al., 2003).

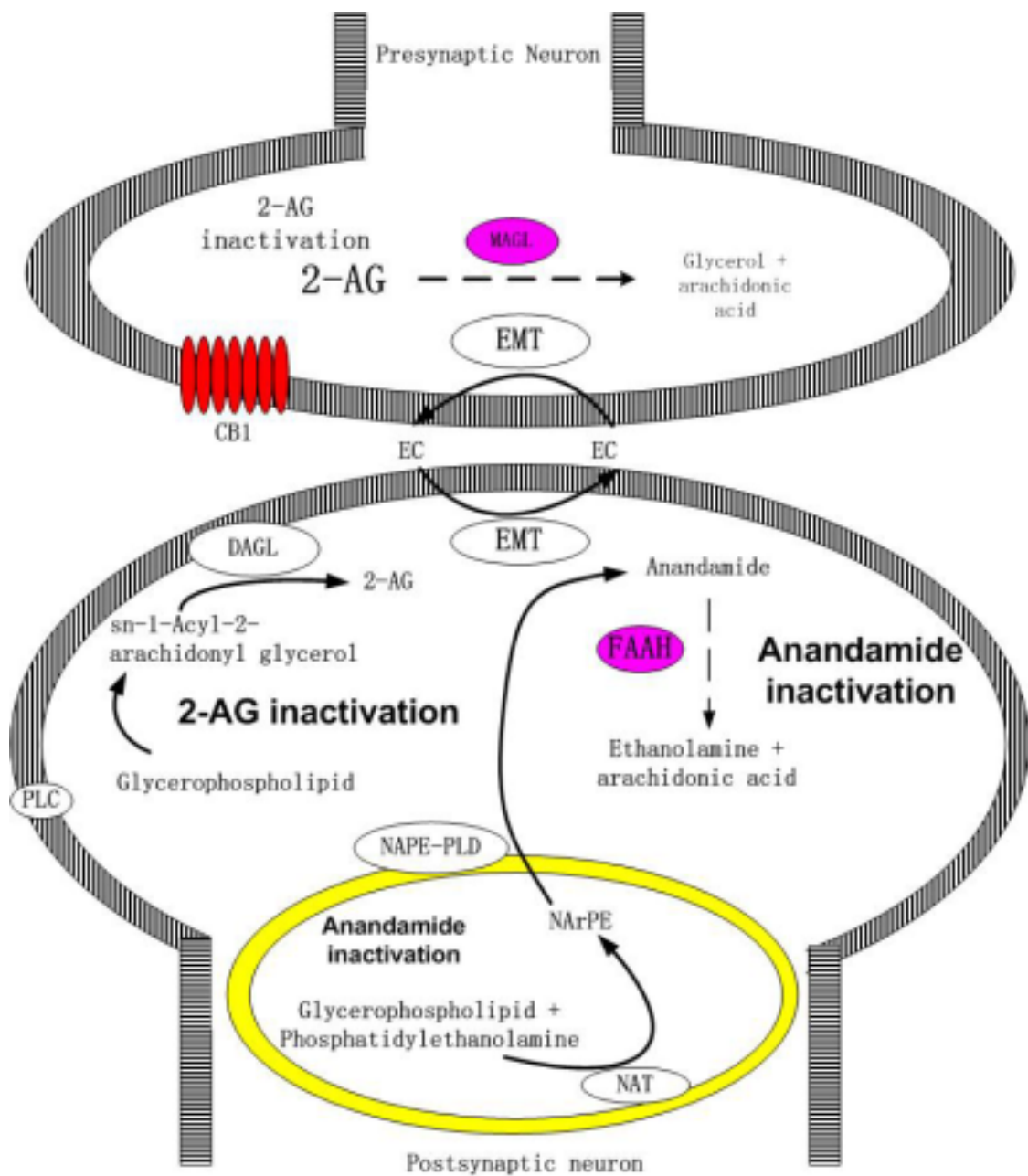


Figure 1-3. Biosynthesis and degradation of endocannabinoid. EC, endocannabinoid. EMT, endocannabinoid membrane transporter. 2-AG, 2-arachidonolglycerol. PLC, phospholipases C. DAGL, sn-1-selective diacylglycerol lipases. MAGL, monoacylglycerol lipases. NAT, N-acyltransferase. NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D. FAAH, fatty acid amide hydrolase. NArPE, N-arachidonoyl-phosphatidylethanolamine (Di Marzo et al., 2004- Copyright permission granted 6/9/2005).

Endocannabinoids are different from other conventional neurotransmitters. Regular neurotransmitters are water-soluble and stored in high concentrations in vesicles. When the neuron depolarizes, an electrical signal at the presynaptic terminals cause neurotransmitter release from the presynaptic terminal which then binds to receptors on postsynaptic neuron (Nicoll and Alger, 2004). Endocannabinoids are synthesized in postsynaptic neurons, and after excitation stimulate various phospholipases. The excitation of the neuron causes depolarization and an influx of calcium ions; postsynaptic calcium then activates enzymes that synthesize endocannabinoids from lipid precursors in the postsynaptic cell. Endocannabinoids then leave the postsynaptic cell and active cannabinoid receptors on the presynaptic membrane of the neurons. G proteins then directly inhibit presynaptic calcium influx. This reduces the probability of the release of synaptic vesicles containing the neurotransmitters GABA or glutamate. Endocannabinoids are removed from the synapse by endocannabinoid transporters into neurons or glial cells, and then broken down by fatty acid amide hydroxylase (FAAH) (Fig 1-4) (Christie and Vaughan, 2001; Wilson and Nicoll, 2002). This signaling, called retrograde, was previously thought to occur only during development of the nervous system; however endocannabinoids participate in this retrograde signaling in adult neurons.

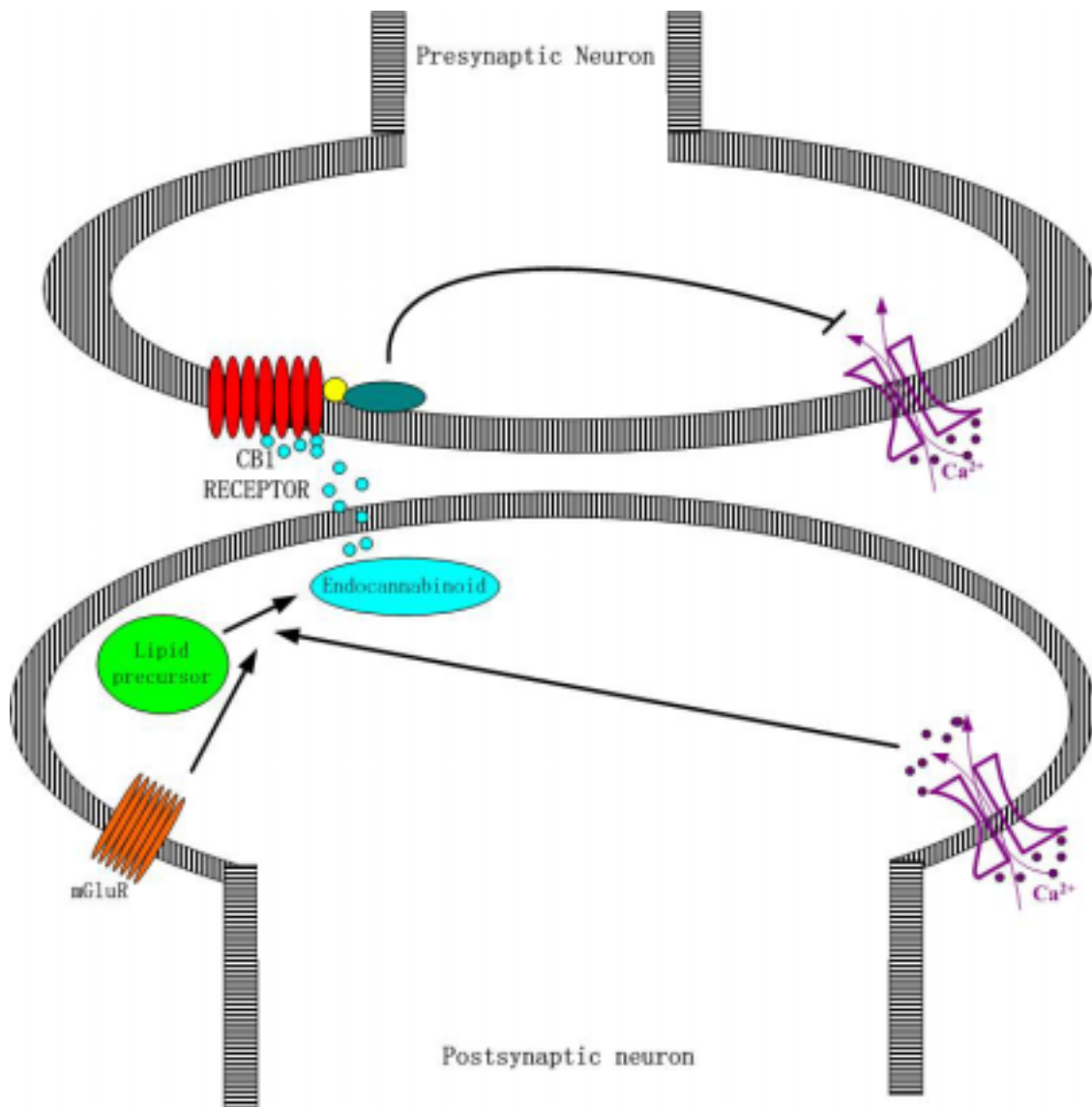


Figure 1-4. Endocannabinoids act as retrograde messages within the brain. Postsynaptic depolarization causes calcium channels to open. The influx of calcium causes endocannabinoid synthesis from lipid precursor. Activation of mGluR may also cause endocannabinoid synthesis. Endocannabinoids leave the postsynaptic cell and bind to the cannabinoid receptors on the presynaptic membrane and activate G protein which inhibits calcium influx. This decreases the release of the neurotransmitter (Wilson and Nicoll, 2002- Copyright permission granted 6/24/2005).

Roles of endocannabinoid system

Food intake

Since 300 AD, marijuana was recommended in India to treat loss of appetite. In the 19th century, it was reported that cannabis could be used to increase stimulation of appetite (Abel, 1975). Following the detection of cannabinoid receptors in the hypothalamus, an area important in food intake regulation, and several researchers provided evidence that the endocannabinoid system was involved in appetite regulation. Indeed, the use of Δ^9 -THC was approved by FDA, and a compound named Dronabinol was approved to stimulate appetite in immunodeficiency syndrome (AIDS) and cancer patients (Mechoulam et al., 1998).

Anandamide increased food intake in rats, while the CB1 antagonist SR141716A inhibited the intake of palatable food (Williams et al., 1999). The effect of THC and anandamide on feeding is dose-dependent with low doses causing stimulation of food consumption and higher doses causing inhibition (Hao et al., 2000). To determine if both CB1 and CB2 receptors are involved in food intake, Trillou et al. (2004) used CB1 cannabinoid receptor knockout mice. Their results indicate that the stimulation of CB1 receptors is a key component in the development of diet-induced obesity, and these receptors and their endogenous ligands are implicated not only in feeding control but also in peripheral metabolic regulation.

Leptin is a primary signal through which the hypothalamus senses nutritional state. Di Marzo et al. (2001) observed that leptin reduced endocannabinoid levels in the hypothalamus but not the cerebellum of rats, and that endocannabinoid levels increased in animals with defective leptin signaling. SR141716SA reduced food intake in normal mice, but had no effect in CB1 knockout mice. These results suggest that the endocannabinoid system contributes to the stimulation of appetite by activating CB1 receptors present in hypothalamus and that it may be a part of the appetite triggering network controlled by leptin.

Although many experiments indicated that endocannabinoids regulate food intake through the central nervous system, evidence suggests that they may also promote feeding via peripheral sites. Gomez et al. (2002) found that peripherally injected anandamide promoted hyperphagia in partially satiated, capsaicin deafferentation rats.

Central administration SR 141716 did not alter this response. Thus, endocannabinoid may regulate food intake through both the central and peripheral nervous system.

Although there is evidence suggesting that endocannabinoids play a role in feeding, the mechanisms are unknown. The effects of endocannabinoids on food intake are related to a direct action at the level of the hypothalamus. Both CB1 receptors and endocannabinoids are present in high levels in the hypothalamus, a brain area associated with feeding. They appear to the release of hypothalamic hormones and peptides, and the regulation of food intake and the pituitary-hypothalamus-adrenal axis (Wenger and Moldrich., 2002).

The endocannabinoid system may also have a relationship with the reward pathway to regulate food intake. Food reward and drug reward pathways may share common components (Harrold and Williams., 2003). CB1 receptors are expressed in areas of the brain such as nucleus accumbens, hippocampus and entopeduncular nucleus, areas either directly involved in hedonic aspects of eating or connected to reward-related brain areas. In addition, cannabinoids appear to interact with known opioidergic reward pathways (Kirkham and Williams., 2001).

Pain

Cannabis was widely used as medicine for pain relief as early as 1500 years ago (Zias et al., 1993). Endocannabinoids and cannabinoid receptors have been reported to exist at various levels in the pain pathways from the peripheral sensory nerve endings to spinal cord and supraspinal centers (Iversen, 2003). This system is parallel but distinct from that involving endorphins and opiate receptors. Systemically administrated THC, anandamide, and 2-AG have anti-nociceptive and anti-hyperalgesic effects in a variety of animal models of acute and inflammatoroty pain, with the effect of THC more efficacious (Hanus et al., 2001; Fride and Mechoulam, 1993; Iversen and Chapman, 2002). As has been shown by Walker et al. (1999), noxious stimulation increased release of anandamide in the periaqueductal grey region of the brainstem, a midbrain area playing a pivotal role in pain perception. Furthermore, after administration of the CB1 receptor antagonist SR141716, hyperalgesia was observed (Richardson et al., 1997). This result suggests that the endocannabinoid system is involved in the maintenance a tonic inhibition of pain.

Not all the anti-nociceptive effect of THC or anandamide is mediated via CB1 receptors. THC anti-nociceptive effects were absent in CB1 receptor-knockout mice, but anandamide continued to show analgesic activity in the hot-plate test, suggesting that the analgesic effects of anandamide may be mediated in part through non-CB1 receptors (Di Marzo et al., 2000). The effects of anandamide might be mediated through the vanilloid VR1 receptor, which is present in primary afferent neurons and known to play an important role in nociceptive responses (Di Marzo et al., 2001).

Opiate receptors are also abundantly distributed in the periaqueductal gray as well as in other areas, where CB1 receptors are found and thought to mediate pain (Richardson et al., 1997). There may be an interaction between cannabinoid and opioid mechanisms.

Motor control

CB1 receptors are expressed in high density in the basal ganglia and cerebral cortex, regions that play an important role in motor control (Sanudo-Pena et al., 2000). Cannabinoids affect motor behavior in a biphasic or even triphasic manner and administration of low doses of THC to rats decreased locomotor activity, while high doses stimulated movements (Sanudo-Pena et al., 2000). However, the CB1 receptor antagonist rimonabant stimulated locomotor activity in mice, tonic activity in the endocannabinoid system that contributes to the control of spontaneous levels of activity (Compton et al., 1996).

Cannabinoids may affect motor control by modifying the release of other neurotransmitters. The cannabinoid system has an effect on GABA release. CB1 receptors express high density in striatal GABAergic medium-spiny projection neurons, and the terminals of glutamatergic projection neurons form the subthalamic nucleus to globus pallidus, entopeduncular nucleus and substantia nigra reticulata, and also are abundant in regions containing the axon terminals of these cells (Iversen, 2003). Cannabinoids might thus be expected to inhibit GABA release in the striatum and GABA and glutamate release in the other nuclei (Sanudo-Pena et al., 1999). Another concept is that the cannabinoid system has a relationship with dopamine release. In 1999, Giuffrida and colleagues showed that the dopamine D2 receptor agonists caused an increase in the anandamide synthesis and release in the striatum. Furthermore, localized administration

of cannabinoids into the nigrostriatal system of the rat counteracted the motor response to D2 receptor agonists (Sanudo-Pena et al., 1998). The anandamide transport blocker AM404 counteracted D2 receptor-mediated responses such as apomorphine-induced yawning (Beltramo et al., 2000). According to the above information, cannabinoid-based medicines may be possible for the treatment of pathological conditions in which the dopamine system is thought to play a role. Such diseases include Parkinson's disease, Huntington's disease, Tourette syndrome, multiple sclerosis and schizophrenia.

Other functions

There are other functions in which cannabinoid system may be involved. CB1 receptors are in high density in the hippocampus, which plays a role in learning and memory formation (Deadwyler et al., 1995). Experiments suggests that too few endocannabinoid receptors or insufficient release of endocannabinods underlie chronic anxiety and post-traumatic stress disorders (Manzanares et al., 2004). The endocannabinoid system also plays a role in the control of intestinal functions. High concentrations of anandamide and CB1 receptors are found in three intestinal disorders in mouse models: small intestine inflammation, cholera-toxin-induced intestinal hypersecretion and diarrhea, and peritonitis-induced paralytic ileus (Izzo et al., 2001; Izzo et al, 2003; Mascolo et al., 2002). Endocannabinoids also control reproductive functions, where anandamide, acting preferentially at CB1 receptors can stimulate embryo implantation at low concentrations and inhibit implantation at higher concentrations (Wang et al., 2003). Endocannabinoids might represent one of the many adaptive responses aimed at counteracting tumour-cell growth (Ligresti et al., 2003).

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CHAPTER II
CENTRAL CANNABINOID REGULATION OF FOOD INTAKE IN
CHICKENS

ABSTRACT

Leghorn and broiler chickens show extreme differences in ingestive and reproductive behavior. Since the goal of my thesis was to determinate the effect of central administration of endocannabinoids on food intake regulation in chickens, the effect of the CB1 agonist 2-arachidonoyl glycerol (2-AG), CB1 antagonist AM251, CB2 agonist JWH015 and CB2 antagonist AM630 on food intake in Leghorns and broilers were investigated. The CB1 agonist 2-AG significantly increased food intake in Leghorns, but had no effect in broilers. The CB2 agonist JWH015 caused a stronger and longer lasting increase in food intake with both Leghorns and broilers. The CB1 antagonist AM251 and CB2 antagonist AM630 significant decreased food intake in both Leghorns and broilers. In addition, the CB1 antagonist appeared to block both the CB1 and CB2 receptors in the chicken brain. In contrast, which pretreatment with the CB2 antagonist AM630 did not block the CB1 or CB2 receptors in Leghorns, in broilers it may block the CB2 receptor in broilers, while not blocking the CB1 receptor. The present studies suggest that the regulation of food intake by endocannabinoids in chickens may differ from that in mammalian species and there are also differences between Leghorn and broilers in cannabinoid system. Furthermore, the CB2 receptor may also be localize in the chicken brain.

Key words: chicken, food intake, intracerebroventricular, CB1 agonist, CB2 antagonis

INTRODUCTION

Cannabis sativa has been cultivated for thousands of years and provides a variety of extracts for medicinal and recreational use. Chemical constituents of cannabis are called cannabinoids. Following the discovery of Δ^9 -tetrahydrocannabinol, which is the major psychoactive ingredient of Cannabis, cannabinoid receptors and their endogenous ligands (endocannabinoids) were also found, thus showing the existence of an endogenous cannabinoid system (Gaoni et al., 1964; Matsuda et al., 1990; Munro et al., 1993; Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995).

Matsuda et al. (1990) cloned the first cannabinoid receptor (CB1) from porcine brain, while the second cannabinoid receptor (CB2) was cloned from the spleen by Munro et al. (1993). Cannabinoid receptors belong to the seven-transmembrane, G protein-coupled receptor family (Howlett et al., 2002). The CB1 receptor is predominantly expressed presynaptically in the cerebral cortex, hippocampus, hypothalamus, cerebellum, basal ganglia, brain stem, spinal cord and amygdala, modulating the release of neurotransmitters including γ -aminobutyric acid (GABA), dopamine, noradrenaline, glutamate and serotonin, and is considered the 'brain-type' cannabinoid receptor (Cota et al., 2003; Nicoll and Alger, 2004; Schlicker et al., 2001). The CB2 receptor is considered the 'peripheral counterpart' because it was detected in rat spleen, mouse spleen and thymus, and is expressed in the immune system (Munro et al., 1993; Schatz et al., 1997). The CB2 receptor is important in cellular and humoral immune responses and is implicated in inflammation and chronic pain (Klein et al., 2003). Although the CB1 receptor is found predominately in the brain, it has also been found in the human B cells, T cells and monocytes and mouse spleen (Schatz et al., 1997). Furthermore, the CB2 has also been localized in brain-derived immune cells (Porter and Felder, 2001).

Following the discovery of cannabinoid receptors in 1992, Devane et al. (1992) discovered a small fatty acid produced in the brain that binds to CB1 receptor and mimics all the activities of marijuana; it was named anandamide. Three years later, another lipid, 2-arachidonoyl glycerol, was discovered and found to be even more abundant in certain brain regions than anandamide (Mechoulam et al., 1995; Sugiura et al., 1995). These two

compounds are considered the major endogenous cannabinoids. In contrast to most other neurotransmitters, endocannabinoids act retrogradally, traveling from the postsynaptic neuron to the presynaptic neuron (Di Marzo et al., 1998). The release of endocannabinoids is triggered by membrane depolarization and calcium influx into the cells, and hydrolyzed by fatty acid amide hydrolase (FAAH) (Di Marzo et al., 1998).

Cannabis-regulated appetite has been known for a long time. During the 19th century, it was observed that Cannabis would stimulate hunger and increase appetite (Abel, 1975). Because the CB1 receptor and endocannabinoids are present in high levels in the hypothalamus, a region known to be involved in food intake, the role of endocannabinoids in appetite has been studied (Harrold et al., 2002).

Most of the present knowledge of endocannabinoid on regulation of food intake has been derived from studies in mammalian species. It is important to determine if endocannabinoids behave like their mammalian equivalents in other species. Little is known about the role of endocannabinoids in food intake regulation in avian species. In the present study, we used CB1 and CB2 agonists and antagonists to investigate the role of endocannabinoids in food intake in Leghorns and broilers.

MATERIALS AND METHODS

Animals

Male day-old Leghorn and broiler chicks were reared in heated batteries with raised wire floor, and continuous lighting. At 3 and 8 weeks-of-age, respectively, the broiler chicks and Leghorn chicks were transferred to individual cages measuring 17.6 x 26.4 x 17.6cm. Each cage was equipped with individual feeders and waters, and continuous lighting. The birds were given free access to a commercial started diet (20% crude protein, 3.0% crude fat, and 2,864 kcal/kg of metabolizable energy) and water for *ad libitum* consumption.

Drugs

The following drugs were purchased from Tocris Cookson, Inc¹. 2-Arachidonylglycerol ((5Z,8Z,11Z,14Z)-5,8,11,14-Eicosatetraenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester), a cannabinoid receptor 1 agonist; AM251(N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide), a cannabinoid receptor 1 antagonist; JWH015 ((2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone), a cannabinoid receptor 2 agonist; AM630 (6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone), a cannabinoid receptor 2 antagonist. These compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at with a desiccant at -20 .

Artificial cerebrospinal fluid (aCSF) consisted of NaCl 0.0013 mol; KCl 0.000038g; CaCl₂ 0.000016mol; MgCl·6H₂O 0.00004mol; NaHCO₃ 0.0023mol; NaHPO₄ 0.000017mol; Ascorbic acid 0.01g/100ml.

Surgery

At 3 and 8 weeks of age, broilers and Leghorns, respectively were anesthetized with iv administration (25mg/kg body weight) of sodium pentobarbital. A 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle for intracerebroventricular (ICV) injection according to the method of Denbow et al. (1981). The injection volume was 10µl in

¹ Tocris Cookson, Inc., 16144 Westwoods Business Park, Ellisville, MO 63021

all experiments. Placement of the cannula into the ventricle was verified the presence of cerebrospinal fluid. A minimum of 3 days recovery allowed for the birds before injection.

Experiment Design

Experiment 1: Effect of ICV injection individual dosage of both CB1 and CB2 agonist and antagonist on Leghorns and broilers.

Eight chicks (8 to 11 week-old Leghorns and 3 to 6 week-old broilers) were divided into two groups and used in a replicated Latin Square design in which birds and days were the blocking factors. DMSO was used as a control for one group, while the remaining treatments received different dosages of either 2-AG, JWH015, AM251 and AM630. Injections were made using a 27-gauge stainless steel injection cannula connected with a 10 μ l Hamilton syringe with a 60cm length of PE-20 tube (Clay Adams). Feed intake and water consumption were recorded at 15 minutes intervals 0~ 180 minutes after injection.

Experiment 2: Effect of ICV injection combine dosage of both CB1 and CB2 agonist and antagonist on Leghorns and broilers.

Using an experimental design similar to Experiment 1, each bird received two 5 μ l injections 15 minutes apart. The treatment consisted of : 1) DMSO followed by DMSO, 2) a CB1 or CB2 antagonist followed by DMSO, 3) DMSO followed by a CB1 or CB2 agonist, and 4) a CB1 or CB2 antagonist followed by a CB1 or CB2 agonist, respectively. The dosages were based on the Experiment1. Feed and water intake were recorded at 15 minutes intervals for 180 minutes after the second injection.

Statistical Analysis

Feed and water intakes were measured as g and ml consumed, respectively, and analysis of variance was performed at each time period. Duncan's multiple range test and non-orthogonal contrasts were used for obtaining all pair comparisons among sample means at each time period. Significance implies $p \leq 0.05$.

RESULTS AND DISCUSSION

ICV injections of the CB1 and CB2 agonists and antagonists in Leghorns and broilers revealed that the CB1 agonist 2-arachidonylglycerol (2-AG) caused a quadratic increase in food consumption from 0 to 30 min after administration ($P < 0.05$) in Leghorns, but had no effect in broilers (Fig 2-1 and 2-2). The CB1 antagonist AM251 caused a quadratic decrease in food consumption from 135 min to 150 min postinjection in SCWL (Fig 2-3), and a quadratic decrease from 0 min to 30 min on broilers (Fig 2-4). The CB2 agonist JWH015 caused a linear increase in food consumption in SCWL (Fig 2-5) and broilers (Fig 2-6). The CB2 antagonist AM630 caused a linear decrease in food intake in both SCWL (Fig 2-7) and broilers (Fig 2-8). Those drugs caused no other apparent affects on behavior, no preening, no vocalization, no locomotion, no sleeping.

The central administration of the CB1 agonist 2-AG potently and dose-dependently stimulated feeding in rats (Kirkham et al., 2002), whereas the peripheral administration of the CB1 antagonist AM251 caused a sustained reduction in daily food intake of rats (Chambers et al., 2004). ICV injections of the CB2 antagonist AM630 failed to block deprivation-induced intake in rats (Werner and Koch, 2003). There are no reports of the effect of the CB2 agonist JWH015 on the food intake. Fowler et al. (2000) investigated the pharmacological properties of brain cannabinoid receptors in 35 day-old Leghorn chickens, and found CB1 receptor recognition sites in the brain having similar pharmacological properties to those in the rat brain. Furthermore, CB2 receptor-like immunoreactivity was expressed in the embryonic chick telencephalon (Fowlet et al., 2000).

In the present study we compare the effects of cannabinoid receptor agonists and antagonists in Leghorns and broilers. Leghorns and broilers show extreme differences in feeding and reproductive behavior. Leghorns have been indirectly selected for slow growth and low body weight as a result of selection for high egg production (Kinney, 1969), while broilers have been genetically selected for rapid muscle and body weight gain (Gous, 1986). The CB1 agonist 2-AG increased food intake in Leghorns but had no effect in broilers. This inconsistency may be due to the differences in localization, affinity or expression of the CB1 receptor meat and egg-type chickens. Central administration of JWH015 caused a more efficacious increase in both Leghorns and

broilers than did the CB1 agonist. Although most studies show that the CB2 receptor is expressed mainly in immune system, it has been also reported to be localized in brain-derived immune cells (Munro et al., 1993; Schatz et al., 1997; Porter and Felder, 2001). The results of our experiments indicate that the CB2 receptor may be localized in the chicken brain, and may also be involved in appetite activity.

Pretreatment with the CB1 antagonist AM251 attenuated the hyperphagic effects of the CB1 agonist 2-AG in Leghorns (Fig 2-9), but had no effect in broilers (Fig 2-10). Similarly, ICV injection of 2-AG alone had no effect in broilers. Interestingly, pretreatment with the CB1 antagonist AM251 attenuated the hyperphagic effects of the CB2 agonist JWH015 in both Leghorns and broilers (Fig 2-15; Fig 2-16) indicating that the CB1 antagonist may block both CB1 and CB2 receptors in the chicken brain. In contrast, pretreatment with the CB2 antagonist AM630 may not block the CB1 or CB2 receptor in Leghorn chickens, but may block the CB2 receptor in broilers. The CB2 antagonist AM630 may not block the CB1 receptor in broilers.

The results summarized as follow (Table 1):

	Leghorn	Broiler
CB1 agonist (2-AG)	↑	↔
CB1 antagonist (AM251)	↓	↓
CB2 agonist (JWH015)	↑	↑
CB2 antagonist (AM630)	↓	↔
CB1 agonist + antagonist	⊙	⊘
CB2 agonist + antagonist	⊘	⊙
CB1 agonist + CB2 antagonist	⊘	⊘
CB2 agonist + CB1 antagonist	⊙	⊙

Table 1. Summarize of results; ↑ increase food intake; ↓ decrease food intake; ↔ No effect on food intake; ⊙ Agonist can be blocked by antagonist ⊘ Agonist can not be blocked by antagonist

In summary, the present studies demonstrated that the avian cannabinoid system may differ from that of mammalian species. The CB2 receptor may be localized in the chicken brain, and there appear to be differences in cannabinoid regulation of food intake between Leghorns and broilers

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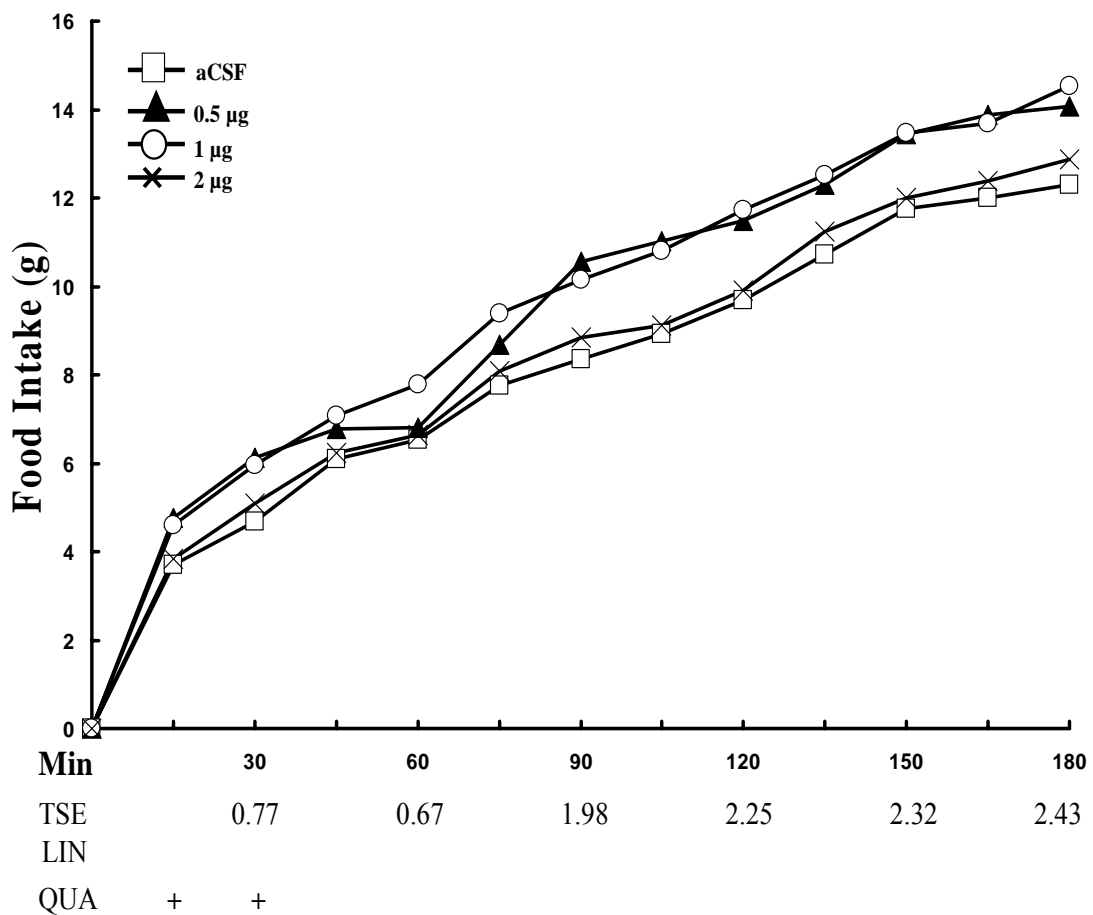


Figure 2-1. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol in Leghorns; artificial cerebrospinal fluid (aCSF); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

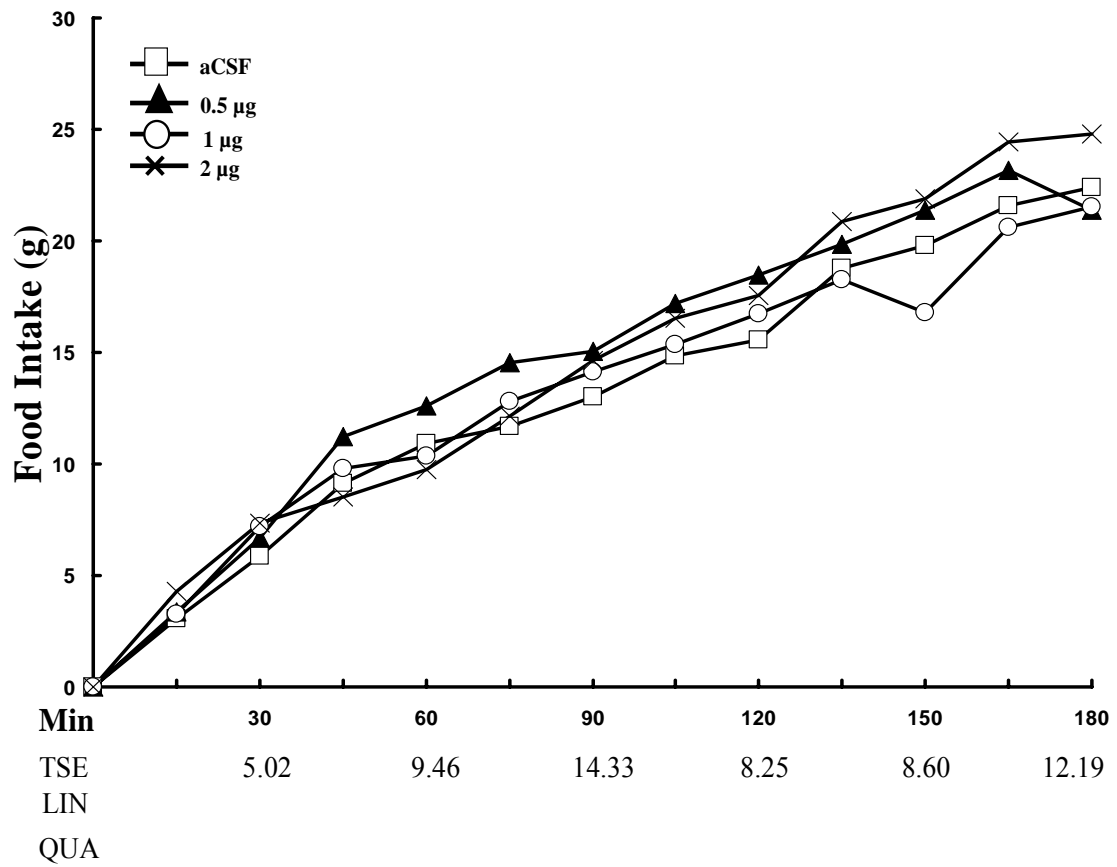


Figure 2-2. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol in broilers; artificial cerebrospinal fluid (aCSF); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

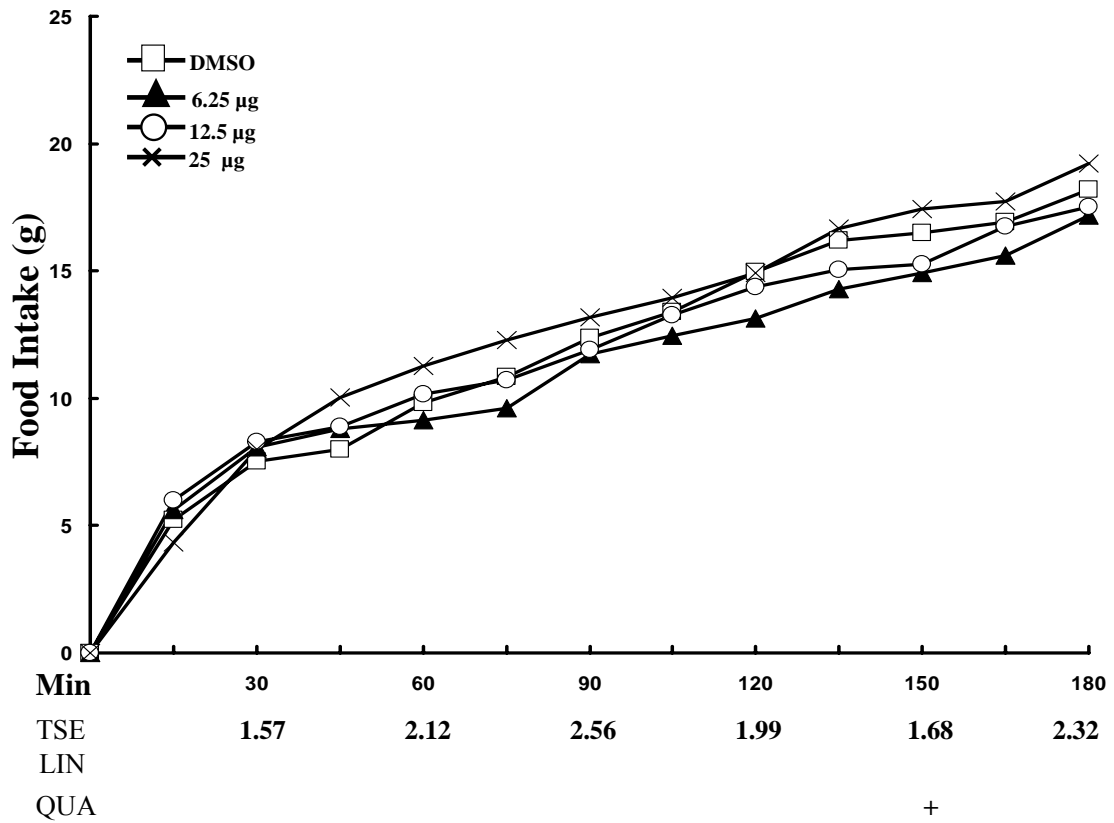


Figure 2-3. Effect of intracerebroventricular injection of CB1 antagonist AM251 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

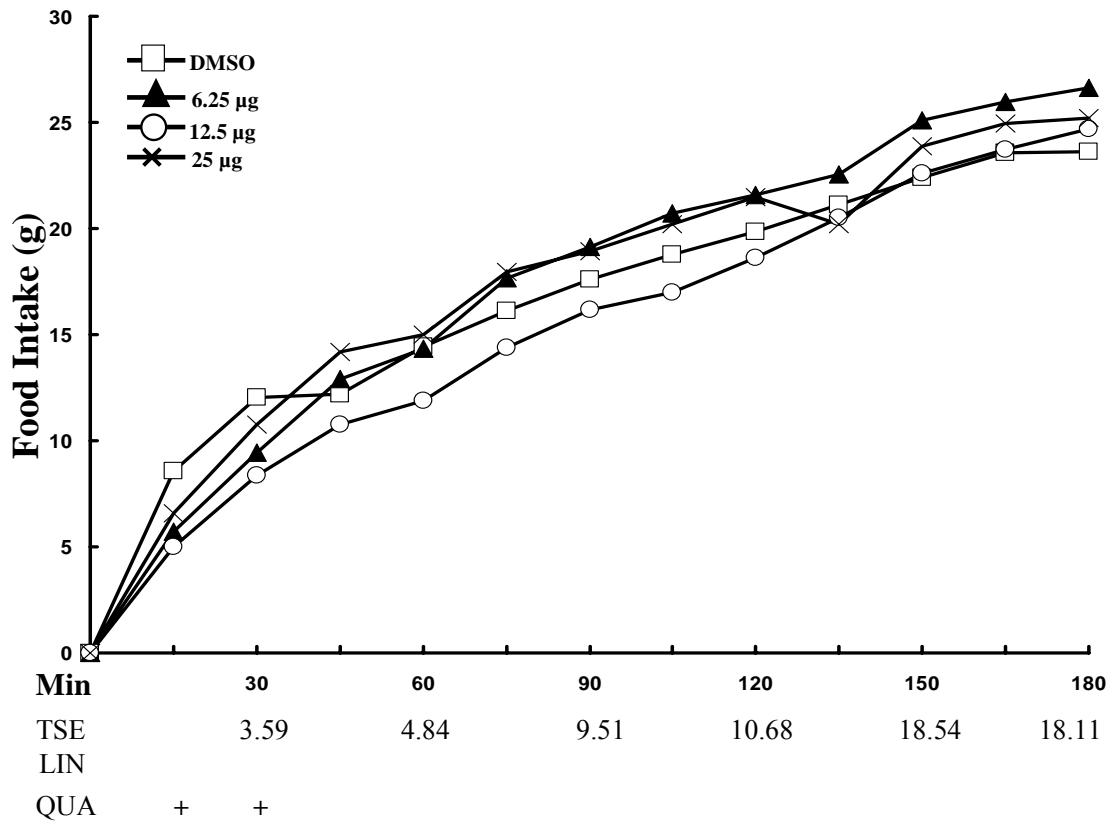


Figure 2-4. Effect of intracerebroventricular injection of CB1 antagonist AM251 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

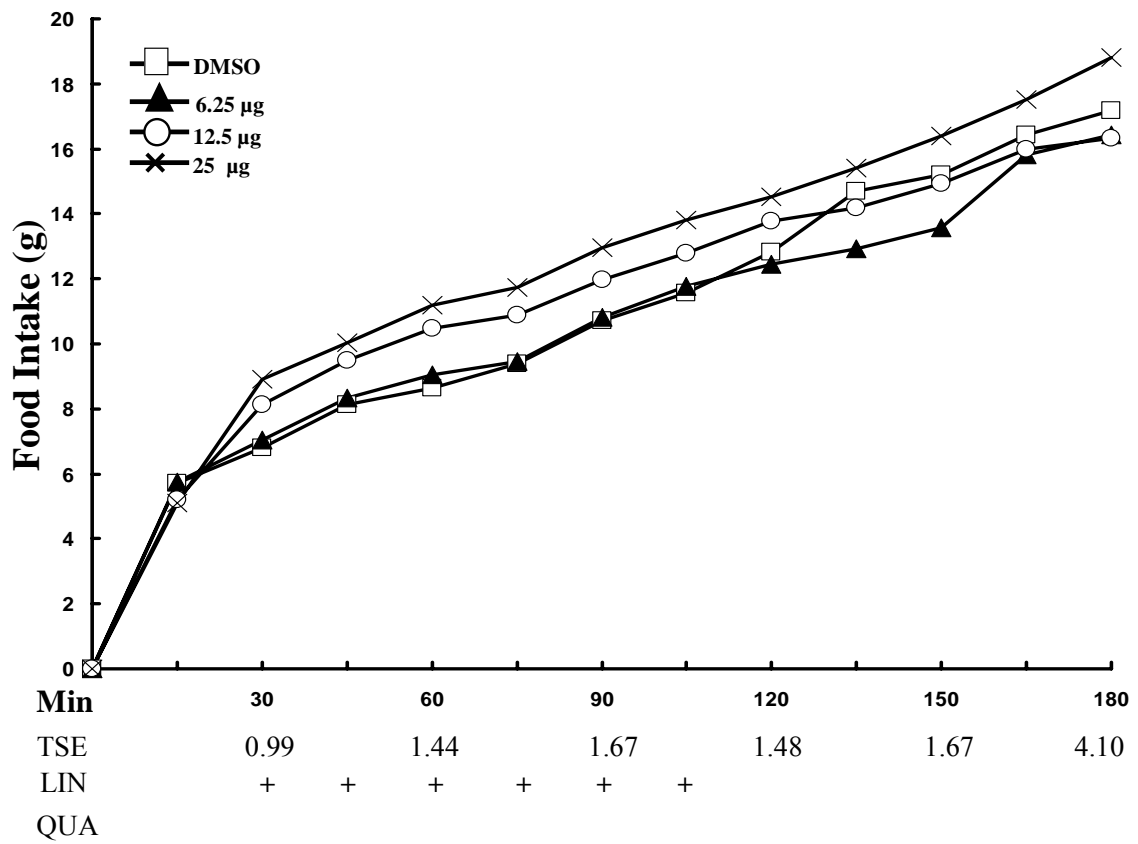


Figure 2-5. Effect of intracerebroventricular injection of CB2 agonist JWH015 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

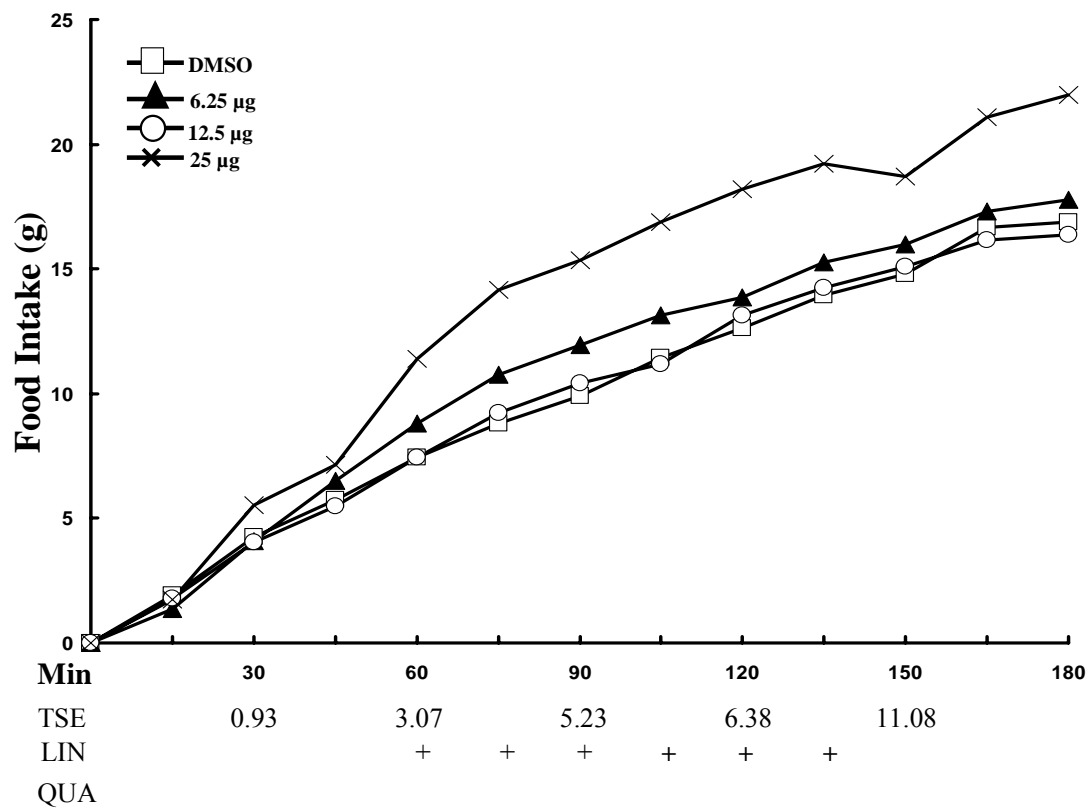


Figure 2-6. Effect of intracerebroventricular injection of CB2 agonist JWH015 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUAD, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

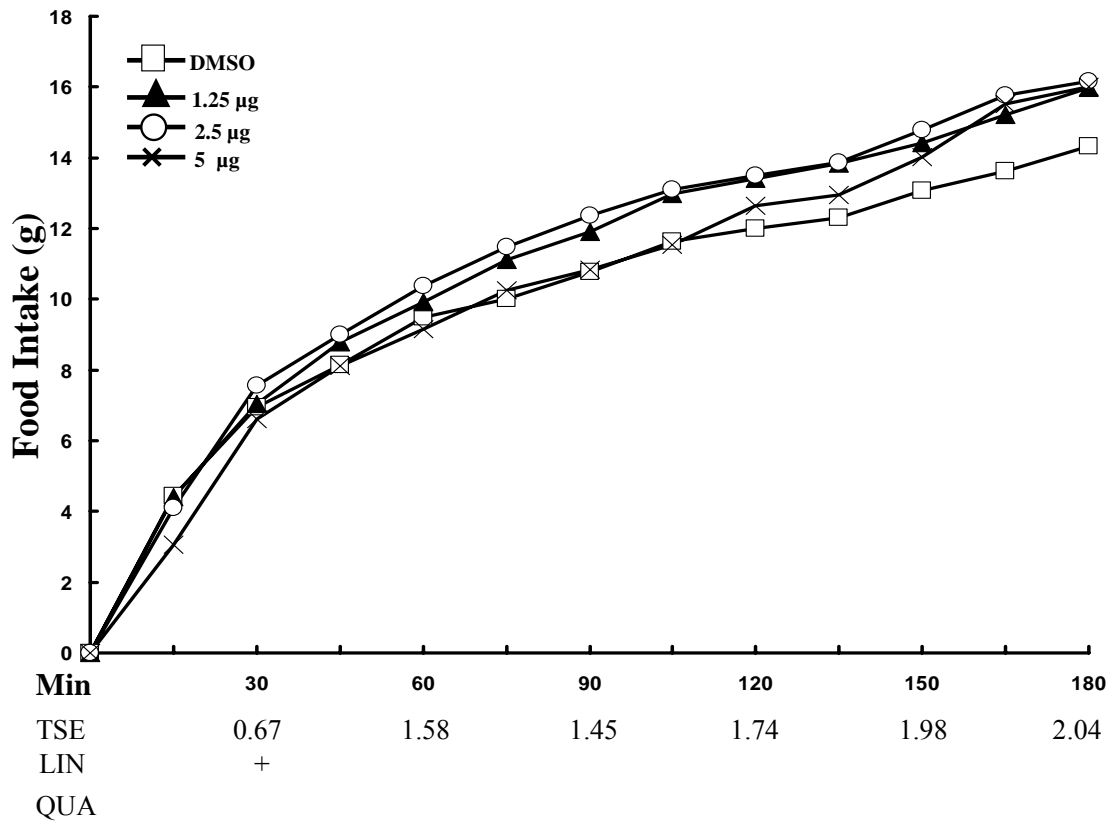


Figure 2-7. Effect of intracerebroventricular injection of CB2 antagonist AM630 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

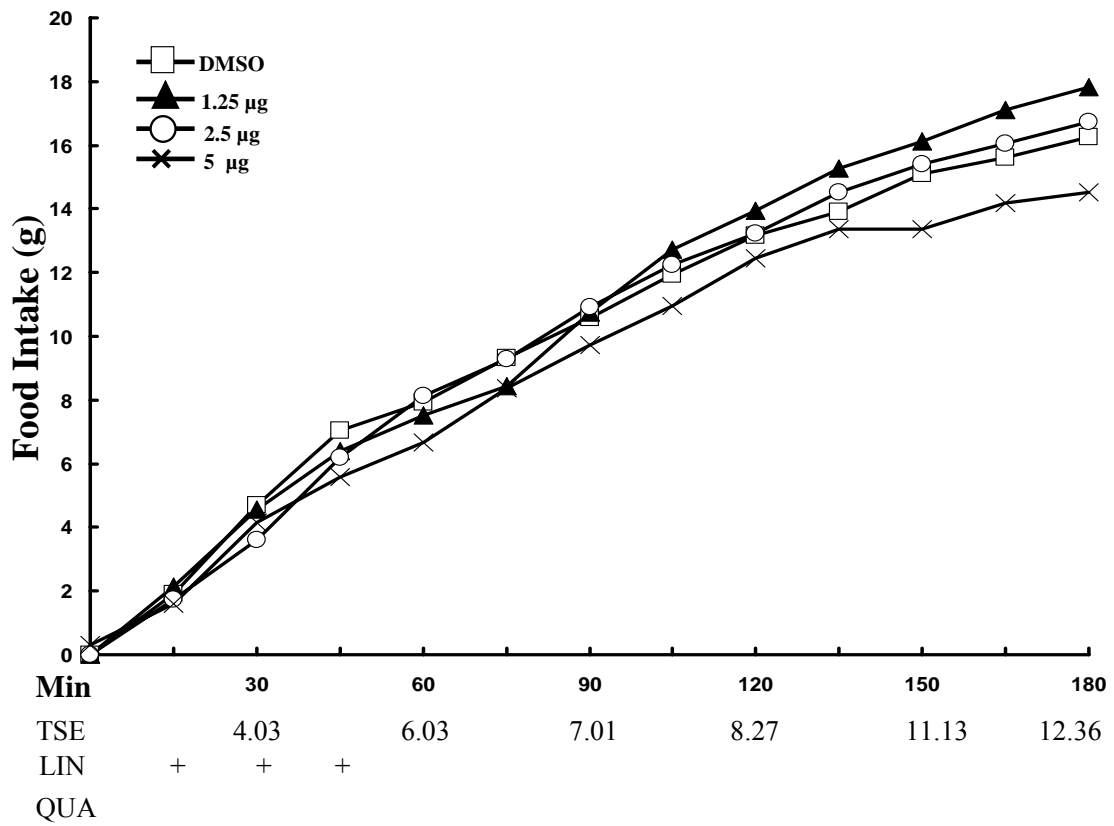


Figure 2-8. Effect of intracerebroventricular injection of CB2 antagonist AM630 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

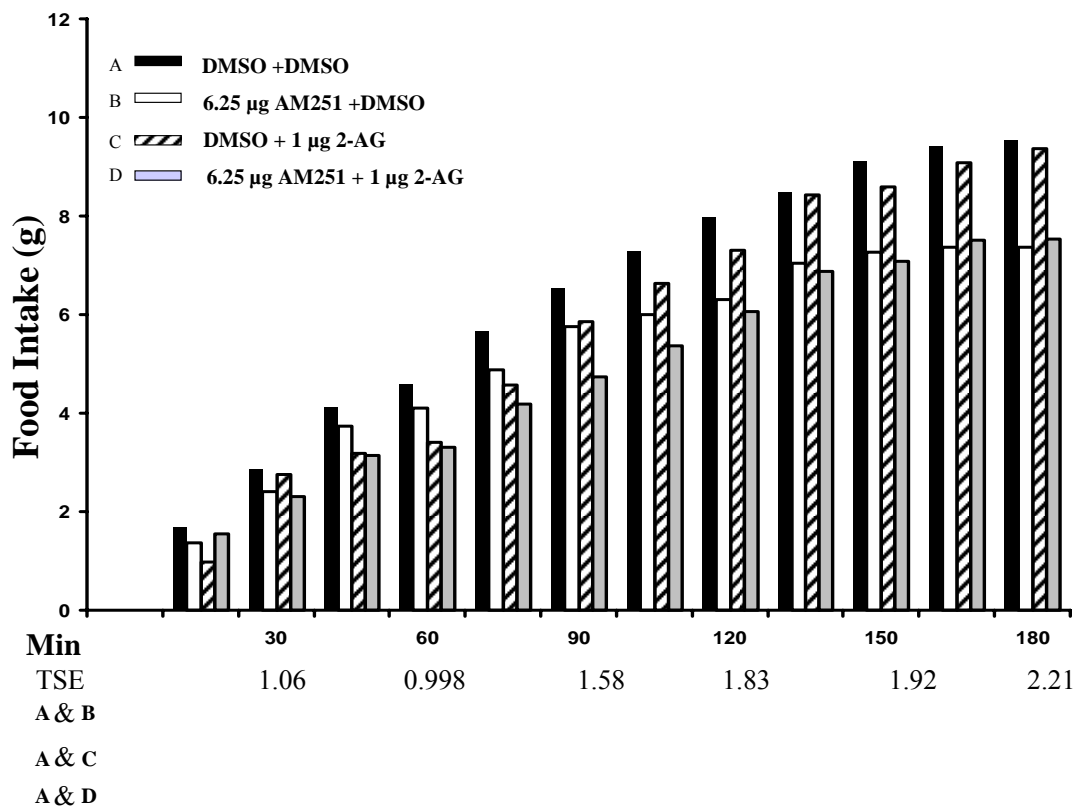


Figure 2-9. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol and CB1 antagonist AM251 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

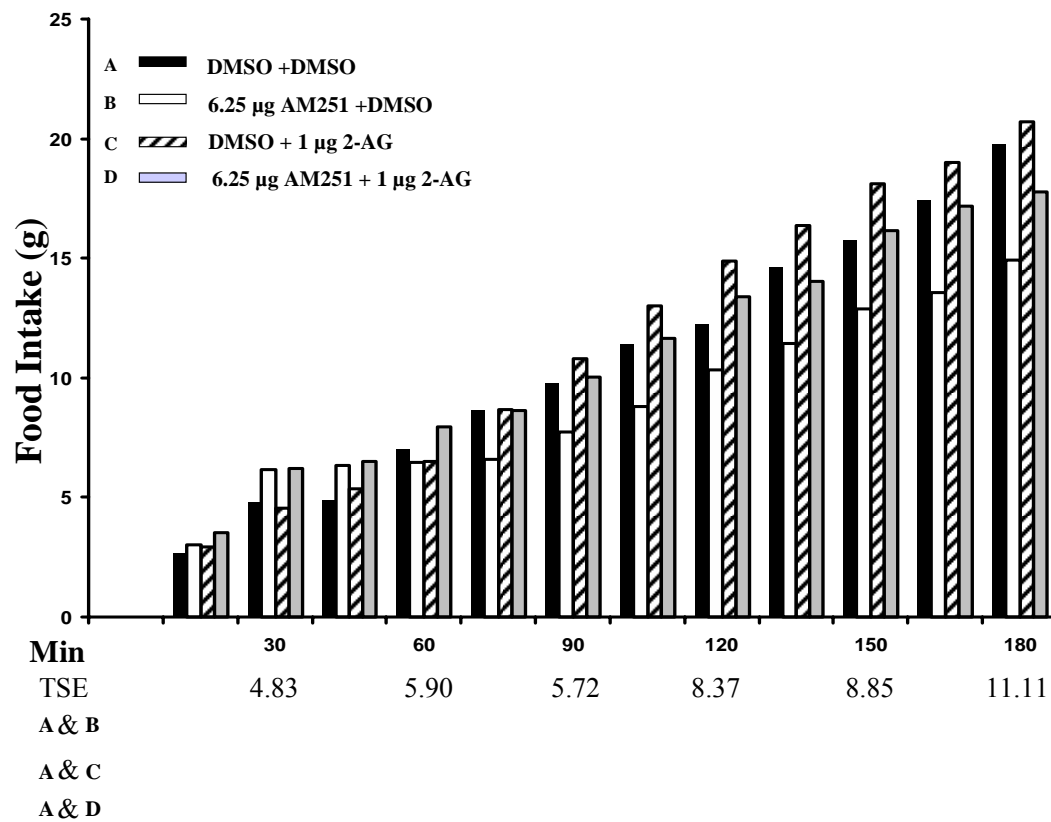


Figure 2-10. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol and CB1 antagonist AM251 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

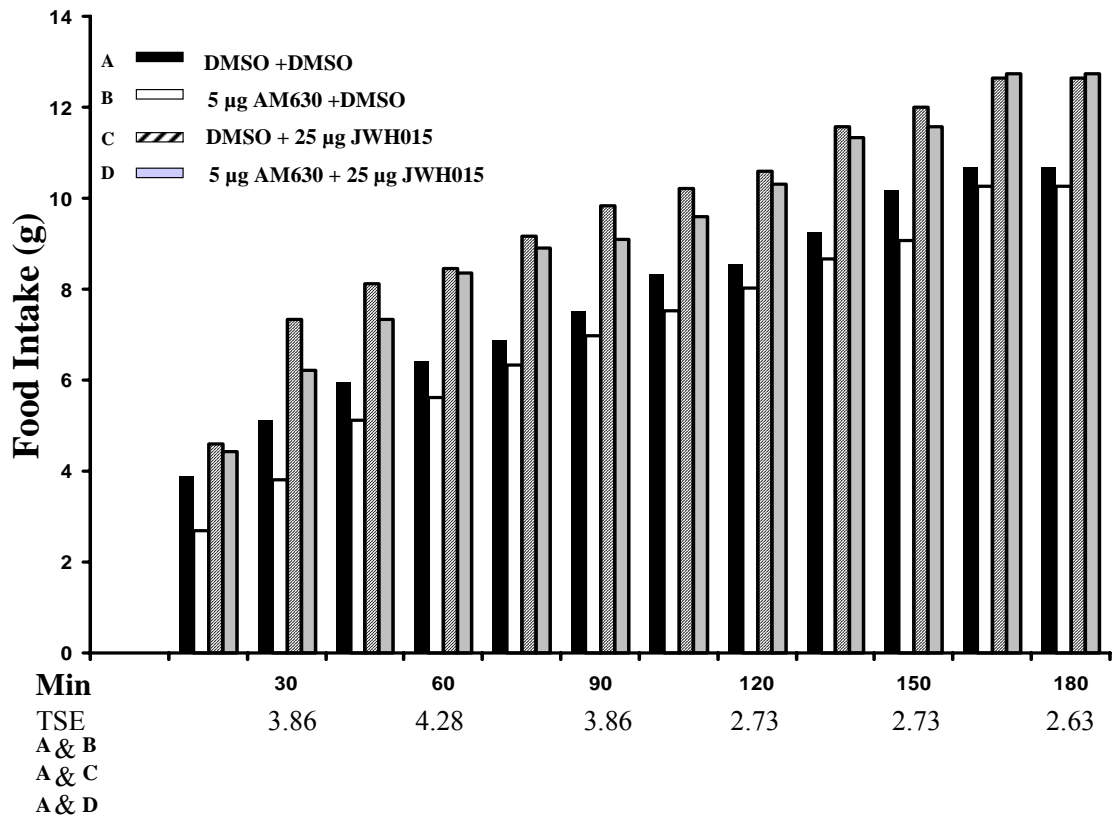


Figure 2-11. Effect of intracerebroventricular injection of CB2 agonist JWH015 and CB2 antagonist AM630 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

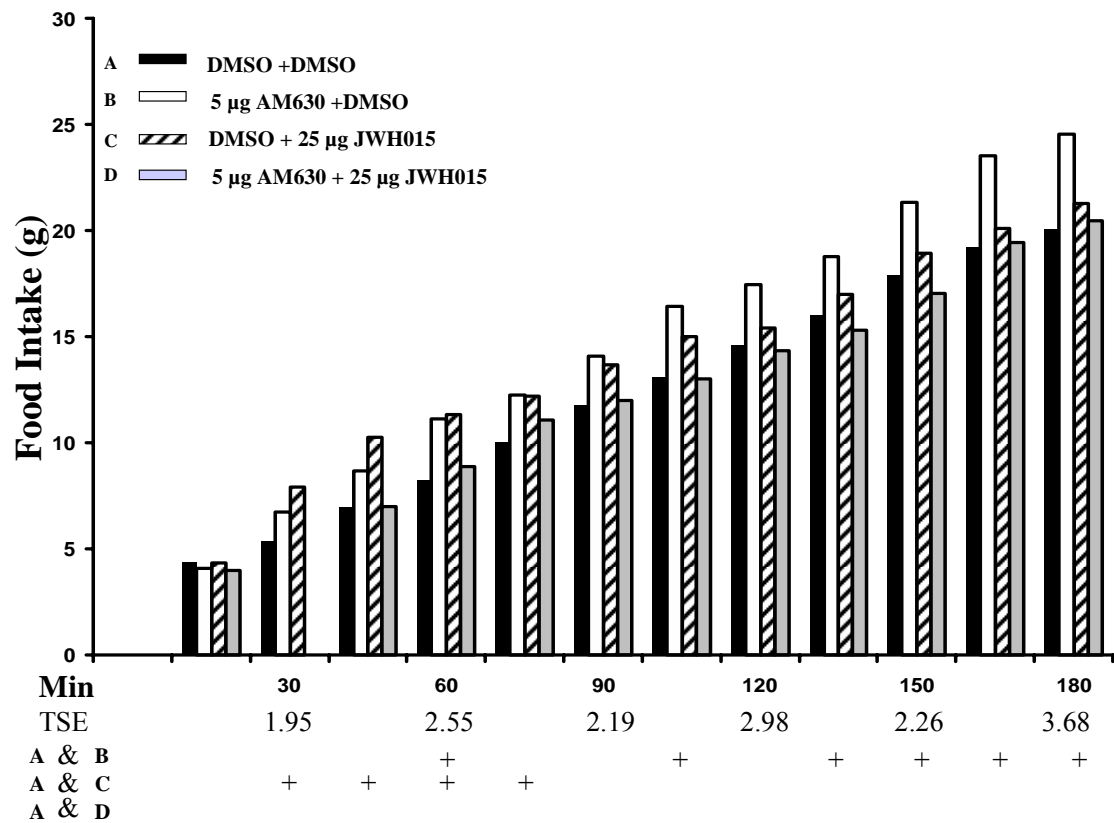


Figure 2-12. Effect of intracerebroventricular injection of CB2 agonist JWH015 and CB2 antagonist AM630 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

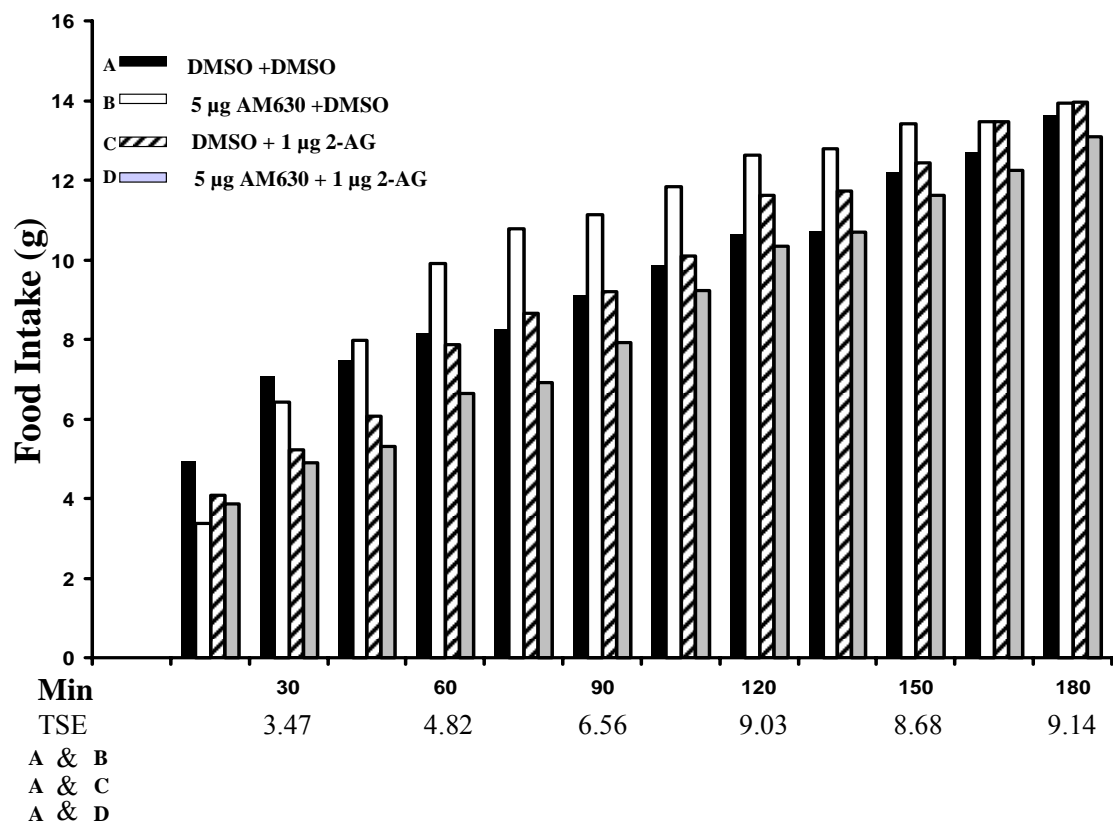


Figure 2-13. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol and CB2 antagonist AM630 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

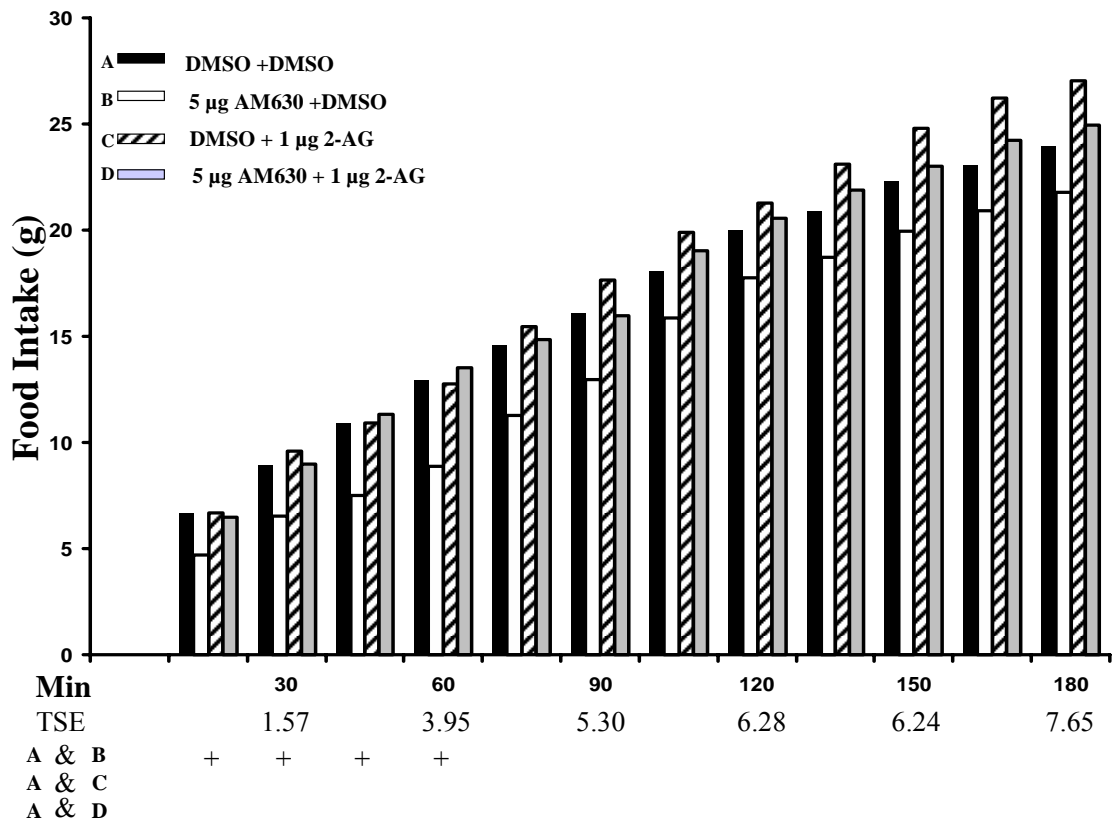


Figure 2-14. Effect of intracerebroventricular injection of CB1 agonist 2-Arachidonylglycerol and CB2 antagonist AM630 in broilers; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

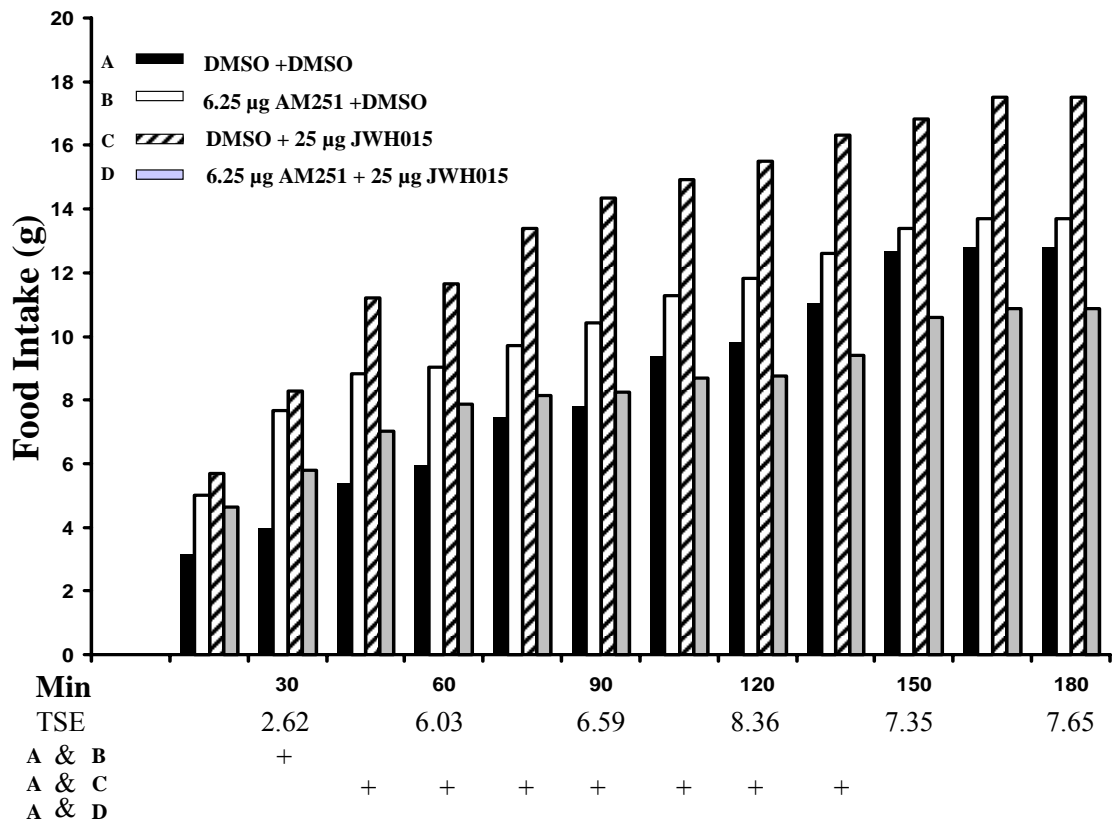


Figure 2-15. Effect of intracerebroventricular injection of CB2 agonist JWH015 and CB1 antagonist AM251 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

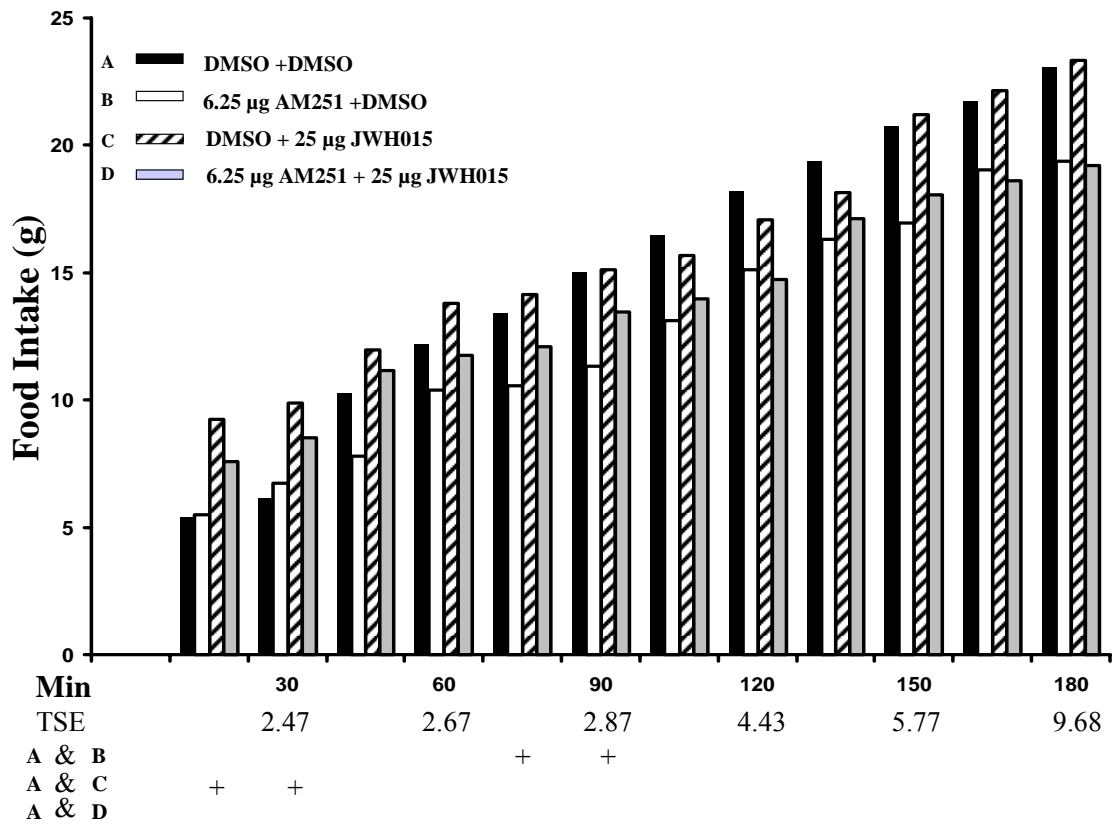


Figure 2-16. Effect of intracerebroventricular injection of CB2 agonist JWH015 and CB1 antagonist AM251 in Leghorns; Dimethyl Sulfoxide (DMSO); LIN, linear contrast; QUA, quadratic contrast; TSE, treatment standard error, +, significant difference ($P \leq 0.05$).

SUMMARY

Since the cloning of the first cannabinoid receptor in 1990, and discovery of the first endocannabinoid 2 years later, much research progress has been made more than a decade. The cannabinoid system is involved in several physiological functions such as regulation of food intake, alleviating pain, and motor control. Among those functions, cannabinoid regulation of food intake has been most studied. Some drugs might soon be on the market, such as rimonabant (SR141716A), a selective CB1 antagonist that might be marketed in the future as an antiobesity drug. New therapeutic drugs might be obtained in the future which selectively inhibitor endocannabinoid biosynthesis and inactivation.

Most information of cannabinoid system has been obtained from mammalian species. It is important to determine whether cannabinoid receptors behave like their mammalian equivalents in other species. In the present study, we found that CB2 receptors may be localized in the chicken brain and play more important role in regulating food intake than the CB1 receptor. This result differs from former information obtained in mammalian species in which the CB2 receptor is considered a peripheral receptor and only found in immune cells. In mammals, only the CB1 receptor is considered as the central receptors, and all studies focus on the effect of the CB1 receptor in food intake regulation.

Since little is known about the avian cannabinoid system, according to the result of the present study, show differences between the mammalian and avian species. We may found some new information of cannabinoid system in the brain.

CURRICULUM VITAE

Jin Zhang

EDUCATION

2003-2005 Virginia Polytechnic Institute and State University

Degree: Master of Science

Special Field: Neurophysiology and Neurochemical Regulation, Department of Animal and Poultry Sciences

1999-2003 China Agricultural University

Degree: Bachelor of Science

Special Field: Department of Animal Science

EXPERIENCE

2003-2005 Intracerebroventricular injection of endocannabinoid regulate food intake in gallus.

(Virginia Polytechnic Institute and State University). This study examines the effects of intracerebroventricular injections of cannabinoid receptor 1 agonist and antagonist, cannabinoid receptor 2 agonist and antagonist on food and water consumption in gallus.

2002-2003 The effect of dwarf (DW) gene to the poultry embryo development (China Agricultural University). This study focuses on the chicken embryo development observation and records the changes, those eggs produced by the chicken that have the DW gene.

2001 Observation pathological change of chronic bronchitis in guinea fowl. After inject the virus of chronic bronchitis, a few weeks dissect the guinea fowls to see the pathological changes (Tokyo University of Agriculture).

AWARDS

2003-2005 John Lee Pratt Graduate Fellowship in Animal Nutrition (Virginia Polytechnic Institute and State University)

2004 JAR Graduate Travel Scholarship (Virginia Polytechnic Institute and State University)

1999-2003 Undergraduate Registration Scholarship (China Agricultural University).

TECHING ASSISTANCE

2004 Embryology course. (Virginia Polytechnic Institute and State University)

2004 Undergraduate mentor (Virginia Polytechnic Institute and State University)

SKILLS

Intracerebroventricular injection (ICV)

Polymerase chain reaction (PCR)

Immunoglobulin G Extraction and Purification

Multiple electrophoretic technique (SDS-PAGE, etc)

Breeding of drosophila monohybrid and dihybrid, sex-linked inheritance.

Gram stain, bacteriolysis, germiculture.

SPECIAL SKILLS

Computer: Extensive experience with Microsoft Office, adobe acrobat, SAS and other
Software

Programming in FORTRAN, BASIC.

Skilled in navigation of the Internet and using browsers

Language: Native Mandarin speaker

English reading, speaking, writing

Limited knowledge of Japanese