# EFFECTS OF DIETARY TYROSINE AND TRYPTOPHAN SUPPLEMENTATION ON IMMUNITY AND BRAIN NEUROTRANSMITTER LEVELS AFTER SRBC INJECTION IN CHICKENS

by

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(ABSTRACT)

This study investigated the effects of dietary tyrosine or tryptophan supplementation on immunity and brain neurotransmitter levels after antigen challenge. Chickens were given 0.1%, 0.5% or 1% supplemental dietary L-tyrosine or L-tryptophan prior to the injection of sheep red blood cells (SRBC). The 0.1% tyrosine supplementation increased primary IgM and secondary IgG titers at some time periods in Leghorns and decreased secondary IgM titers at Day 5 in broilers, while 0.1% tryptophan addition decreased Leghorn secondary IgM titers and increased secondary IgG titers at Day 9 and broiler secondary IgM titers at Day 9. The phytohemagglutinin (PHA) wattle response in Leghorns and broilers and resistance of Leghorns to *E. coli* challenge were not affected with the 0.1% supplemental level. With higher levels, 0.5% tyrosine supplementation increased Leghorn primary IgM titers at Day 11. In broilers, 0.5 and 1% tryptophan supplementation decreased secondary total antibody titers at Day 2, while the secondary IgM titers at Day 6 with the 0.5% tyrosine supplementation were higher than those with 1% tyrosine or tryptophan supplementation. The 0.5% or 1% tryptophan supplementation also lowered stressed broiler primary IgM titers at Day 3. After pooling the titer results within two dietary supplemental levels (0.5% and 1%) of a given amino acid, the tyrosine treatment appeared to suppress antibody response in unstressed broilers, but not stressed ones, while tryptophan displayed a suppressive trend in broilers under both situations. Supplementation with 0.5% or 1% dietary tyrosine did not alter brain catecholamine or serotonin (5-HT) levels in Leghorns. In contrast, 0.5% or 1% dietary tryptophan supplementation generally increased 5-HT, and its metabolite 5-HIAA, and 5-HIAA/5-HT ratios in the diencephalon, telecephalon, and brain stem in Leghorns. Tryptophan

supplementation caused a dose-response increase in 5-HT and 5-HIAA levels of the brain stem. The results demonstrated that 0.5-1% dietary tryptophan supplementation suppressed broiler, but not Leghorn, antibody response, and the mechanism was probably via enhancing 5-HT synthesis and release. The results also suggested that lower levels (0.1-0.5%) of dietary tyrosine supplementation may enhance immune response in Leghorns and stressed broilers, but ingesting large quantity of tyrosine, occurred in broilers, suppresses the response.

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*TABLE OF CONTENTS*

APPENDICES

## *LIST OF TABLES*

## *LIST OF FIGURES*

*Chapter 1*

# LITERATURE REVIEW

## Effects of Brain Catecholamines and Serotonin on Neuroimmunomodulation

It has been understood for some time that psychological factors, such as stressors, decrease immune function and increase the incidence of disease, thus, leading to the conclusion that the brain is able to influence the activity of the immune system (Dunn, 1989). Not all stressors, however, cause suppression of the immune response; some even increase it. For example, continued auditory stimulation enhanced the lymphocyte response to mitogens (Monjan and Collector, 1977). Accordingly, the effect of stress on the immune system may depend on how the brain reacts to the stressor. The disparate effects of stressors may involve different neurohormonal responses.

There are two available routes by which the brain can signal the immune system. These include the neuroendocrine route and the autonomic nerves (Felen *et al.*, 1988). The neuroendocrine route involves an activation of the hypothalamo-hypophyseal-adrenocortical (HPA) axis resulting in the secretion of adrenal glucocorticoids and neuropeptides (Bateman *et al.*, 1989). The autonomic nerves, on the other hand, directly innervate immune organs (Felten *et al.*, 1988). Much attention has been focused on the effect of the neuroendocrine route on the immunological response.

The immediate products resulting from activation of the HPA axis: *i.e.* adrenocorticotrophic hormone (ACTH), $\beta$-endorphin, and a number of other peptides may participate in neuroimmunomodulation (reviewed by Bateman *et al.*, 1989). $\beta$-Endorphin enhanced the proliferative response of splenic lymphocytes to T-cell mitogens (Gilman *et al.*, 1982) and natural killer cell activity (Carr *et al.*, 1986), whereas ATCH inhibited spleen cell antibody response against both T-cell dependent and T-cell independent antigens (Johnson *et al.*, 1982). Glucocorticoids, whose release is stimulated by ACTH, inhibited the production of IL-2 (Kelso *et al.*, 1984), which is an important T-cell growth factor. Furthermore, glucocorticoids decreased cell

growth and number in LPS-stimulated lymphocytes (Roess *et al.*, 1982), though considerable species differences existed in the glucocortoid sensitivity of B lymphocytes (Bateman *et al.*, 1989). Catecholamines and serotonin appeared able to influence the activity of the HPA axis, as will be discussed below.

Corticotrophic-releasing factor (CRF) is elaborated primary by the paraventricular nucleus (PVN) and is transported to the pituitary gland via the hypothalamo-hypophyseal portal system (Bateman *et al*, 1989). There, it stimulates ACTH release. The endorphins and other proopiomelanocortin (POMC)-derived peptides may also be secreted in response to CRF stimulation (Bateman *et al*, 1989).

The injection of norepinephrine (NE) and epinephrine into the paraventricular nucleus (PVN) can stimulate CRF release, and catecholaminergic inputs in this area originate in the brain stem (Plotsky *et al.*, 1989). However, the secretion of ACTH is not controlled only by CRF. Vasopressin (Whitnall, 1988) and oxytocin (Gibbs, 1986) are also important ACTH secretagogues. Clarke *et al.* (1989) proposed that the inputs from the brain stem provide information about the physiological state, while the descending inputs from the limbic system provide information predominantly about the emotional state. The inputs from the limbic system are oxytocinergic and vasopressinergic, with some axons extenting to the pituitary (Swanson *et al.*, 1983). Accordingly, vasopressin and oxytocin in the limbic system may play a more important role in release of ACTH in stress than catecholamines do in the PVN.

Moreover, brain catecholamines have also been shown to participate in inhibition of ACTH release. Ganong *et al.* (1977) found that a variety of catecholamine-releasing drugs, including amphetamine, methamphetamine and dihydroxyphenylalanine (L-dopa), caused similar inhibition. Furthermore, the inhibitory effect of L-dopa could be blocked by the decarboxylase inhibitor benserazide. He also reported that clonidine inhibited ACTH secretion, and the $\alpha$-adrenergic blocker phenoxybenzamine prevented the effect of clonidine when administrated into the third cerebroventricle. On the other hand, the $\beta$-adrenoceptor antagonist prazosin and the tyrosine hydroxylase inhibitor $\alpha$-methyl-p-tyrosine increased the hypothalamic CRF content and the plasma ACTH concentration, and exaggerated the HPA axis response to stress (Buckingham

*et al.*, 1987). Injection of 6-hydroxydopamine (6-OHDA) into the third cerebroventricle also caused a significant rise of the plasma cortisol level during stress (Domanski *et al.*,1985). Therefore, it appears that the catecholaminergic neurons located outside the brain stem may play an inhibitory role in the HPA axis by decreasing CRF release.

Dopamine (DA) also participates in the regulation of the HPA axis. Sapun-Malcolm *et al.* (1986) reported that dopamine had an inhibitory effect in the control of pituitary released $\beta$-endorphin-like immunoreactivity. This result was confirmed by Loeffler (1988) who reported that dopamine inhibited POMC gene expression in the intermediate lobe of the pituitary. The pituitary secretion of these hormones was increased during periods of physical and psychological stress, and, therefore, contributed to stress-induced immunosuppression (reviewed by Cannon *et al.*, 1986).

Further evidence shows that catecholaminergic neurons participate in immune regulation. Cross *et al.* (1986) demonstrated that injection of 6-OHDA into the cisterna magna resulted in marked inhibition of the antibody response to SRBC, and inhibition was not related to changes in circulating corticosterone. Immunizing rats with SRBC caused decreased turnover of norepinephrine (NE) in the hypothalamus (Besedovsky *et al.*, 1983). Conversely, Carlson *et al.* (1987) reported an increased turnover of NE in the PVN, but not other sites of the hypothalamus, following inoculation with SRBC. Probably the strongest evidence, which was reported by Saphier (1989), was that an increase in antibody titers was accompanied by a rise in NE levels in the preoptic area/anterior hypothalamus (PO/AH), and an increase in plasma corticosterone concentration occurred following a large decrease of NE levels in the PO/AH during the primary response to SRBC. In addition, in an extensive series of experiments, Devoino *et al.* (1987) found that administration of L-dopa, the precursor of dopamine, enhanced the immune response, an effect believed to be mediated by the central nervous system.

In contrast to the catecholamines, indoleamines appear to stimulate the HPA axis. Serotonin was reported to possess a stimulatory role in the control of CRF secretion (Holmes *et al.*, 1982). Furthermore, it has been shown to exert a stimulatory effect on the pituitary release of $\beta$-endorphin-like immunoreactivity, with dopamine and serotonin neurons appearing to exert

reciprocal and independent control of pituitary β-endorphin secretion *in vivo* (Sapum-Malcom *et al.*, 1986).

Serotonin plays an inhibitory role in the immune system. Devoino and Ilyutchenok (1968) reported that increasing brain serotonin (5-HT) levels by giving daily injections of 5-hydroxytryptophan (5-HPT), the precursor of serotonin, decreased delayed-type hypersensitivity in a dose-dependent manner. Likewise, increasing the brain serotonin level, using either monoamine oxidase (MAO) inhibitors or 5-HPT, resulted in suppression of humoral immunity (Devoino *et al.*, 1973; 1987) and transplantation immunity (Draskoci *et al.*, 1964; Pierpaoli *et al.*, 1978). The immunological deficits seen after increasing brain 5-HT were a result of decreased IgM antibody titers in the primary response, and a lack of a secondary IgG response (Devoino *et al.*, 1973).

Conversely, decreasing brain serotonin appeared to increase the immune response. Lesioning the midbrain raphe nucleus, the major location of serotoninergic cell bodies, resulted in a two-fold increase in antibody titers to bovine serum albumin (reviewed by Devoino *et al.*, 1987). Injections of the tryptophan hydroxylase inhibitor p-chlorophenylalanine also resulted in an enhanced immune response (Devoino *et al.*, 1987).

The catecholamines and serotonin can also influence the immune response peripherally. In chickens, injection of serotonin 30 min. prior to antigen injection decreased the IgM and IgG plaque-forming cell response against SRBC (Gray *et al.*, 1986). However, the peripheral role of catecholamines appeared less clear. Besedovesky *et al.* (1979) showed that norepinephrine suppressed the *in vitro* anti-SRBC response of murine spleen cells, whereas dopamine enhanced the response. Conversely, Kouassi *et al.* (1988) found that the catecholamines dopamine and norepinephrine exerted stimulatory and inhibitory effects, respectively, on murine polyclonal B-cell activation.

## Effect of Tryptophan Availability on Serotonin Synthesis and Physiological Activities

Hydroxylation of tryptophan to 5-hydroxytryptophan, catalyzed by the enzyme tryptophan hydroxylase (TpOH), is the rate limiting step in the synthesis of serotonin. The factors regulating the activity of TpOH are not fully understood, but availability of tryptophan can influence the reaction (Sved, 1983). The enzyme was reported to be unsaturated (Kaufman, 1974) since brain tryptophan levels are about 20 $\mu$M, well below the 50 $\mu$M Km of TpOH. End product inhibition does not appear to operate, at least at physiological levels of serotonin (Cooper *et al.*, 1978). Increasing brain tryptophan levels elevated brain serotonin concentration (Ashcroft *et al.*, 1964; Weber *et al.*, 1965), and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Curzon *et al.*, 1974; Green *et al.*, 1975). On the other hand, decreasing tryptophan availability has an opposite effect (Fernstrom *et al.*, 1975). Studies using HPLC with electrochemical detection to measure serotonin and 5-HIAA confirmed the above conclusion (Marsden *et al.*, 1980; Reinhard *et al.*, 1980) Using the rate at which brain serotonin accumulates after inhibition of monoamine oxidase (MAO), the enzyme that breaks drown the indoleamine, also provided evidence that the rate of serotonin synthesis varied directly with the brain tryptophan level (Knott *et al.*, 1974). This effect occured in regions containing primarily serotoninergic cell bodies as well as in regions containing terminals of serotoninergic neurons.

Administration of tryptophan can also influence physiological activities. Tryptophan administration consistently reduced sleep latency, and in some studies, also increased total sleep time (Griffiths *et al.*, 1972; Hartmann *et al.*, 1977). Sved *et al.* (1982) found that tryptophan injection lowered blood pressure in spontaneously hypertensive rats. The action of tryptophan could be blocked by the serotonin receptor antagonist metergoline, or by the inhibitor of serotonin synthesis, p-chlorophenylalanine. Regarding anterior pituitary function, Sapun *et al.* (1981) provided evidence that $\beta$-endorphin release from the pituitary was increased by the administration of tryptophan, as well as by drugs that enhanced serotoninergic neurotransmission.

Data on the effect of tryptophan administration on blood ACTH and corticosterone levels is conflicting, with some laboratories reporting a decrease (Woolf *et al.*, 1977) and others reporting an increase (Modlinger *et al.*, 1979). Kennett and Joseph (1981) examined the effect of valine injection on the stress-induced elevation of serum corticosterone levels. Valine injection suppressed the stress-induced rise in serum corticosterone and in brain 5-HIAA. They argued that valine prevented the increase in brain serotonin synthesis, since valine and tryptophan use the same transport system to enter the brain.

## Effect of Tyrosine Supplement on Catecholamine Synthesis and Stress

The initial reaction in the biosynthesis of DA, NE, and epinephrine is hydroxylation of tyrosine to form L-dopa. This is the rate-limiting step in catecholamine synthesis. The availability of tyrosine have been proposed as one of the regulators of the rate at which tyrosine is hydroxylated, and, thus, catecholamines synthesized (Sved, 1983). Tyrosine hydroxylase (TOH) is mostly, but not fully, saturated with tyrosine *in vivo* (Kaufman, 1975), which may limit the regulatory effect of tyrosine availability on catecholamine synthesis. Studies on the effect of increasing brain tyrosine showed that DA and NE levels were not changed (Dairman, 1979; Sved, 1983). However, Acworth *et al.* (1988) recently demonstrated that tyrosine administration caused a short-lived dose-related increase in extracellular DA levels in the nigrostriatum, as well as a non dose-related increase in 3, 4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA), the major metabolites of dopamine. On the other hand, high frequency firing markedly affected the responsiveness of the neurons to added tyrosine (Weiner *et al.*, 1978). Tyrosine increased DA synthesis within neurons with high spontaneous firing frequencies and busting activities (Bannon *et al.*, 1983).

Although the relationship between tyrosine loading and catecholamine synthesis is not significant under normal physiological conditions, administration of tyrosine reduced behavioral and neurochemical deficits in stressful situations. Banderet *et al.* (1989) reported that tyrosine

significantly decreased symptoms, adverse moods, and performance impairments in subjects who exhibited average or greater responses to environmental conditions. There are other experiments showing that tyrosine, given acutely or in the diet, protected rodents from both the neurochemical and behavioral effects of acute stressors such as tail shock or cold exposure (Brady *et al.*, 1980; Lehnert *et al.*, 1984).

In addition, stress can affect the metabolism of catecholamines. TOH activity was decreased in the septal area of tree shrews exposed to a chronic social stress (the visual presence of a dominant animal that subjugated them). In contrast, animals that were able to avoid the visual presence of the victor survived much longer, and those animals showed increased activity of TOH in the limbic septum and adrenal glands (Raab *et al.*, 1980). Stress also caused an increase in NE turnover and decreased NE in the locus ceruleus, hypothalamus, and hippocampus (Lehnert *et al.*, 1984), and even resulted in depletion of NE, and perhaps dopamine, in catecholaminergic neurons (Stone, 1975). During stressful situations, highly active catecholaminergic neurons may require additional precursor so that catecholamine synthesis can keep pace with the increased turnover of neurotransmitters (Milner *et al.*, 1985). Further evidence supporting the concept that dietary tyrosine can enhance the immune response during stress was done by Reinstein *et al.* (1985). They found that tyrosine supplementation suppressed the rise in plasma corticosterone following acute stress in rats.

## Objectives

Dietary supplementation of amino acids, particularly tryptophan, can increase respective neurotransmitters. Catecholamines appear to enhance the immune response, whereas 5-HT appears to inhibit the response. In addition, feed-grade amino acids have recently become available making it practical to provide these in commercial diets. Therefore, the objective of this study was to determine what effect dietary manipulation of endogenous serotonin and catecholamines has on immunity.

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*Chapter 2*

# EFFECTS OF DIETARY TRYOSINE AND TRYPTOPHAN SUPPLEMENTATION ON IMMUNITY AND BRAIN NEUROTRANSMITTER LEVELS AFTER SRBC INJECTION

## Introduction

Serotonin and catecholamines function as neurotransmitters and are synthesized from the amino acids tryptophan and tyrosine, respectively. Since the precursors of the neurotransmitters are supplied to the body via the diet, diet-induced alterations in plasma levels of the amino acids may affect brain serotonin and catecholaminic synthesis. It has become apparent that ingesting increased quantities of these amino acids, under certain circumstances, increases respective brain neurotransmitter levels (Sved, 1983; Milner *et al.*, 1987) . Furthermore, administration of the precursors can alter physiological activities and behavior of animals including sleep, blood pressure, hormone release from the pituitary, and the stress response (Sved, 1983; Reinstein *et al.*, 1985; Milner *et al.,* 1985; 1987).

Recently, studies on neuroimmunomodulation indicated that the brain participates in the regulation of the immune response (Roszman, *et al.,* 1988; Saphier, 1988; Bateman *et al.,* 1989; Rabin *et al.* 1989). Alterations in brain indoleamines and catecholamines influenced the ability of an animal to respond to an antigen challenge. Devoino *et al.* (1987) found that the dopamine precursor L-Dopa enhanced the antibody response to SRBC, an effect believed to be mediated by the central nervous system. On the other hand, lesioning catecholaminergic neurons by injecting 6-OHDA into the cisterna magna resulted in marked inhibition of the antibody response to SRBC (Cross *et al.*, 1986). In contrast, increasing brain serotonin levels by giving daily injections of 5-hydroxytryptophan (5-HPT), the precursor of serotonin, or by using monoamine oxidase inhibitors, decreased delayed-type hypersensitivity in a dose-dependent manner (Devoino and Ilyutchenok, 1968), suppressed humoral immunity (Devoino *et al.*, 1973; 1987) and transplantation

immunity (Draskoci *et al.*, 1964; Pierpaoli *et al.*, 1978). On the other hand, decreasing brain serotonin levels by lesioning the raphe nucleus increased antibody titers (reviewed by Devoino *et al.*, 1987).

Stress was found to alter the metabolism of catecholamines and indoleamines (reviewed by Stone, 1975; Anisman, 1978; Dunn *et al.*, 1984; Glavin, 1985). It increased NE turnover and decreased, or even depleted, NE content if sufficiently severe (Stone, 1975; Glavin, 1985), while the content of 5-HT was generally unchanged although the 5-HT turnover increased (Morgan *et al.*, 1975; Joseph *et al.*, 1981; Kennett *et al.*, 1981). Stressors are well-known immune suppressors (Dunn, 1989). Alteration in brain neurotransmitter levels, especially the depletion during stress, probably results in changes in immunoregulation. Thus, highly active catecholaminergic neurons may require additional precursor so that catecholamine synthesis can keep pace with increased turnover (Milner *et al.*, 1987). Reinstein *et al.* (1985) successfully suppressed the increase in plasma corticosterone of stressed rats by supplying tyrosine in the diet.

Given the fact that diet has an effect on brain neurotransmitters and that brain neurotransmitters can influence the immune response, it appears possible that diet may be able to influence disease resistance. To date, little if any research has focussed on the possible role of diet in enhancing animal production through its possible effect on the immune response. The purpose of this study was to investigate whether such a dietary supplementation could affect an immune response.

## Materials and Methods

Leghorns and broilers were obtained on the day of hatch from a commercial hatchery, weighed, wing-banded, and randomly placed in heated batteries with 10 birds per pen until three weeks of age. The chickens were then moved to wire-floor batteries with 5 birds per pen. The birds were provided a starter diet (Appendix B) for the first three weeks, after which they were fed a grower diet (Appendix B). The starter diet was calculated to contain 3134 Kcal ME/Kg, 21.3% crude protein, 0.84% tyrosine and 0.24% tryptophan, and the grower diet was 3160 Kcal ME/Kg,

19.7% crude protein, 0.77% tyrosine and 0.22% tryptophan. Feed and water were provided *ad libitum* and lighting was continuous. This study consisted of five experiments.

*Experiment 1*

This experiment was to investigate the effect of 0.1% dietary tyrosine and tryptophan supplementation on humoral and cell-mediated immunity, as well as disease resistance. At three weeks of age, twenty one pens of male Leghorns were randomly assigned to one of 3 dietary treatments as follows: 1) birds fed a control grower diet, 2) birds fed a grower diet with 0.1% tyrosine supplementation, and 3) birds fed a grower diet with 0.1% tryptophan supplementation. Two pens in each group were injected with 1 ml of a 5% SRBC suspension, and two pens with 0.5 ml of a 0.5% SRBC suspension. To investigate the secondary immune response, the birds were inoculated again with the same dose 14 days after the first injection. Blood samples (0.3-0.4 ml) were taken via the brachial vain every two days starting 3 days after injection. The blood was allowed to clot, and the serum used to measure antibody titers as briefly described below.

Total antibody titers and 2-mercaptoethanol resistant (MER) titers to SRBC were measured using the microtiter procedure (Wegman *et al.*, 1966). The MER titers were determined as a measure of immunglobulin G (IgG) levels, and 2-mercaptoethanol sensitive (MES) titers were associated with immunoglobulin M (IgM) levels, obtained by subtracting MER titers from total titers. Titers were expressed as $\log_2$ of the reciprocal of the highest dilution in which there was hemagglutination. MES and MER titers will be referred to as IgG and IgM titers, respectively.

The same birds were used for the phytohemagglutinin (PHA) wattle response (McCorkle *et al.*, 1979). One wattle was injected with 0.1 ml of 100 $\mu$g/0.1 ml PHA (pH 7.5), while the other wattle injected with 0.1 ml of saline, served as a control. The thickness of each wattle was measured by a micrometer before and 16 hours after the injection. The fold differences were calculated by dividing the wattle thickness post injection with the thickness before injection.

At 5 weeks of age, the other three pens of birds in each group were injected with 0.1 ml of a $10^{-4}$ dilution of *E. coli* via the posterior thoracic air sac. The *E. coli* was from a 24-hour incubated serotype of KI in tryptose broth. Body weights were recorded at 24, 48, 72 and 96 hr after the challenge. Following the final weighing, the birds were killed via cervical dislocation and

scored for air sac and heart lesions as described by Collins and Siegel (1987). Relative weight loss was calculated by dividing the weight loss by body weight before each weight loss, and the scores of lesion were transformed using the formula $(\text{score} + 1)^{1/2}$.

*Experiment 2*

This experiment was similar to Experiment 1 with the exception that male broilers were used instead of Leghorns. Twelve pens of broilers (four pens per treatment) were given the same dietary treatments and injected with 1 ml of a 5% or 0.5 ml of a 0.5% SRBC suspension. The birds injected with the 5% SRBC suspension were inoculated again 14 days after the first injection. Antibody titers were measured using the procedure described above. The PHA wattle response was also conducted on these birds using the same procedure as that in the Experiment 1.

*Experiment 3*

This experiment was designed to examine the effect of higher tyrosine and tryptophan supplementation levels on humoral immunity using a lower injection dose of SRBC. At 4 weeks of age, 7 pens of Leghorns were randomly assigned to each of 5 dietary treatments including: 1) grower diet with 0.5% supplemental tyrosine, 2) grower diet with 1% supplemental tyrosine, 3) grower diet with 0.5% supplemental tryptophan, 4) grower diet with 1% supplemental tryptophan, and 5) grower diet only. These diets were fed for one week after which the birds returned to control grower diet. Feed intake and body weight gain were measured while the birds were fed the amino acid supplemented diets.

The Leghorns were inoculated intravenously with 0.1 ml of a 0.5% SRBC suspension one day after starting dietary treatments. The same birds were inoculated again 14 days after the primary inoculation. Blood samples were collected from the brachial vein of 10 birds per group every two days starting 3 days and 2 days after injection of the primary and secondary inoculation, respectively, for measuring antibody titers. The remaining birds were used for measuring brain neurotransmitter levels. Five chickens in each group were randomly sacrificed by cervical dislocation at days 2, 4, 6, 8, and 10 after the first SRBC injection. The brains were quickly removed, placed on dry ice, and sectioned into four brain parts including telencephalon,

diencephalon, brain stem, and cerebellum. The brain parts were frozen at -70$^0$C for further analysis.

Brain catecholamines, serotonin and their metabolite concentrations were measured by high performance liquid chromatography with electrochemical detection (HPLC-EC) using a modified procedure of Myers *et al.* (1986). Briefly, brain parts were homogenized in 0.05 M $HClO_4$ with 3,4 dihydroxybenylamine (DHBA) added as an internal standard. Using a Valco injector, 100 ul samples were loaded into the HPLC-EC system consisting of a pump (Model SM-909; Anspec, Ann Arbor, Michigan), C-18 column (Bioanalytical Systems, Inc., 250 X 4.6 mm, 5 $\mu$ Biophase), amperometric detector (Model 400, Princeton Applied Res. Corp., Princeton, NJ) and Chromatochart, a computerized data collection system (Interactive Microware, Inc., State College, PA). The mobile phase contained 0.12 M citric acid, 350 mg/l NaOH, 150 mg/l Na-EDTA and 200 mg disodium octyl sulfate, 86 ml acetonitrile and 1.8 ml of triethanolamine adjusted to a pH of 3.1. The mobile phase was run through an inline degasser (Model ERC-3001; ERMA Inc., Tokyo, Japan).

*Experiment 4*

This experiment was designed to examine the effect of a higher level dietary amino acid supplementation on antibody response in broilers. Fifty male broilers were assigned to each of the 5 dietary treatments, and the experimental procedure was the same as that of Experiment 3. Briefly, ten birds (two pens) per group were inoculated with the SRBC suspension, and serum samples were collected for antibody titration. Feed intakes and body weight gain during the supplemental period were also measured.

*Experiment 5*

Since stress is known to affect the immune response, this experiment was designed to investigate the interaction of dietary amino acid supplementation and stress on humoral immunity. Immobilization stress, in addition to dietary treatment, was used in the experiment. Ten (two pens) male broilers 4 weeks of age were randomly assigned to one of each of following treatments: 1) stressed and fed 0.5% supplemental tyrosine, 2) stressed and fed 1% supplemental tyrosine, 3) stressed and fed 0.5% supplemental tryptophan, 4) stressed and fed 1% supplemental tryptophan,

5) stressed and fed grower diet, and 6) fed grower diet without stress. The birds were given the supplemental diets for 10 days, then returned to the control grower diet. The immobilization stress was conducted by restraining both wings and legs for three hours just prior to the first SRBC injection. The SRBC inoculation and the collection of blood samples for measuring antibody titers was done as described for Exp. 1. Feed intake and body weight gain were measured during the dietary supplemental period.

The antibody titers, brain neurotransmitter levels, relative body weight loss, transformed lesion scores, fold differences of wattle thickness, feed intakes, and body weight gain were analyzed by analysis of variance using the Statistical Analysis System (SAS Inst. Inc., 1985) for a completely randomized design. The model was:

$$Y_{ij} = \mu + T_j + e_{ij}$$

Where $\mu$ is the mean of $Y_{ij}$, T equals the effect of the treatments and $e_{ij}$ is the random variation unique to each group. If significant treatment effects were observed, then the means were separated using Duncan's new multiple range test (Duncan, 1955). Significance implies $p \leq 0.05$.

## Results

The dietary addition of 0.1% tyrosine increased the primary IgM titers at Day 9 (Table 1) and secondary IgG titers at Days 3 and 9 in Leghorns injected with 0.5% SRBC (Table 2). No differences in titers were observed in Leghorns injected with 5% SRBC. The addition of 0.1% supplemental tryptophan increased secondary IgG titers and decreased secondary IgM titers at Day 9 in Leghorns injected with 5% SRBC suspension (Table 2). In broilers, 0.1% tyrosine supplementation decreased secondary IgM titers at Day 5 with 5% SRBC injection, and 0.1% tryptophan supplementation increased the IgM titers at Day 9 (Table 4), whereas the primary antibody response was not affected by the tryptophan supplementation (Table 3).

Neither 0.1% tyrosine or tryptophan supplementation affected the PHA wattle response in Leghorns or broilers (Table 5). The relative weight loss (Table 6) and transformed lesion score (Table 7) of Leghorns in response to the *E. coli* challenge were also not changed by the dietary supplementation.

With higher supplemental levels, 0.5% tyrosine supplementation significantly enhanced the primary IgM response at Day 11 in Leghorns (Table 8). Antibody titers at other time periods were not significantly affected by 0.5% or 1% tyrosine or tryptophan supplementation.

In broilers, the primary antibody titers were not significantly affect by 0.5% or 1% tryptophan supplementation (Table 9). However, both levels of added tryptophan significantly decreased the secondary IgM titers at Day 2 (Table 9). The secondary total antibody titers at Day 6 with 1% tyrosine or tryptophan supplementation were significantly lower than those with 0.5% tyrosine supplementation.

The stress increased the primary IgM titers at Day 3 in broilers, while 0.5% or 1% tryptophan supplementation significantly lowered the titers (Table 10). The primary IgM titers at Day 3 with the 1% tyrosine supplementation were significantly lower than those stressed as well as with 0.5% tyrosine dietary supplementation or stressed only. The secondary IgM titers at Day 2 with 0.5% tryptophan supplementation were also lower that those with 1% tryptophan supplementation.

Since there were no significant differences in antibody titers and brain neurotransmitter levels between the two levels (0.5% and 1%) of amino acid supplementation, except at a few time periods, the titers within the two dietary supplemental levels of each amino acid were pooled. The antibody titers after grouping were statistically analyzed to determine if there were effects due to the amino acid supplementation.

Tyrosine supplementation caused an increase in the Leghorn primary IgM titers at Day 11 (Fig. 2), but it decreased broiler primary total antibody titers at Day 7 (Fig. 1). The restraint stress increased primary total antibody titers at Day 3 (Fig. 7) and IgM titers at Days 2, 5, 7 in the broilers (Fig. 8). The increase in IgM titers was also found in the stressed broilers fed the tyrosine supplemented diet, but not the tryptophan added diet (Fig. 8).

The antibody titers of the Leghorns remained affected by the tryptophan supplementation (Fig. 1-6); nevertheless, the addition of tryptophan decreased the primary total titers at Days 7 and 9 (Fig. 2) and IgM titers at Day 9 (Fig. 3), secondary IgM titers at Day 2 (Fig. 5), and IgG titers at Day 8 (Fig. 6) in broilers. It also decreased primary IgM titers at Days 3, 9 (Fig. 8) and secondary total titers at Day 8 (Fig. 7), and IgG titers at Day 6 (Fig. 9) in stressed broilers.

In Leghorn chicks, dietary tryptophan supplementation increased 5-HT, 5-HIAA levels, and 5-HIAA to 5-HT ratios in the brains. The addition of 1% dietary tryptophan significantly increased 5-HT at Days 4 and 6, 5-HIAA levels at Days 2, 4, and 6, and their ratios at Day 2 post-inoculation in the brain stem (Table 11). It also increased 5-HT levels at Day 2, 5-HIAA levels at Days 2, 4, and 6, and the 5-HIAA/5-HT ratios at Days 4 and 6 in the diencephalon (Table 12). The 5-HIAA levels and the ratios were increased in the telecephalon at Days 2 and 6 (Table 13). There were no changes in 5-HIAA or 5-HT levels and their ratios in the cerebellum (Table 14). The addition of 0.5% trptophan also increased 5-HT levels at Day 2, 5-HIAA levels and 5-HIAA/5-HT ratios at Days 4 and 6 in the brain stem (Table 11); the 5-HIAA levels and the ratios at Day 4 in the diencephalon (Table 12) and at Day 6 in the telecephalon (Table 13), and the 5-HIAA/5-HT ratios at Day 6 in the cerebellum (Table 14). There were dose-dependent increases in 5-HT and 5-HIAA levels in the brain stem (Table 11), though the higher tryptophan addition caused significant increases in more measurements.

Dietary supplementation of tryptophan also influenced brain catecholamine levels. Both tryptophan supplementation levels increased NE in the brain stem at Days 2 and 4 post inoculation (Table 15); it also increased epinephrine in the brain stem and diencephalon at day 2 (Table 15, 16), and DA in the diencephalon at Day 4 (Table 16). Brain DA concentrations were increased dose-dependently at Day 6 in the diencephalon and at Day 4 in the telencephalon.

Tyrosine supplementation had no effect on brain catecholamine levels (Table 15-18) except for a significant increase in L-dopa levels in the diencephalon at Day 4 (Table 16). Brain 5-HT were also not affected by tyrosine supplementation (Table 11-14). However, 1% tyrosine supplementation increased 5-HIAA levels in the diencephalon at Day 4 (Table 12) and the ratios of 5-HIAA to 5-HT in the brain stem (Table 11), telencephalon (Table 13), and cerebellum (Table

14) at Day 6. It also decreased the ratios in the cerebellum at Day 2 (Table 14). No significant changes occurred in 5-HT and 5-HIAA levels, or their ratios with 0.5% tyrosine supplementation (Table 11-14).

Feed intake and body weight gain were not significantly influenced by the 0.5% or 1% dietary amino acid addition during the supplementation period; however, the daily average feed consumption of broilers was twice that of Leghorns (Table 19, 20).

## Discussion

The present study demonstrated that dietary tryptophan supplementation significantly increased brain 5-HT and 5-HIAA levels, as well as the ratios of 5-HIAA to 5-HT. The increase in 5-HT levels probably reflects an increase in the synthesis of 5-HT from tryptophan, since the rate limiting enzyme in this conversion, tryptophan hydroxylase, was estimated to be only 50% saturated under normal conditions (Fernstrom *et al.*, 1971; Young *et al.*, 1977). As 5-HIAA is the major metablite of 5-HT, the 5-HIAA level can be used as a measurement of 5-HT turnover (Reinhard *et al.*, 1977). The increase of 5-HIAA concentration and its ratio to 5-HT indicates an increase in the activity of serotoninergic neurons. These results are similar to those seen in rats (Fernstrom *et al.*, 1971; Sved, 1983). Other evidence shows that tryptophan loading increased extracellular serotonin (Schwartz *et al* , 1990) and enhanced serotonin release (Schaechter *et al.*, 1989). It has been suggested that alterations in tetrahydrobipterin ($BH_4$) availability after tryptophan loading may serve as a mechanism by which brain tryptophan hydroxylation, and, therefore, serotonin turnover, can be regulated (Bengtsson *et al.*, 1991).

In the brain stem, tryptophan supplementation caused dose-dependent increases in 5-HT and 5-HIAA levels. The tryptophan requirement for chickens has been reported to be no greater than 0.16% of a diet containing 3,200Kcal ME/Kg (Smith *et al.*, 1987), which is far below the supplementation levels used in this study. When supplementing the diet with 0.1 to 0.3% tryptophan, 5-HT and 5-HIAA levels were increased dose-dependently in turkeys (Hobbs, 1989). Therefore, it can be concluded that the 0.5% of tryptophan supplementation in the basal diet

(Appendix B) of poultry does not saturate tryptophan hydroxylase and allows for incremental increases in 5-HT synthesis and its turnover. Tryptophan binding to albumin may slow down the substrate saturation of tryptophan hydroxylase, because such binding lowers the free tryptophan concentration in the plasma. The percentage of serum free tryptophan ranges from 10-50% in the serum (Fernstrom *et al.*, 1976). The free tryptophan can cross the blood-brain barrier, while albumin-bound tryptophan requires a catalyst-enhanced dissociation of tryptophan from its albumin binding site within brain microcirculation before being transported into the brain (Pardridge *et al.*, 1987).

Tryptophan supplementation was also found to occasionally increase brain catecholamine levels in selected brain regions, a result similar to that observed in turkeys (Hobbs, 1989). While the mechanism is not known, there is evidence of morphological connections and interrelationships between serotoninergic and catecholaminergic systems in their neurotransmitter concentration, turnover, and release (Samanin *et al.*, 1975). In addition, serotoninergic systems have an inhibitory influence on catecholamine function (Andrews *et al.*, 1978; Dray *et al.*, 1978). Such evidence suggests that the increase of catecholamine levels may result from increasing the inhibitory effect of the serotoninergic system on catecholaminergic release and, thus, reducing the amount of catecholamines broken down by MAO and thereby increasing the storage of catecholamines.

The failure of tyrosine addition to the diet to increase brain levels of catecholamines in the present study is also in agreement with a previous report that systemic administration of tyrosine, in general, does not increase the synthesis or brain levels of catecholamines and their major metabolites (Carlsson *et al.*, 1972). This may be primarily due to the 75% saturation of tyrosine hydroxylase with its substrate, tyrosine, under basal conditions (Gibson *et al.*, 1978). However, using brain microdialysis, it was recently found that tyrosine administration increased DA release with minor elevations in the levels of DOPAC and HVA in the striatum, but the rise in DA was short-lived (Acworth *et al.*, 1988). Unlike tyrosine supplementation, L-dopa administration was found to increase brain DA level (Sved, 1983). Accordingly, substrate saturation and end-product inhibition of tyrosine hydroylase may play major roles in maintaining stable catecholamine levels during normal physiological condition.

Moreover, tyrosine supplementation occasionally increased the ratios of 5-HIAA/5-HT. The mechanism is unknown; however, tyrosine, unlike tryptophan, supplementation did not actually increase 5-HT or 5-HIAA levels, which may not necessarily indicate that the supplementation increased serotonin turnover.

Although the tryptophan treatment significantly suppressed broiler antibody titers at only a few time periods, there was a nearly significant ($p \leq 0.10$) decrease in the primary antibody response in stressed and unstressed broilers. This suggests that the tryptophan supplementation played a suppressive role in antibody production under both physiological and stressful conditions in broilers, but not Leghorns.

Previously, serotonin has been demonstrated to cause an inhibition of immune function via action in the central nervous system. Increases in brain serotonin caused by various chemicals including MAO inhibitors, 5-hydroxytryptophan, and reserpine, resulted in inhibition of immunogenesis (Draskoci *et al.*, 1964; Devoino *et al.*, 1968; 1973; Pierpaoli *et al.*, 1978; Boranic *et al.*, 1984). The inhibition in immunological function included the humoral antibody response, delayed type hypersensitivity, and transplantation immunity in different mammals with different antigens, including SRBC. On the other hand, reducing the level of serotonin by tryptophan hydroxylase blockade, or lesioning of the raphe nuclei was observed to increase rosette forming cells to bovine serum albumin (BSA) and SRBC (reviewed by Devoino *et al.*, 1987).

It has been reported that serotoninergic terminals in the hypothalamus activated secretion of ACTH-releasing hormone (reviewed by Devoino *et al.*, 1987) and serotonin stimulated CRF secretion from the hypothalamus *in vitro* (Jones *et al.*, 1976). On the other hand, hypophysectomy or pituitary stalk destruction prevented stimulation of the immune response after lesioning of the serotoninergic raphe nuclei, as well as the inhibition of the immune response after increasing serotonin levels (Devoino *et al.*, 1987). Furthermore, adrenalectomy not only prevented the inhibition of the immune response, but stimulated it after 5-hydroxytrptophan injection (Devoino *et al.*, 1987). According to the evidence above, Devoino *et al.*, (1987) suggested that the serotoninergic system has an inhibitory effect on the immune response which is modulated via the HPA axis. Their work using syngenic transfer of cells from bone marrow, spleen, and thymus

demonstrated that the inhibition of serotoninergic system in immune response by activating antigen-nonspecific suppression.

Although peripheral levels of serotonin were not measured in the current study, plasma levels have been found to increase following dietary supplementation of tryptophan in turkeys (Hobbs, 1989). Thus, a peripheral role for serotonin in the immune response should not be neglected. Systemic administration of serotonin, which can not pass the blood brain barrier, prior to inoculation resulted in suppression of IgM and IgG plaque-forming cells in response to SRBC (Cross *et al.*, 1986; Gray *et al.*, 1988). The finding that receptors for neurotransmitters are present on the surface membrane of lymphocytes and monocytes (Eliseeva *et al.*, 1982) also supports that serotonin is capable of influencing immune function peripherally.

In the present study, lower (0.1 and 0.5%) dietary tyrosine supplementation increased Leghorn and stressed broiler immune response. According to previous reports, dopaminergic neurons in the striatum were found to have a stimulating role on the immune response. Increasing endogenous DA levels by 3,4-dihydroxyphenylalanine adminstration stimulated antibody formation (reviewed by Devoino *et al.*, 1987). A similar result was observed with phenamine, a drug that attenuates the release of catecholamines from storage and inhibits their reuptake (Devoino *et al.*, 1987). Furthermore, activation of the dopaminergic system by increasing DA receptor activity or decreasing serotonin levels caused a more intense immune reaction which was mediated via the hypothalamus-hypophysis-thymus axis (Devoino *et al.*, 1987).

Tyrosine supplementation failed to alter catecholamine levels. However, both 0.5% and 1% supplemental tyrosine, instead of increasing catecholamine levels, had an effect on maintaining a stable DA level in the brain stem of Leghorns, while DA levels fluctuated significantly in other groups (Table 15). This indicates that tyrosine supplementation may have a facilitating role on the catecholaminergic system, eliciting a stimulative effect on the immune response, thus, causing increases in the primary IgM titers in Leghorns.

Since brain catecholamine levels remained unchanged following dietary tyrosine supplementation treatment in Leghorns, it is assumed that this was also true in broilers. The suppressive effect of tyrosine supplementation in broilers, but not Leghorns, may be due to the

peripheral catecholamines (not measured). Because of significant differences in feed intake behavior between Leghorns and broilers, the broiler peripheral catecholamine levels may greatly differ from that of Leghorns. Tyrosine availability has been shown to affect peripheral catecholamine synthesis. Administration of oral tyrosine to human subjects increased urinary levels of three catecholamines and their major metabolites (Agharanya *et al.*, 1981; Alonso *et al.*, 1982). The evidence suggests that the plasma levels of catecholamines may increase after the oral administration. The increasing levels of catecholamines may result in immune suppression. Johnson *et al.* (1981) reported a suppression of the proliferation response of mouse spleen cells to LPS in the presence of NE, and Crary *et al.* (1983) found decreases in mitogen responsiveness of mononuclear cells from peripheral blood after epinepherine administration in humans. There was also a negative relationship between adrenaline and the T helper cell/T suppressor cell ratio (Landmann *et al.*, 1984). In the current study, 0.1% tyrosine supplementation enhanced Leghorn antibody response, and 0.5% tyrosine supplementation resulted in higher antibody response than 1% tyrosine supplementation. Accordingly, ingesting very large quantity of dietary tyrosine due to high feed intake or high supplementation level caused immune suppression probably via the effect of high peripheral catecholamines.

The stimulating effect of restraint stress on the immune response may have resulted from the activation of catecholaminergic neurons, especially those in the striatum, which enhanced DA synthesis and release. This may have resulted in enhancing the immune response via stimulation of the hypothalmus-hypophysis-thymus axis as stated previously. The following evidence supports this hypothesis. Immobilization stress significantly enhanced L-dopa accumulation in the striatum after decarboxylase inhibition (Algeri *et al.*, 1988). Footshock and restraint stress resulted in a significant increase in DOPAC to DA ratios in the striatum (Dunn, 1988). Moreover, tyrosine administration increased DA synthsis and release in rats given stress (Gibson *et al.*, 1978; Lehnert *et al.*, 1984).

Furthermore, the immobilization stress used in the current study may not have been strong enough to cause depletion of brain catecholamines, which usually occurs in severe stress and may result in behavioral deficits (Lehnert *et al.*, 1984; Stone, 1975) and immune suppression. As stated

previously, stress activates catecholamine neurons and enhances catecholamine synthesis and release, including firing rate. An increase in firing frequency of the neurons causes allosteric changes in tyrosine hydroxylase, thereby increasing its affinity for its cofactor $BH_4$ and reducing its susceptibility to end-product inhibition (Lovenberg *et al.*, 1975). Thus, the immune stimulative effect due to increased activity of the catecholaminergic system by stress and tyrosine supplementation may overcome the immune suppressive effect of increasing peripheral catecholamines which probably occurred in unstressed broilers. The stress stimulating effect on the immune response was not found in broilers fed the tryptophan supplemented diets which must be prevented by the suppressive effect of the tryptophan supplementation as described above.

There were no significant differences in *E. coli* challenge and PHA wattle response between the dietary treatments. The *E. coli* challenge has been used as an index of resistance to disease (Gross and Siegal, 1983), and the wattle swelling response to mitogen stimulation shown to be an indicator of cell-mediated immunity in chickens (Klesius *et al.*, 1977). Due to the great deviation in the observations, it may be too early to conclude that cell-mediated immunity and disease resistance were not affected by the dietary treatments.

Disparate results within the same dietary treatment were found between broilers and Leghorns. Leghorns appeared to be more resistant to dietary tryptophan supplementation than broilers. Tyrosine dietary supplementation enhanced Leghorn immnue response, but suppressed the broiler response. Tryptophan supplementation decreased antibody titers in broilers, but not Leghorns. The reason for these varying responses is unclear. Similar phenomena were reported in broilers and Leghorns responding to ICV injection of biogenic amines in feed intake and drinking (Denbow *et al.*, 1982; 1983). On the other hand, it is well-known that Leghorns possess a higher response to antigen challenges and much lower feed intake than broilers, which may contribute to the disparate results.

**Summary**

The purpose of the present study was to determine if dietary supplementation with tyrosine or tryptophan affected brain neurotransmitter levels and humoral immune response in chickens. A basal diet supplemented with 0.1%, 0.5% or 1% of tyrosine or tryptophan were given to chickens at 3-4wks of age. The chickens were injected with SRBC suspension one Day after the supplementation and 14 Days after the first injection. Brain neurotransmitter levels and antibody titers were measured using HPLC-EC and the microtiter procedure, respectively, at different time intervals following the dietary treatment and SRBC injection.

The dietary addition of 0.1% tyrosine increased the primary IgM and secondary IgG titers at some time periods, while 0.1% tryptophan increased the secondary IgG titers and decreased IgM titers at Day 9 in Leghorns. In broilers, 0.1% tyrosine supplementation decreased secondary IgM titers at Day 5 and 0.1% tryptophan dietary addition increased the IgM titers at Day 9. The PHA wattle response and resistance to *E. Coli* challenge were not different between the dietary treatments.

The addition of 0.5% tyrosine significantly enhanced the Leghorn primary IgM response at Day 11 and broiler secondary total antibody response at Day 6. In broilers, secondary total antibody titers at Day 6 with 1% tyrosine supplementation were significantly lower than those with 0.5% tyrosine supplementation. The primary IgM titers at Day 3 with 1% tyrosine supplementation were also significantly lower that those stressed as well as with 0.5% tyrosine supplementation or stressed only. Both 0.5% or 1% tryptophan addition resulted in suppression of the broiler secondary IgM titers at Day 2 and stressed broiler primary IgM titers at Day 3 but had not effect on Leghorn immune response. When the titers within the two supplemental levels of a given amino acid were pooled, tyrosine supplementation appeared to enhance the antiboby production in stressed broilers and unstressed Leghorns and decreased antibody titers in unstressed broilers, while tryptophan showed a suppressive effect on stressed and unstressed broilers antibody response.

Dietary supplementation with 0.5% and 1% Tyrosine had no effect on brain catecholamine levels in Leghorns, though it significantly increased L-dopa levels. Serotonin was also not

influenced by tyrosine supplementation. However, 1% tyrosine altered the 5-HIAA levels and the 5-HIAA/5-HT ratios. Dietary tryptophan supplementation significantly increased 5-HT, 5-HIAA levels, and their ratios in all brain parts at some time periods during the dietary supplementation period except in the cerebellum. There were dose-dependent increases in 5-HT and 5-HIAA levels in the brain stem, although 1% tryptophan supplementation caused more significant increases.

These results demonstrate that 0.5-1% dietary tryptophan supplementation suppressed the antibody response in broilers, which is probably via enhancing serotonin synthesis and release. They also suggest that lower (0.1-0.5%) dietary tyrosine supplementation probably enhances the immmune response in Leghorns and stressed broilers, but ingesting large quantity of tyrosine which occurred in broilers suppress the response.

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Table 1. Primary anti-SRBC antibody titers of male Leghorns fed 0.1% supplemental dietary tyrosine or tryptophan[1,2,3]

| Measurement | Dietary Treatment | Days Post Inoculation 3 | 5 | 7 | 9 |
|---|---|---|---|---|---|
| | | 5% SRBC Inoculation | | | |
| Total | Contorl | 2.6±0.8 | 6.0±0.8 | 4.1±1.5 | 3.6±1.2 |
| | 0.1% Tyr | 2.3±0.9 | 5.5±1.3 | 3.3±1.3 | 3.1±0.9 |
| | 0.1% Trp | 2.1±0.6 | 5.4±0.8 | 3.6±1.3 | 2.9±0.9 |
| IgG | Control | 0 | 0.3±0.5 | 0.7±0.9 | 0.9±0.9 |
| | 0.1% Tyr | 0 | 0.1±0.3 | 0.5±0.8 | 0.6±0.8 |
| | 0.1% Trp | 0 | 0 | 1.2±1.2 | 1.6±0.7 |
| IgM | Control | 2.6±0.8 | 5.7±0.7 | 3.4±1.6 | 2.7±1.3 |
| | 0.1% Tyr | 2.3±0.9 | 5.4±1.3 | 2.8±1.5 | 2.5±0.8 |
| | 0.1% Trp | 2.1±0.6 | 5.4±0.8 | 2.4±2.1 | 2.3±0.9 |
| | | 0.5% SRBC Inoculation | | | |
| Total | Control | 1.5±0.8 | 3.6±1.4 | 4.2±1.4 | 3.2±1.5 |
| | 0.1% Tyr | 2.3±1.3 | 4.8±1.8 | 4.7±1.2 | 4.4±0.7 |
| | 0.1% Trp | 1.5±0.7 | 4.6±1.9 | 4.3±1.5 | 4.0±1.5 |
| IgG | Control | 0 | 0.2±0.4 | 1.3±0.9 | 1.1±0.8 |
| | 0.1% Tyr | 0 | 0.1±0.3 | 0.9±0.8 | 1.1±0.7 |
| | 0.1% Trp | 0 | 0 | 0.8±0.8 | 0.9±0.7 |
| IgM | Control | 1.5±0.8 | 3.4±1.6 | 2.9±1.2 | 2.1±1.2[b] |
| | 0.1% Tyr | 2.3±1.3 | 4.7±1.9 | 3.8±1.3 | 3.3±0.9[a] |
| | 0.1% Trp | 1.5±0.7 | 4.6±1.9 | 3.5±1.5 | 3.1±1.2[ab] |

[1] The birds were given the treated diets one day before SRBC injection, and the supplementation diets were given throughout the experiment.

[2] The values represent means ± SE.

[a,b] Means within a row with different superscript are significantly different ($p \leq 0.05$).

Table 2. Secondary anti-SRBC antibody titers of male Leghorns fed 0.1% dietary supplemental tyrosine or tryptophan[1,2,3]

| Measurement | Dietary Treatment | Days Post Inoculation | | | |
|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 9 |
| | | 5% SRBC Inoculation | | | |
| Total | Contorl | 5.1 ± 0.6 | 7.6 ± 1.1 | 6.4 ± 0.8 | 6.4 ± 0.8 |
| | 0.1% Tyr | 5.6 ± 1.3 | 7.9 ± 1.4 | 7.0 ± 0.8 | 7.1 ± 0.7 |
| | 0.1% Trp | 5.1 ± 1.0 | 7.8 ± 0.9 | 6.4 ± 0.5 | 6.3 ± 0.8 |
| IgG | Control | 2.4 ± 1.0 | 3.8 ± 1.5 | 3.4 ± 1.3 | 2.3 ± 0.7[b] |
| | 0.1% Tyr | 2.7 ± 0.8 | 4.5 ± 1.5 | 3.8 ± 1.1 | 2.9 ± 1.1[ab] |
| | 0.1% Trp | 2.4 ± 0.5 | 4.2 ± 1.2 | 3.6 ± 1.0 | 3.6 ± 0.8[a] |
| IgM | Control | 2.7 ± 1.1 | 3.8 ± 1.5 | 3.0 ± 1.2 | 4.1 ± 0.9[a] |
| | 0.1% Tyr | 2.9 ± 1.4 | 3.4 ± 1.3 | 3.2 ± 1.4 | 4.2 ± 1.0[a] |
| | 0.1% Trp | 2.7 ± 0.9 | 3.6 ± 1.2 | 2.8 ± 1.0 | 2.7 ± 1.3[b] |
| | | 0.5% SRBC Inoculation | | | |
| Total | Control | 4.5 ± 1.1 | 7.6 ± 1.1 | 6.9 ± 1.0 | 6.1 ± 1.3 |
| | 0.1% Tyr | 4.4 ± 1.4 | 7.7 ± 1.3 | 6.7 ± 0.8 | 5.8 ± 1.3 |
| | 0.1% Trp | 4.7 ± 1.1 | 7.5 ± 1.6 | 6.7 ± 1.3 | 5.9 ± 1.5 |
| IgG | Control | 1.2 ± 0.6[b] | 3.1 ± 0.9 | 2.9 ± 1.2 | 3.0 ± 0.8[b] |
| | 0.1% Tyr | 2.0 ± 0.8[a] | 3.9 ± 1.4 | 3.7 ± 1.3 | 4.2 ± 1.4[a] |
| | 0.1% Trp | 1.8 ± 0.6[ab] | 3.3 ± 0.9 | 3.5 ± 0.8 | 3.2 ± 0.9[b] |
| IgM | Control | 3.3 ± 1.3 | 4.5 ± 1.4 | 4.0 ± 1.6 | 3.2 ± 1.8 |
| | 0.1% Tyr | 2.4 ± 1.3 | 3.8 ± 1.4 | 3.0 ± 1.2 | 1.6 ± 1.2 |
| | 0.1% Trp | 2.9 ± 1.3 | 4.2 ± 1.8 | 3.2 ± 1.6 | 2.7 ± 1.3 |

[1] The birds were given the treated diets one day before SRBC injection, and the supplementation diets were given throughout the experiement.

[2] The values represent means ± SE.

[a,b] Means within a row with different superscript are significantly different ($\leq 0.05$).

Table 3. Primary anti-SRBC antibody titers of male broilers fed 0.1% dietary supplemental tyrosine or tryptophan[1,2,3]

| Measurement | Dietary Treatment | Days Post Inoculation | | | | |
|---|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 9 | 11 |
| | | 5% SRBC Inoculation | | | | |
| Total | Contorl | 2.3 ± 1.6 | 5.7 ± 1.3 | 5.3 ± 1.1 | 3.8 ± 0.9 | 2.8 ± 1.0 |
| | 0.1% Tyr | 2.3 ± 1.4 | 6.2 ± 0.8 | 5.5 ± 0.7 | 4.3 ± 0.8 | 3.2 ± 0.6 |
| | 0.1% Trp | 3.1 ± 0.7 | 6.3 ± 1.4 | 5.8 ± 1.0 | 4.0 ± 1.1 | 3.0 ± 0.9 |
| IgG | Control | 0 | 0 | 1.3 ± 1.2 | 0.6 ± 1.0 | 0.4 ± 0.8 |
| | 0.1% Tyr | 0 | 0 | 1.8 ± 1.0 | 0.9 ± 1.2 | 0 |
| | 0.1% Trp | 0 | 0 | 1.4 ± 1.3 | 0.9 ± 1.2 | 0.3 ± 0.9 |
| IgM | Control | 2.3 ± 1.6 | 5.7 ± 1.3 | 4.0 ± 1.1 | 3.2 ± 1.0 | 2.4 ± 1.3 |
| | 0.1% Tyr | 2.3 ± 1.4 | 6.2 ± 0.8 | 3.7 ± 0.9 | 3.4 ± 1.2 | 3.2 ± 0.6 |
| | 0.1% Trp | 3.1 ± 0.7 | 6.3 ± 1.4 | 4.4 ± 1.1 | 3.1 ± 1.4 | 2.7 ± 1.1 |
| | | 3 | 5 | 6 | 7 | 9 |
| | | 0.5% SRBC Inoculation | | | | |
| Total | Control | 2.3 ± 0.5 | 5.5 ± 1.8 | 6.6 ± 1.2 | 5.6 ± 1.7 | 4.4 ± 1.8 |
| | 0.1% Tyr | 2.2 ± 0.4 | 5.2 ± 0.9 | 6.1 ± 1.4 | 5.9 ± 0.7 | 4.8 ± 0.9 |
| | 0.1% Trp | 2.0 ± 0.0 | 5.5 ± 0.8 | 5.8 ± 1.4 | 4.6 ± 1.6 | 3.8 ± 1.3 |
| IgG | Control | 0.7 ± 0.8 | 0.4 ± 0.8 | 0.7 ± 1.0 | 0.8 ± 1.4 | 0.2 ± 0.7 |
| | 0.1% Tyr | 0.6 ± 0.8 | 0.4 ± 0.8 | 0.5 ± 1.1 | 0.5 ± 1.1 | 0 |
| | 0.1% Trp | 0.4 ± 0.8 | 0 | 0.4 ± 0.8 | 0.7 ± 1.2 | 0.2 ± 0.6 |
| IgM | Control | 1.6 ± 0.8 | 0.4 ± 0.8 | 5.9 ± 1.2 | 4.7 ± 1.8 | 4.2 ± 1.7 |
| | 0.1% Tyr | 1.6 ± 0.8 | 0.4 ± 0.8 | 5.6 ± 1.4 | 5.4 ± 1.0 | 4.8 ± 0.9 |
| | 0.1% Trp | 1.6 ± 0.8 | 0 | 5.4 ± 1.4 | 3.9 ± 1.5 | 3.6 ± 1.1 |

[1] The birds were given the treated diets one day before SRBC injection, and the supplementation diets were given throughout the experiment.

[2] The values represent means ± SE.

[3] No significant differences between the treatments ($P \leq 0.05$).

Table 4. Secondary anti-SRBC antibody titers of male broilers fed 0.1% dietary supplemental tyrosine or tryptophan[1,2,3]

| Measurement | Dietary Treatment | Days Post Inoculation | | | |
|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 9 |
| | | 5% SRBC Inoculation | | | |
| Total | Contorl | 5.6 ± 2.6 | 5.1 ± 2.0 | 4.9 ± 1.3 | 3.4 ± 2.2 |
| | 0.1% Tyr | 4.9 ± 1.5 | 5.0 ± 1.6 | 4.4 ± 2.0 | 3.7 ± 2.1 |
| | 0.1% Trp | 6.2 ± 2.1 | 5.5 ± 2.1 | 5.1 ± 1.7 | 4.5 ± 1.6 |
| IgG | Control | 1.9 ± 1.2 | 3.1 ± 1.3 | 2.6 ± 0.9 | 2.5 ± 1.4 |
| | 0.1% Tyr | 2.1 ± 1.3 | 3.2 ± 1.9 | 1.8 ± 1.1 | 2.3 ± 1.3 |
| | 0.1% Trp | 2.0 ± 1.6 | 3.1 ± 1.7 | 2.9 ± 1.6 | 2.2 ± 1.6 |
| IgM | Control | 3.7 ± 2.3 | 2.9 ± 1.1[a] | 2.1 ± 1.2 | 0.9 ± 1.0[b] |
| | 0.1% Tyr | 2.8 ± 1.0 | 1.8 ± 0.4[b] | 2.6 ± 1.3 | 1.4 ± 1.0[b] |
| | 0.1% Trp | 4.2 ± 1.6 | 2.4 ± 0.7[ab] | 2.2 ± 1.1 | 2.3 ± 0.8[a] |

[1] The birds were given the treated diet one day before SRBC injection and the supplementation diets were given throughout the experiment.

[2] The values represent means ± SE.

[a,b] Means with different superscripts are significantly different($p \leq 0.05$).

Table 5. Fold differences of wattle thicknesses as affected by phytohemagglutinin injection in Leghorns and broilers fed 0.1% tyrosine or tryptophan supplemental diets[1,2]

| Dietary Treatment | Fold Difference | |
|---|---|---|
| | Leghorns | Broilers |
| Controll | 3.655 | 2.152 |
| 0.1% Tyrosine | 3.909 | 2.147 |
| 0.1% Tryptophan | 4.272 | 2.258 |

[1] The values represent means ± SE.

[2] No significant difference between the treatments ($P \leq 0.05$).

[3] Fold differces = (wattle diameter after PHA injection)/(wattle diameter before PHA injection).

Table 6. Relative body weight changes (%) in response to injection of *E. coli* into air sac of Leghorns fed 0.1% tyrosine or tryptophan supplemented diets[1,2,3]

| | Days Post *E. Coli* Injection | | | |
|---|---|---|---|---|
| Dietary Treatment | 1 | 2 | 3 | 4 |
| Controll | -4.10 ± 5.02 | 0.14 ± 3.29 | 0.16 ± 3.24 | 0.48 ± 5.07 |
| 0.1% Tyrosine | -3.72 ± 4.55 | 1.93 ± 2.93 | 2.96 ± 2.51 | -0.24 ± 2.10 |
| 0.1% Tryptophan | -2.79 ± 4.60 | 1.21 ± 3.64 | 1.25 ± 3.44 | -1.50 ± 3.77 |

[1] The values represent means ± SE.

[2] No significant difference between the treatments ($P \leq 0.05$).

[3] Relative body weight changes were calculated by dividing body weight lossby body weight before each weight loss.

Table 7. Transformed scores of lesion after *E. coli* injection in Leghorns fed diets supplemented with 0.1% tyrosine or tryptophan[1,2,3]

| Dietary Treatment | Transformed Score of Lesion |
|---|---|
| Controll | 1.954 ± 0.673 |
| 0.1% Tyrosine | 1.826 ± 0.611 |
| 0.1% Tryptophan | 1.828 ± 0.700 |

[1] The values represent means ± SE.

[2] No significant difference between the treatments ($P \leq 0.05$).

[3] The transformed scores are from the formula of $(\text{score} + 1)^{1/2}$.

Table 8. Anti-SRBC antibody titers of male Leghorns fed varying levels of added tyrosine and tryptophan in the diets from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| | | Primary Antibody Response | | | | |
| Total | 3 | 1.4±1.1 | 1.5±0.5 | 1.4±0.8 | 1.1±1.2 | 0.8±0.8 |
| | 5 | 6.9±1.1 | 6.7±0.9 | 7.4±0.8 | 6.9±0.6 | 6.7±1.1 |
| | 7 | 7.1±1.5 | 7.5±1.1 | 7.6±1.0 | 7.6±0.8 | 6.7±1.2 |
| | 9 | 5.3±0.8 | 6.1±0.9 | 6.0±0.8 | 5.8±0.9 | 5.7±1.4 |
| | 11 | 3.3±0.8 | 4.0±0.9 | 3.9±1.1 | 3.8±1.0 | 3.8±1.3 |
| IgG | 3 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 0.9±0.3 | 0.9±0.7 | 0.6±0.7 | 0.5±0.5 | 0.9±0.9 |
| | 7 | 1.7±0.5 | 1.6±0.5 | 2.1±0.6 | 1.9±0.3 | 2.1±1.0 |
| | 9 | 1.5±0.7 | 1.6±0.8 | 1.7±0.7 | 2.1±0.6 | 1.4±0.7 |
| | 11 | 1.2±0.4 | 0.9±0.7 | 1.3±0.7 | 1.3±0.5 | 1.9±1.2 |
| IgM | 3 | 1.4±1.1 | 1.5±0.5 | 1.4±0.8 | 1.1±1.2 | 0.8±0.8 |
| | 5 | 6.0±0.9 | 5.8±1.0 | 6.8±1.1 | 6.4±0.5 | 5.8±0.6 |
| | 7 | 5.4±1.3 | 5.9±1.3 | 5.5±1.4 | 5.7±1.1 | 4.6±1.2 |
| | 9 | 3.8±0.8 | 4.5±1.0 | 4.3±0.9 | 3.7±1.3 | 4.3±1.2 |
| | 11 | 2.1±0.7[b] | 3.1±1.1[a] | 2.6±1.1[ab] | 2.5±0.7[ab] | 1.9±0.7[b] |
| | | Secondary Antibody Response | | | | |
| Total | 2 | 2.3±0.7 | 2.4±0.7 | 2.5±0.8 | 2.2±0.8 | 2.4±0.5 |
| | 4 | 7.5±1.2 | 7.0±1.8 | 7.8±0.9 | 7.0±0.9 | 6.7±0.7 |
| | 6 | 8.1±1.0 | 7.6±1.1 | 8.3±1.1 | 7.7±0.9 | 7.5±1.0 |
| | 8 | 6.4±0.8 | 6.2±1.5 | 6.9±0.7 | 6.2±1.1 | 5.8±1.0 |
| IgG | 2 | 0.9±0.3 | 0.7±0.5 | 0.7±0.5 | 0.4±0.5 | 0.8±0.4 |
| | 4 | 2.9±0.9 | 2.4±0.7 | 2.6±0.5 | 2.2±0.4 | 2.6±0.5 |
| | 6 | 3.1±0.7 | 3.0±0.7 | 3.5±0.5 | 3.3±0.5 | 3.2±0.4 |
| | 8 | 2.9±0.3 | 2.7±0.7 | 2.8±0.6 | 2.9±0.3 | 2.5±0.7 |
| IgM | 2 | 1.4±0.7 | 1.7±0.9 | 1.8±0.8 | 1.8±1.1 | 1.6±0.5 |
| | 4 | 4.6±1.1 | 4.6±1.7 | 5.2±0.8 | 4.8±1.0 | 4.1±0.9 |
| | 6 | 5.0±1.3 | 4.6±0.7 | 4.8±0.8 | 4.3±0.9 | 4.3±1.2 |
| | 8 | 3.5±1.0 | 3.6±1.1 | 4.1±0.7 | 3.3±1.0 | 3.4±1.2 |

[1] The birds were given the treated diet one day before SRBC injection(0.5%, 0.1ml), and the supplementated diets were given for 7 days.

[2] The values represent means ± SE.

[3] The number of the days indicates days after SRBC injection.

[a,b] Means within a row with different superscripts are significantly different ($p \leq 0.05$).

Table 9. Anti-SRBC antibody titers of male broilers fed varying levels of added tyrosine and tryptophan in the diets from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| | | Primary Antibody Response | | | | |
| Total | 3 | 0.6±0.7 | 0.2±0.4 | 0.5±0.7 | 0.4±0.8 | 0.3±0.5 |
| | 5 | 4.4±1.1 | 4.0±1.5 | 4.5±1.2 | 4.4±1.3 | 3.7±1.7 |
| | 7 | 6.0±1.1 | 4.9±1.5 | 4.9±1.4 | 4.8±1.3 | 4.7±0.9 |
| | 9 | 4.5±1.4 | 3.6±1.1 | 3.3±1.1 | 3.2±1.3 | 3.4±1.0 |
| IgG | 3 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 | 0 | 0.1±0.3 |
| | 7 | 0.4±0.7 | 0 | 0.1±0.3 | 0.1±0.3 | 0.6±0.8 |
| | 9 | 0.4±0.7 | 0.1±0.3 | 0 | 0.4±0.5 | 0.4±0.7 |
| IgM | 3 | 0.6±0.7 | 0.2±0.4 | 0.5±0.7 | 0.4±0.8 | 0.3±0.5 |
| | 5 | 4.4±1.1 | 4.0±1.5 | 4.5±1.2 | 4.4±1.3 | 3.6±1.5 |
| | 7 | 5.6±0.8 | 4.9±1.5 | 4.8±1.4 | 4.7±1.2 | 4.1±1.3 |
| | 9 | 4.1±1.0 | 3.5±1.0 | 3.3±1.1 | 2.8±1.3 | 3.0±0.8 |
| | | Secondary Antibody Response | | | | |
| Total | 2 | 1.4±0.5[a] | 1.5±0.5[a] | 1.7±0.9[a] | 0.8±0.8[b] | 0.7±0.6[b] |
| | 4 | 6.2±1.4 | 6.6±1.3 | 5.6±1.4 | 5.4±1.4 | 5.3±1.1 |
| | 6 | 5.8±1.2[ab] | 6.4±0.8[a] | 5.4±0.7[b] | 5.6±1.1[ab] | 5.0±0.5[b] |
| | 8 | 4.9±1.6 | 4.6±0.8 | 4.2±1.0 | 4.3±1.0 | 3.9±0.5 |
| IgG | 2 | 0 | 0 | 0.1±0.3 | 0 | 0 |
| | 4 | 1.4±0.5 | 1.1±1.0 | 0.8±0.8 | 0.7±1.1 | 0.8±1.0 |
| | 6 | 1.8±0.7 | 1.9±1.0 | 1.4±0.7 | 1.2±1.3 | 1.4±0.7 |
| | 8 | 2.0±0.9 | 2.0±0.9 | 1.3±0.7 | 1.0±1.3 | 1.1±0.9 |
| IgM | 2 | 1.4±0.5[ab] | 1.5±0.5[a] | 1.6±1.1[a] | 0.8±0.8[b] | 0.7±0.6[b] |
| | 4 | 4.8±1.2 | 5.5±0.7 | 4.8±1.2 | 4.8±0.8 | 4.5±1.4 |
| | 6 | 4.0±1.0 | 4.5±0.7 | 4.0±0.9 | 4.3±0.7 | 3.6±0.8 |
| | 8 | 2.9±1.8 | 2.6±0.7 | 2.9±0.8 | 3.3±0.7 | 2.8±1.0 |

[1] The birds were given the treated diet one day before SRBC injection(0.5%, 0.1ml), and the supplementated diets were given for 7 days.

[2] The values represent means ± SE.

[3] The number of the days indicates days after SRBC injection.

[a,b] Means within a row with different superscripts are significantly different ($p \leq 0.05$).

Table 10. Anti-SRBC antibody titers of stressed male broilers fed varying levels of added tyrosine and tryptophan in the diets from 27 to 37 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | Controll | Stress | Stress + 0.5% Tyr | Stress + 1% Tyr | Stress + 0.5% Trp | Stress + 1% Trp |
| | | | | Primary Antibody Response | | | |
| Total | 3 | 0.7±0.5$^{bc}$ | 1.3±0.5$^{a}$ | 1.0±0.5$^{ab}$ | 0.3±0.7$^{cd}$ | 0.1±0.3$^{d}$ | 0$^{d}$ |
| | 5 | 5.2±1.3 | 5.9±1.1 | 5.9±0.6 | 5.4±0.8 | 4.8±0.6 | 5.4±0.8 |
| | 7 | 5.8±1.5 | 6.4±1.1 | 6.6±1.2 | 6.0±0.9 | 5.2±1.4 | 5.8±1.1 |
| | 9 | 4.7±1.6 | 5.1±0.9 | 5.3±1.1 | 4.7±1.1 | 4.2±1.8 | 3.9±1.5 |
| IgG | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 0.1±0.3 | 0.1±0.3 | 0 | 0 | 0 | 0 0 |
| | 7 | 0.8±1.0 | 0.5±0.7 | 0.3±0.5 | 0.1±0.3 | 0.2±0.4 | 0.4±0.5 |
| | 9 | 0.6±1.0 | 0.2±0.4 | 0.3±0.5 | 0.1±0.3 | 0.1±0.3 | 0.5±0.7 |
| IgM | 3 | 0.7±0.5$^{bc}$ | 1.3±0.5$^{a}$ | 1.0±0.5$^{ab}$ | 0.3±0.7$^{cd}$ | 0.1±0.3$^{d}$ | 0$^{d}$ |
| | 5 | 5.1±1.4 | 5.8±0.9 | 5.9±0.6 | 5.4±0.8 | 4.8±0.6 | 5.4±0.8 |
| | 7 | 5.0±1.3 | 5.9±1.0 | 6.3±0.9 | 5.9±1.0 | 5.0±1.3 | 5.4±1.0 |
| | 9 | 4.1±1.4 | 4.9±0.7 | 5.0±0.8 | 4.6±1.1 | 4.1±1.7 | 3.4±1.6 |
| | | | | Secondary Antibody Response | | | |
| Total | 2 | 1.0±0.5 | 1.2±0.4 | 1.2±0.4 | 1.3±0.5 | 1.0±0.7 | 1.4±0.7 |
| | 4 | 6.8±1.3 | 6.7±1.3 | 6.2±1.0 | 6.7±1.2 | 5.9±1.8 | 6.2±1.4 |
| | 6 | 7.1±1.3 | 6.8±1.3 | 6.6±0.7 | 7.0±1.0 | 6.3±1.4 | 6.8±1.6 |
| | 8 | 6.4±1.0 | 5.8±1.1 | 5.6±0.7 | 5.7±1.0 | 5.2±1.1 | 5.2±1.0 |
| IgG | 2 | 0.1±0.3 | 0 | 0 | 0 | 0.2±0.4 | 0 |
| | 4 | 1.7±0.9 | 1.4±1.1 | 1.4±1.0 | 1.1±0.7 | 1.3±1.1 | 1.4±0.5 |
| | 6 | 3.0±0.7 | 2.8±0.9 | 2.2±0.9 | 2.2±1.1 | 2.1±1.0 | 2.2±0.9 |
| | 8 | 2.3±0.7 | 1.9±1.0 | 1.9±1.1 | 1.8±1.0 | 1.6±1.3 | 1.7±0.7 |
| IgM | 2 | 0.9±0.3$^{bc}$ | 1.2±0.4$^{abc}$ | 1.2±0.4$^{abc}$ | 1.3±0.5$^{ab}$ | 0.8±0.4$^{c}$ | 1.4±0.7$^{a}$ |
| | 4 | 5.1±1.0 | 5.3±1.4 | 4.8±0.8 | 5.6±1.2 | 4.6±1.3 | 4.8±1.3 |
| | 6 | 4.1±1.4 | 4.0±1.1 | 4.4±0.8 | 4.8±1.0 | 4.2±1.2 | 4.6±1.6 |
| | 8 | 4.1±1.3 | 3.9±1.4 | 3.7±1.1 | 3.9±1.3 | 3.6±0.7 | 3.5±1.3 |

[1] The birds were given the treated diet one day before SRBC injection(0.5%, 0.1ml), and the supplementated diets were given for 10 days.

[2] The values represent means ± SE.

[3] The number of the days indicates days after SRBC injection.

[a,b,c,d] Means within a row with different superscripts are significantly different ($p \leq 0.05$).

Table 11. 5-HIAA, 5-HT levels (ng/g), and their ratio in the brain stem of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| 5-HT | 2 | 1038.8 ± 160.8$^{b}$ | 934.6 ± 234.7$^{b}$ | 981.8 ± 101.3$^{b}$ | 1268.7 ± 68.8$^{a}$ | 1430.7 ± 183.1$^{a\ x}$ |
| | 4 | 1119.8 ± 177.5 | 1363.6 ± 445.3 | 1164.0 ± 140.1 | 1366.0 ± 374.0 | 1500.0 ± 321.8$^{x}$ |
| | 6 | 1154.1 ± 204.6$^{abc}$ | 977.4 ± 59.3$^{bc}$ | 907.4 ± 315.6$^{c}$ | 1211.1 ± 223.4$^{ab}$ | 1380.1 ± 83.6$^{a\ x}$ |
| | 8 | 1167.6 ± 178.4 | 1003.7 ± 212.4 | 932.5 ± 115.8 | 941.2 ± 149.5 | 1055.5 ± 76.6$^{y}$ |
| | 10 | 991.8 ± 154.7 | 1062.4 ± 252.2 | 1116.7 ± 180.4 | 1115.7 ± 112.2 | 1230.7 ± 246.9$^{xy}$ |
| 5-HIAA | 2 | 341.2 ± 62.2$^{bc}$ | 292.3 ± 53.5$^{c}$ | 272.3 ± 56.4$^{c\ y}$ | 404.7 ± 61.2$^{b}$ | 547.0 ± 28.5$^{a\ x}$ |
| | 4 | 281.4 ± 61.2$^{c}$ | 313.8 ± 101.1$^{c}$ | 343.7 ± 38.9$^{bc\ x}$ | 473.9 ± 127.0$^{ab}$ | 562.7 ± 148.0$^{a\ x}$ |
| | 6 | 273.1 ± 48.4$^{b}$ | 238.7 ± 46.1$^{b}$ | 261.6 ± 60.0$^{b\ y}$ | 377.0 ± 49.2$^{a}$ | 433.9 ± 37.9$^{a\ y}$ |
| | 8 | 300.4 ± 54.7 | 343.3 ± 75.0 | 365.2 ± 35.2$^{x}$ | 342.2 ± 105.9 | 315.1 ± 45.8$^{z}$ |
| | 10 | 289.2 ± 47.6 | 348.9 ± 57.0 | 333.8 ± 37.0$^{x}$ | 295.2 ± 69.6 | 314.2 ± 92.9$^{z}$ |
| 5-HIAA/5-HT | 2 | 0.332 ± 0.068 | 0.339 ± 0.147 | 0.276 ± 0.047$^{y}$ | 0.319 ± 0.048 | 0.386 ± 0.038$^{x}$ |
| | 4 | 0.252 ± 0.045$^{cd}$ | 0.233 ± 0.040$^{d}$ | 0.300 ± 0.060$^{bc\ y}$ | 0.352 ± 0.060$^{ab}$ | 0.373 ± 0.029$^{a\ xy}$ |
| | 6 | 0.238 ± 0.033$^{c}$ | 0.244 ± 0.043$^{bc}$ | 0.300 ± 0.056$^{ab\ y}$ | 0.315 ± 0.038$^{a}$ | 0.316 ± 0.043$^{a\ yz}$ |
| | 8 | 0.265 ± 0.073 | 0.371 ± 0.190 | 0.397 ± 0.065$^{x}$ | 0.377 ± 0.152 | 0.300 ± 0.056$^{z}$ |
| | 10 | 0.301 ± 0.092 | 0.336 ± 0.056 | 0.302 ± 0.025$^{y}$ | 0.271 ± 0.092 | 0.260 ± 0.071$^{z}$ |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.

[2] The values represent means ± standard error.

[3] The number of the days indicates days after SRBC injection.

[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).

[x,y,z] Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 12. 5-HT and 5-HIAA levels (ng/g) and their ratio in the diencephalon of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| 5-HT | 2 | 1099.9 ± 292.5 | 1096.7 ± 161.8$^{x}$ | 1151.1 ± 380.7$^{x}$ | 1401.1 ± 425.2$^{x}$ | 1235.0 ± 139.0$^{y}$ |
| | 4 | 969.7 ± 185.4$^{b}$ | 983.4 ± 132.8$^{b\ xy}$ | 1003.32 ± 190.5$^{b\ xy}$ | 1163.5 ± 81.6$^{ab\ xy}$ | 1235.0 ± 139.0$^{a\ y}$ |
| | 6 | 1257.5 ± 92.0$^{bc}$ | 1140.8 ± 221.6$^{c\ x}$ | 1150.3 ± 111.4$^{c\ x}$ | 1416.2 ± 105.8$^{ab\ x}$ | 1587.4 ± 155.0$^{a\ x}$ |
| | 8 | 1010.9 ± 238.2 | 978.1 ± 149.3$^{xy}$ | 971.9 ± 66.3$^{xy}$ | 1054.6 ± 131.8$^{y}$ | 1079.8 ± 163.3$^{y}$ |
| | 10 | 819.3 ± 190.8 | 763.5 ± 181.0$^{y}$ | 766.1 ± 106.3$^{y}$ | 773.6 ± 156.3$^{z}$ | 836.4 ± 145.8$^{z}$ |
| 5-HIAA | 2 | 322.8 ± 104.3$^{ab}$ | 223.0 ± 48.4$^{b}$ | 239.6 ± 89.0$^{b}$ | 315.6 ± 112.7$^{ab\ yz}$ | 465.5 ± 154.0$^{a\ x}$ |
| | 4 | 261.6 ± 64.7$^{c}$ | 304.1 ± 49.6$^{bc}$ | 361.1 ± 46.6$^{ab}$ | 411.6 ± 57.0$^{a\ xy}$ | 403.3 ± 59.5$^{a\ xy}$ |
| | 6 | 299.7 ± 103.3$^{b}$ | 311.0 ± 80.9$^{b}$ | 355.0 ± 73.6$^{ab}$ | 447.8 ± 118.5$^{ab\ x}$ | 477.1 ± 122.7$^{a\ x}$ |
| | 8 | 270.9 ± 63.8 | 317.7 ± 15.7 | 338.5 ± 45.2 | 314.1 ± 56.5$^{yz}$ | 312.1 ± 32.8$^{y}$ |
| | 10 | 250.4 ± 46.0 | 289.8 ± 99.8 | 286.9 ± 30.5 | 273.0 ± 41.8$^{z}$ | 321.0 ± 18.5$^{y}$ |
| 5-HIAA/5-HT | 2 | 0.295 ± 0.073$^{ab}$ | 0.205 ± 0.046$^{b\ z}$ | 0.213 ± 0.048$^{b\ y}$ | 0.250 ± 0.047$^{b\ y}$ | 0.373 ± 0.100$^{a}$ |
| | 4 | 0.270 ± 0.045$^{c}$ | 0.311 ± 0.047$^{bc\ xy}$ | 0.406 ± 0.055$^{a\ x}$ | 0.353 ± 0.035$^{ab\ x}$ | 0.316 ± 0.045$^{bc}$ |
| | 6 | 0.237 ± 0.074 | 0.272 ± 0.049$^{yz}$ | 0.309 ± 0.062$^{x}$ | 0.314 ± 0.066$^{xy}$ | 0.299 ± 0.063 |
| | 8 | 0.269 ± 0.024 | 0.334 ± 0.073$^{xy}$ | 0.347 ± 0.030$^{x}$ | 0.298 ± 0.035$^{xy}$ | 0.293 ± 0.047 |
| | 10 | 0.324 ± 0.103 | 0.373 ± 0.037$^{x}$ | 0.379 ± 0.060$^{x}$ | 0.360 ± 0.069$^{x}$ | 0.393 ± 0.073 |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.
[2] The values represent means ± standard error.
[3] The number of the days indicates days after SRBC injection.
[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).
[x,y,z] Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 13. 5-HT, 5-HIAA levels (ng/g), and their ratio in the telencephalon of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| 5-HT | 2 | 1339.9 ± 86.9$^{x}$ | 1285.6 ± 155.8$^{y}$ | 1243.5 ± 65.3$^{xy}$ | 1467.4 ± 78.3$^{x}$ | 1487.9 ± 223.7$^{x}$ |
| | 4 | 1179.5 ± 99.5$^{y}$ | 1247.4 ± 283.6$^{y}$ | 1186.3 ± 194.4$^{xyz}$ | 1233.3 ± 177.5$^{y}$ | 1521.6 ± 289.2$^{x}$ |
| | 6 | 1119.3 ± 74.7$^{y}$ | 1030.9 ± 47.4$^{z}$ | 1009.3 ± 93.4$^{yz}$ | 1136.5 ± 128.8$^{yz}$ | 1075.7 ± 222.1$^{y}$ |
| | 8 | 1542.2 ± 251.0$^{x}$ | 1551.2 ± 152.9$^{x}$ | 1441.5 ± 189.8$^{x}$ | 1667.0 ± 189.9$^{x}$ | 1623.4 ± 383.9$^{x}$ |
| | 10 | 1005.8 ± 146.3$^{y}$ | 914.3 ± 164.2$^{z}$ | 966.1 ± 310.8$^{z}$ | 963.8 ± 247.9$^{z}$ | 851.8 ± 202.4$^{y}$ |
| 5-HIAA | 2 | 129.4 ± 49.0$^{bc}$ | 121.6 ± 24.8$^{bc}$ | 103.4 ± 15.0$^{c}$ | 153.2 ± 9.2$^{b\,x}$ | 199.8 ± 32.9$^{a\,x}$ |
| | 4 | 102.6 ± 19.6 | 116.2 ± 8.8 | 143.3 ± 81.5 | 170.8 ± 54.9$^{x}$ | 149.8 ± 45.3$^{y}$ |
| | 6 | 97.4 ± 8.9$^{b}$ | 95.3 ± 5.8$^{b}$ | 120.7 ± 17.8$^{ab}$ | 138.8 ± 22.6$^{a\,xy}$ | 129.0 ± 25.1$^{a\,yz}$ |
| | 8 | 104.9 ± 20.9 | 110.1 ± 10.4 | 115.8 ± 8.9 | 109.2 ± 11.2$^{yz}$ | 97.6 ± 23.9$^{z}$ |
| | 10 | 98.0 ± 9.3 | 99.9 ± 14.1 | 95.6 ± 13.2 | 98.6 ± 16.5$^{z}$ | 95.3 ± 14.2$^{z}$ |
| 5-HIAA/5-HT | 2 | 0.097 ± 0.041$^{b}$ | 0.096 ± 0.024$^{b}$ | 0.083 ± 0.011$^{b}$ | 0.105 ± 0.006$^{ab}$ | 0.134 ± 0.024$^{a\,x}$ |
| | 4 | 0.088 ± 0.018 | 0.097 ± 0.022 | 0.131 ± 0.099 | 0.147 ± 0.079 | 0.098 ± 0.024$^{xy}$ |
| | 6 | 0.088 ± 0.013$^{b}$ | 0.092 ± 0.004$^{ab}$ | 0.120 ± 0.021$^{a}$ | 0.124 ± 0.027$^{a}$ | 0.122 ± 0.019$^{a\,x}$ |
| | 8 | 0.069 ± 0.006$^{abc}$ | 0.074 ± 0.005$^{ab}$ | 0.082 ± 0.014$^{a}$ | 0.066 ± 0.006$^{bc}$ | 0.058 ± 0.010$^{c\,y}$ |
| | 10 | 0.099 ± 0.018 | 0.113 ± 0.031 | 0.113 ± 0.063 | 0.112 ± 0.047 | 0.122 ± 0.053$^{x}$ |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.

[2] The values represent means ± standard error.

[3] The number of the days indicates days after SRBC injection.

$^{a,b,c}$ Means within a row with different superscripts are significantly different ($P \leq 0.05$).

$^{x,y,z}$ Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 14. 5-HT, 5-HIAA levels, and their ratios of in the cerebellum of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| 5-HT | 2 | 221.1 ± 41.8 | 204.8 ± 40.8 | 220.2 ± 67.2 | 239.8 ± 70.5 | 304.7 ± 30.2 |
| | 4 | 215.8 ± 63.1 | 359.8 ± 258.7 | 316.2 ± 101.5 | 204.6 ± 73.8 | 329.5 ± 125.1 |
| | 6 | 427.6 ± 175.0 | 242.9 ± 44.1 | 240.3 ± 77.0 | 281.7 ± 78.2 | 358.9 ± 127.0 |
| | 8 | 267.3 ± 82.9 | 198.5 ± 76.2 | 199.2 ± 63.4 | 330.2 ± 99.7 | 411.7 ± 278.2 |
| | 10 | 282.9 ± 92.2 | 265.4 ± 105.9 | 226.9 ± 82.3 | 351.2 ± 114.3 | 297.2 ± 59.2 |
| 5-HIAA | 2 | 66.9 ± 10.4$^{ab}$ | 42.2 ± 14.8$^{bc}$ | 33.4 ± 11.0$^{c}$ | 59.2 ± 18.6$^{abc\ y}$ | 82.7 ± 19.2$^{a\ xy}$ |
| | 4 | 41.2 ± 23.8 | 40.7 ± 10.4 | 53.1 ± 16.2 | 31.2 ± 7.5$^{y}$ | 48.7 ± 18.7$^{y}$ |
| | 6 | 57.0 ± 21.7 | 47.0 ± 15.7 | 65.2 ± 21.9 | 56.8 ± 15.3$^{y}$ | 67.9 ± 20.7$^{y}$ |
| | 8 | 81.9 ± 52.4 | 61.6 ± 8.0 | 74.7 ± 38.9 | 100.5 ± 34.4$^{x}$ | 103.4 ± 33.9$^{x}$ |
| | 10 | 45.4 ± 18.1 | 44.9 ± 14.1 | 49.0 ± 18.2 | 59.5 ± 20.9$^{y}$ | 58.6 ± 13.2$^{y}$ |
| 5-HIAA/5-HT | 2 | 0.264 ± 0.082$^{a}$ | 0.184 ± 0.037$^{ab\ y}$ | 0.139 ± 0.017$^{b\ z}$ | 0.248 ± 0.045$^{a\ xy}$ | 0.276 ± 0.069$^{a\ x}$ |
| | 4 | 0.169 ± 0.047 | 0.113 ± 0.044$^{y}$ | 0.175 ± 0.061$^{yz}$ | 0.165 ± 0.055$^{y}$ | 0.135 ± 0.058$^{y}$ |
| | 6 | 0.137 ± 0.015$^{b}$ | 0.190 ± 0.033$^{ab\ y}$ | 0.246 ± 0.063$^{a\ y}$ | 0.207 ± 0.059$^{a\ xy}$ | 0.197 ± 0.043$^{ab\ xy}$ |
| | 8 | 0.374 ± 0.373 | 0.327 ± 0.123$^{x}$ | 0.365 ± 0.103$^{x}$ | 0.325 ± 0.136$^{x}$ | 0.296 ± 0.089$^{x}$ |
| | 10 | 0.170 ± 0.061 | 0.177 ± 0.046$^{y}$ | 0.218 ± 0.051$^{yz}$ | 0.171 ± 0.035$^{y}$ | 0.200 ± 0.044$^{xy}$ |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.
[2] The values represent means ± standard error.
[3] The number of the days indicates days after SRBC injection.
[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).
[x,y,z] Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 15. L-Dopa and catecholamine levels (ng/g) in the brain stem of male Leghorns fed varying levels of added diatary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| L-Dopa | 2 | 466.5± 67.1$^{x}$ | 447.6± 42.9$^{x}$ | 473.7± 33.8$^{x}$ | 458.7± 95.8$^{x}$ | 484.0± 42.2$^{x}$ |
| | 4 | 354.0± 95.8$^{xyz}$ | 332.4±118.6$^{xy}$ | 398.9± 91.1$^{x}$ | 449.8± 44.3$^{x}$ | 428.8± 57.6$^{xy}$ |
| | 6 | 381.0±221.0$^{xy}$ | 315.6±119.6$^{y}$ | 267.4±118.4$^{y}$ | 406.8± 79.1$^{x}$ | 315.2±115.6$^{yz}$ |
| | 8 | 216.0± 41.2$^{ab\ yz}$ | 136.1± 32.3$^{b\ z}$ | 260.7± 18.6$^{a\ y}$ | 155.4±109.9$^{b\ y}$ | 187.8± 56.2$^{ab\ z}$ |
| | 10 | 172.7± 86.6$^{z}$ | 182.8±100.0$^{z}$ | 180.3± 64.6$^{y}$ | 153.5± 98.2$^{y}$ | 206.5±154.1$^{z}$ |
| Dopamine | 2 | 131.9± 34.4$^{xy}$ | 153.7± 27.6 | 165.7± 12.2 | 159.1± 17.2$^{x}$ | 172.4± 25.1$^{x}$ |
| | 4 | 134.3± 21.5$^{xy}$ | 168.5± 29.4 | 148.6± 9.9 | 142.7± 36.5$^{xy}$ | 142.6± 11.9$^{xy}$ |
| | 6 | 149.6± 16.2$^{x}$ | 158.3± 3.5 | 155.7± 25.0 | 157.0± 18.0$^{x}$ | 167.6± 44.4$^{x}$ |
| | 8 | 157.2± 17.8$^{x}$ | 146.1± 22.8 | 145.6± 22.2 | 151.4± 15.9$^{x}$ | 144.2± 20.7$^{xy}$ |
| | 10 | 109.6± 21.6$^{y}$ | 129.9± 26.3 | 139.2± 24.0 | 115.7± 20.7$^{y}$ | 111.7± 14.0$^{y}$ |
| Norepinephine | 2 | 876.9±102.9$^{c\ x}$ | 772.1± 96.1$^{c\ x}$ | 859.5± 85.9$^{c\ x}$ | 1011.1±105.7$^{b\ x}$ | 1161.3 ± 102.3$^{a\ x}$ |
| | 4 | 667.0±222.5$^{b\ xy}$ | 698.9±100.0$^{ab\ x}$ | 705.3±127.2$^{ab\ y}$ | 901.3±171.0$^{a\ x}$ | 909.2±107.8$^{a\ y}$ |
| | 6 | 743.9±223.9$^{xy}$ | 638.2±155.0$^{xy}$ | 682.3±120.6$^{y}$ | 836.8±130.5$^{x}$ | 808.7±247.4$^{y}$ |
| | 8 | 569.0±167.3$^{y}$ | 668.9±100.9$^{x}$ | 581.2±141.8$^{y}$ | 569.2±127.7$^{y}$ | 541.9± 69.6$^{z}$ |
| | 10 | 507.7±170.7$^{y}$ | 507.6± 86.2$^{y}$ | 551.8± 47.4$^{y}$ | 563.7± 88.4$^{y}$ | 505.3± 89.3$^{z}$ |
| Epinephine | 2 | 137.5± 16.9$^{b}$ | 137.7± 8.3$^{b\ y}$ | 144.8± 26.1$^{b}$ | 213.1± 31.6$^{a\ xy}$ | 221.1 ± 47.6$^{a}$ |
| | 4 | 140.6± 9.8 | 181.5± 31.9$^{x}$ | 122.2± 35.3 | 309.9±177.0$^{x}$ | 280.8±208.2 |
| | 6 | 146.2± 28.2 | 139.2± 22.7$^{y}$ | 162.3± 77.2 | 200.2± 45.5$^{xy}$ | 284.2±198.1 |
| | 8 | 115.5± 25.5 | 118.3± 29.1$^{y}$ | 94.8± 30.9 | 120.1± 26.1$^{y}$ | 139.7± 52.1 |
| | 10 | 135.3± 24.5 | 124.9± 32.4$^{y}$ | 147.4± 27.2 | 131.5± 40.8$^{y}$ | 144.9± 16.4 |

1 The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.
2 The values represent means ± standard error.
3 The number of the days indicates that the samples were collected after the days of SRBC injection.
a,b,c Means within a row with different superscripts are significantly different ($P \leq 0.05$).
x,y,z Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 16. L-Dopa and catecholamine levels (ng/g) in the diencephalon of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2]

| Measurement | Days[3] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| L-Dopa | 2 | 351.5±276.5$^{y}$ | 86.6± 71.0$^{y}$ | 219.5± 95.7$^{y}$ | 283.6±126.7$^{y}$ | 213.7± 52.3$^{y}$ |
| | 4 | 74.0± 12.8$^{c\,z}$ | 122.6± 34.0$^{b\,y}$ | 167.7± 44.6$^{a\,y}$ | 80.5± 21.3$^{c\,z}$ | 59.9± 10.3$^{c\,z}$ |
| | 6 | 106.4± 69.3$^{z}$ | 119.8± 71.5$^{y}$ | 180.6± 82.3$^{y}$ | 90.6± 41.8$^{z}$ | 82.6± 19.4$^{z}$ |
| | 8 | 781.3±164.2$^{x}$ | 860.9±187.2$^{x}$ | 691.2±123.8$^{x}$ | 786.1±222.6$^{w}$ | 742.1±101.7$^{w}$ |
| | 10 | 568.4±209.0$^{xy}$ | 747.1±197.0$^{x}$ | 560.6±122.5$^{x}$ | 476.0± 98.8$^{x}$ | 541.4± 98.4$^{x}$ |
| Dopamine | 2 | 332.7±246.6 | 271.5± 60.2 | 242.5± 82.3$^{x}$ | 386.6±127.5$^{x}$ | 488.1±150.8$^{x}$ |
| | 4 | 201.2± 61.5$^{c}$ | 187.7± 28.1$^{c}$ | 176.7± 40.4$^{c\,xy}$ | 309.9±104.7$^{b\,xy}$ | 470.9± 84.6$^{a\,x}$ |
| | 6 | 241.9±113.1$^{b}$ | 194.5± 72.7$^{b}$ | 198.5± 24.9$^{b\,xy}$ | 262.5± 42.6$^{b\,yz}$ | 405.7± 27.3$^{a\,x}$ |
| | 8 | 215.1± 17.3 | 223.7± 71.9 | 248.9± 19.0$^{x}$ | 225.8± 37.7$^{yz}$ | 259.9± 38.0$^{y}$ |
| | 10 | 189.6± 62.8 | 189.6± 55.5 | 163.0± 46.2$^{y}$ | 176.5± 44.8$^{z}$ | 149.7± 7.7$^{y}$ |
| Norepinephine | 2 | 540.3±165.0$^{z}$ | 471.8± 71.0$^{y}$ | 490.1± 57.4$^{z}$ | 576.8±131.7$^{z}$ | 533.7±142.5$^{z}$ |
| | 4 | 690.4±151.7$^{yz}$ | 736.0±123.3$^{y}$ | 740.1±229.4$^{yz}$ | 691.4± 68.1$^{yz}$ | 667.2± 93.8$^{z}$ |
| | 6 | 793.2±237.4$^{yz}$ | 679.2±142.0$^{y}$ | 736.4± 97.8$^{yz}$ | 715.9± 85.9$^{yz}$ | 683.9± 76.3$^{z}$ |
| | 8 | 1256.4±265.9$^{x}$ | 1324.7±300.2$^{x}$ | 1212.4±422.7$^{x}$ | 1049.1±199.2$^{x}$ | 1256.5±153.4$^{x}$ |
| | 10 | 921.1±158.0$^{y}$ | 1247.3±358.4$^{x}$ | 978.7±257.1$^{xy}$ | 897.7±244.6$^{xy}$ | 936.6±175.8$^{y}$ |
| Epinephine | 2 | 93.7± 18.9$^{b\,y}$ | 105.0± 8.8$^{b\,z}$ | 112.7± 31.0$^{b}$ | 264.0±189.3$^{a}$ | 253.5$^{a\,x}$ * |
| | 4 | 146.9± 43.1$^{y}$ | 166.0± 42.0$^{yz}$ | 140.4± 47.3 | 175.4± 22.1 | 145.9± 30.8$^{y}$ |
| | 6 | 173.0± 53.8$^{y}$ | 113.6± 31.0$^{yz}$ | 147.9± 36.9 | 136.6± 32.5 | 150.0± 16.6$^{y}$ |
| | 8 | 298.8±114.7$^{x}$ | 301.2±133.0$^{xy}$ | 373.4±344.5 | 218.8± 49.9 | 254.3± 56.2$^{x}$ |
| | 10 | 191.5± 41.2$^{y}$ | 429.8±285.0$^{x}$ | 283.2±235.2 | 183.4± 22.9 | 205.5± 20.6$^{xy}$ |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.
[2] The values represent means ± standard error.
[3] The number of the days indicates days after SRBC injection.
* Only one observation in the measurement.
[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).
[w,x,y,z] Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 17. L-Dopa and norepinephine levels (ng/g) in the telencephalon of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2,3]

| Measurement | Days[4] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| Dopamine | 2 | 479.8± 90.4 | 617.9±128.0 | 590.6±102.0 | 609.4± 85.9 | 596.4± 97.3$^{yz}$ |
| | 4 | 587.5± 64.1$^{b}$ | 604.2±111.5$^{b}$ | 531.5±107.7$^{b}$ | 627.9±253.8$^{b}$ | 901.3±181.9$^{a\,x}$ |
| | 6 | 545.8±142.2 | 616.43±169.9 | 558.8±136.8 | 570.9±125.8 | 578.1±223.9$^{y}$ |
| | 8 | 604.1±151.3 | 474.4± 85.5 | 466.2±186.8 | 399.7±120.2 | 666.8±190.5$^{yz}$ |
| | 10 | 558.1±121.2 | 496.0± 17.8 | 443.6± 52.5 | 455.9± 83.6 | 477.6± 58.2$^{y}$ |
| Norepinephine | 2 | 195.2± 33.4$^{y}$ | 237.7± 51.4 | 216.6± 13.7 | 202.4± 17.3 | 249.3$^{*}$ |
| | 4 | 225.0± 24.9$^{y}$ | 233.1± 46.4 | 286.2±136.0 | 281.3±107.5 | 251.3± 58.4 |
| | 6 | 293.9± 55.4$^{x}$ | 270.2± 65.7 | 233.6± 32.2 | 232.6± 46.7 | 205.5± 46.9 |
| | 8 | 253.3± 55.2$^{xy}$ | 278.4±101.4 | 219.3± 63.8 | 238.9± 36.9 | 241.4± 38.2 |
| | 10 | 258.4± 38.7$^{y}$ | 257.5± 48.5 | 247.7± 49.0 | 253.1± 33.0 | 262.2± 41.4 |

1 The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.

2 The values represent means ± standard error.

3 The peaks of L-dopa and norepinephrine was not picked up by HPLC-EC.

4 The number of the days indicates days after SRBC injection.

$^{a,b}$ Means within a row with different superscripts are significantly different ($P \leq 0.05$).

$^{x,y,z}$ Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

$^{*}$ Only one observation in the measurement.

Table 18. Norepinephine in the cerebellum of male Leghorns fed varying levels of added dietary tyrosine and tryptophan from 27 to 34 days of age[1,2,3]

| Measurement | Days[4] | Dietary Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| Norepinephine | 2 | 199.4± 75.9[y] | 321.2± 53.4 | 376.7±139.1 | 304.1± 89.2 | 265.8 ± 50.5 |
| | 4 | 206.2± 59.3[y] | 339.0± 78.4 | 255.2± 80.6 | 233.7± 81.0 | 227.0± 42.4 |
| | 6 | 187.6± 65.2[y] | 310.7±148.1 | 288.6±101.8 | 322.3±109.5 | 285.8±144.6 |
| | 8 | 312.5± 44.3[y] | 312.6±113.9 | 232.8± 48.6 | 257.4± 73.1 | 263.4± 54.7 |
| | 10 | 390.4±142.8[x] | 340.3± 52.0 | 347.3± 11.6 | 312.2±118.6 | 412.2±167.7 |

[1] The birds were given the treated diet one day before SRBC injection, and the dietary supplementation lasted for 7 days.
[2] The values represent means ± standard error.
[3] Only peak of norepinephrine was picked up by HPLC-EC
[4] The number of the days indicates days after SRBC injection.
[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).
[x,y,z] Means within a column of measurement with different superscripts are significantly different ($P \leq 0.05$).

Table 19. Daily feed intake and body weight gain (g) of Leghorns and broilers during the dietary supplementation period[1,2]

| Measurement | Dietary Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 0.5% Tyr | 1% Tyr | 0.5% Trp | 1% Trp |
| | | | Leghorns | | |
| Feed intake | 59.6 ± 0.6 | 59.9 ± 4.0 | 59.1 ± 2.0 | 61.0 ± 3.2 | 58.6 ± 2.2 |
| Body weight gain | 40.1 ± 15.6 | 45.4 ± 10.2 | 48.6 ± 9.1 | 48.3 ± 5.6 | 47.4 ± 3.3 |
| | | | Broilers | | |
| Feed intake | 123.9 ± 8.0 | 128.8 ± 5.0 | 131.7 ± 1.0 | 128.1 ± 0.9 | 131.4 ± 3.2 |
| Body weight gain | 56.3 ± 4.4 | 57.3 ± 5.9 | 59.6 ± 1.9 | 55.6 ± 0.6 | 56.9 ± 0 |

[1] The values represent Means ± SE.

[2] No significant difference between the treatment at the level of $P \leq 0.05$.

Table 20. Daily feed intake and body weight gain (g) of stressed broilers during the dietary supplementation period[1,2,3]

| Measurement | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| | Controll | Stress | Stress + 0.5% Tyr | Stress + 1% Tyr | Stress + 0.5% Trp | Stress + 1% Trp |
| Feed intake | 1159.5 ± 13.5 | 128.4 ± 5.4 | 126.9 ± 5.4 | 122.4 ± 4.3 | 129.9 ± 4.7 | 121.7 ± 3.3 |
| Body weight gain | 58.3 ± 8.6 | 61.4 ± 4.5 | 62.0 ± 1.7 | 61.0 ± 2.0 | 63.0 ± 2.8 | 56.5 ± 2.1 |

[1] The broilers were tied both legs and wings for thress hours.
[2] The values represent means ± SE.
[3] No significant difference between the treatments ($p \leq 0.05$).

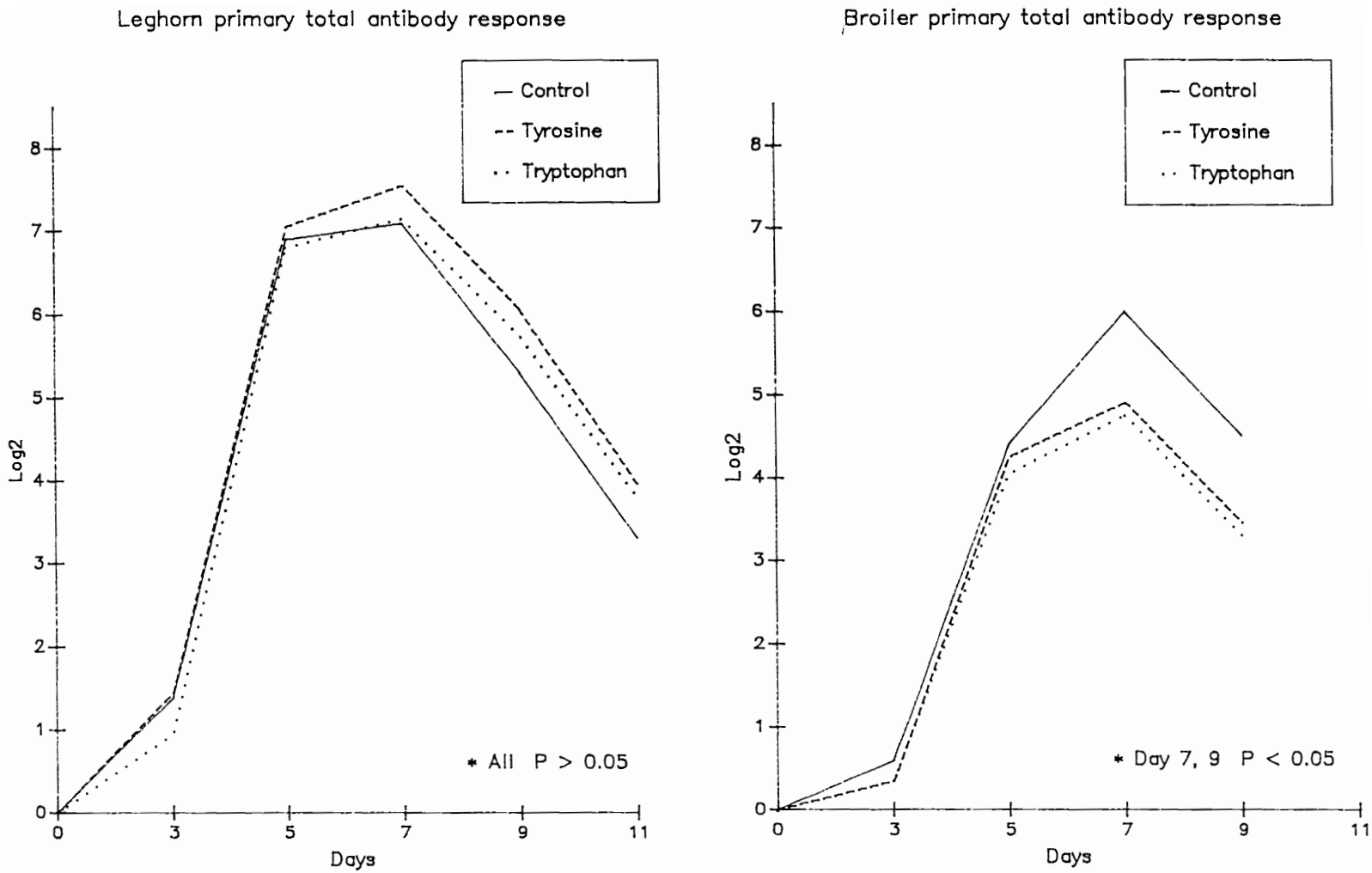


Figure 1. Primary total antibody titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

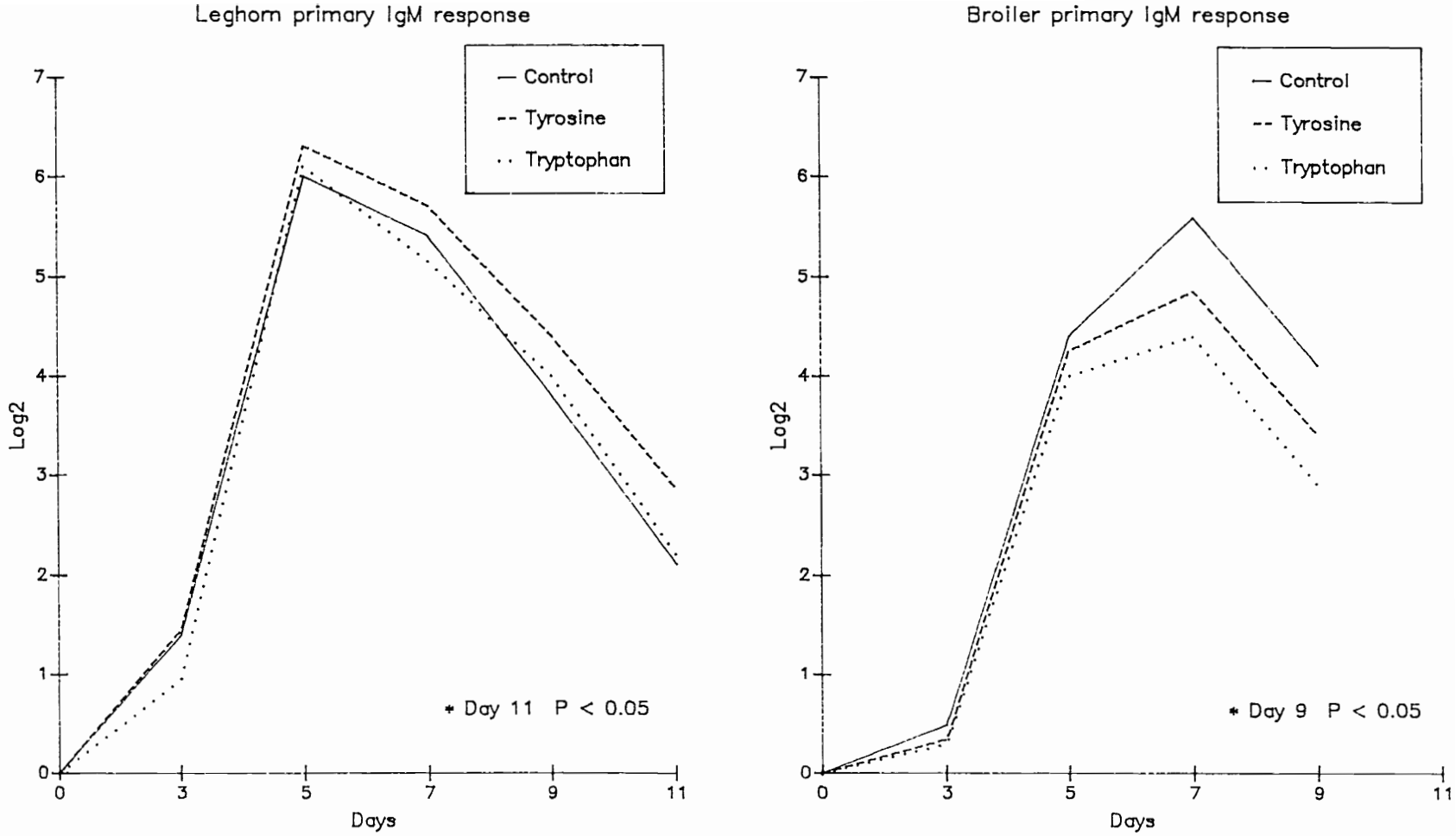


Figure 2. Primary IgM titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

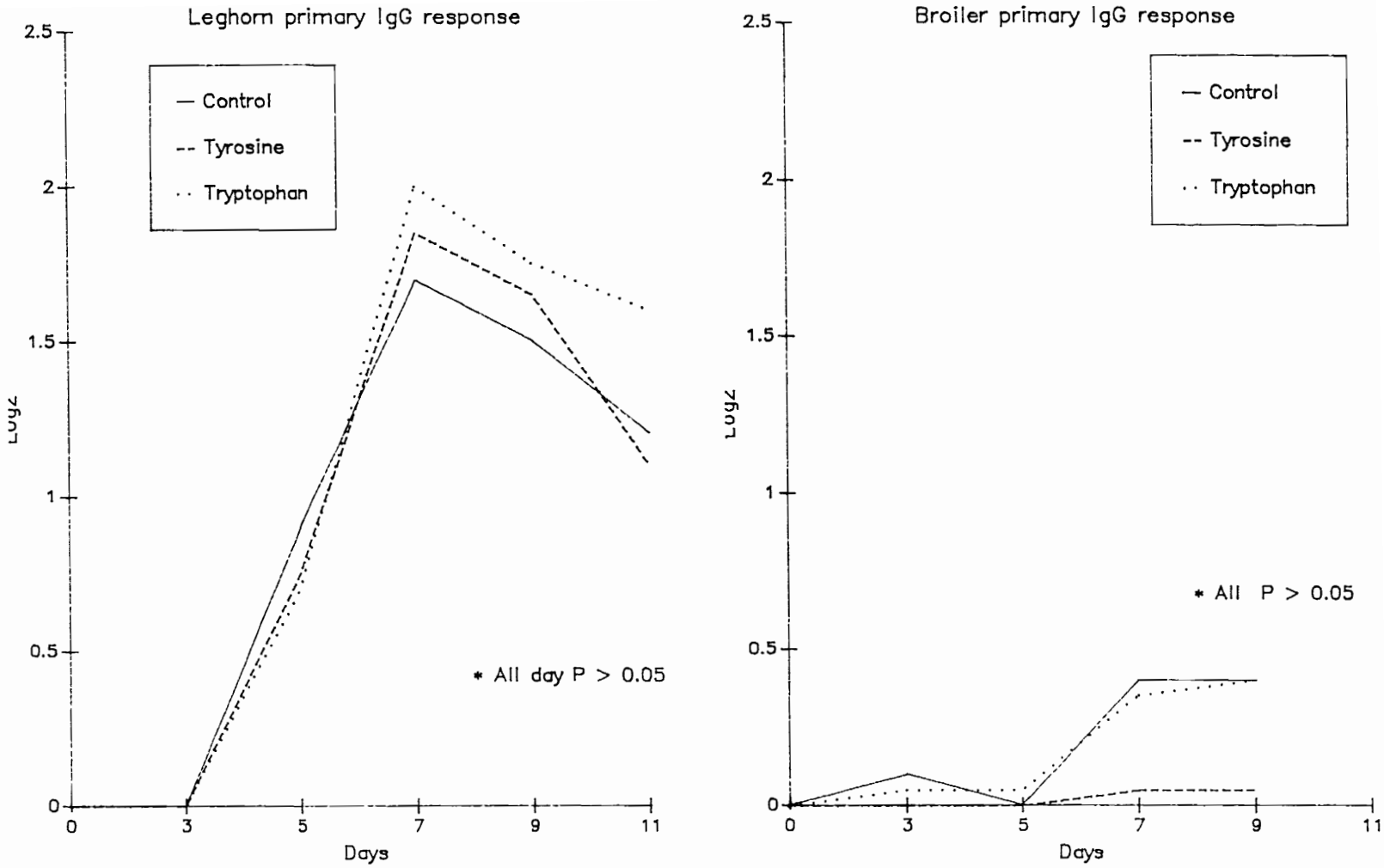


Figure 3. Primary IgG titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

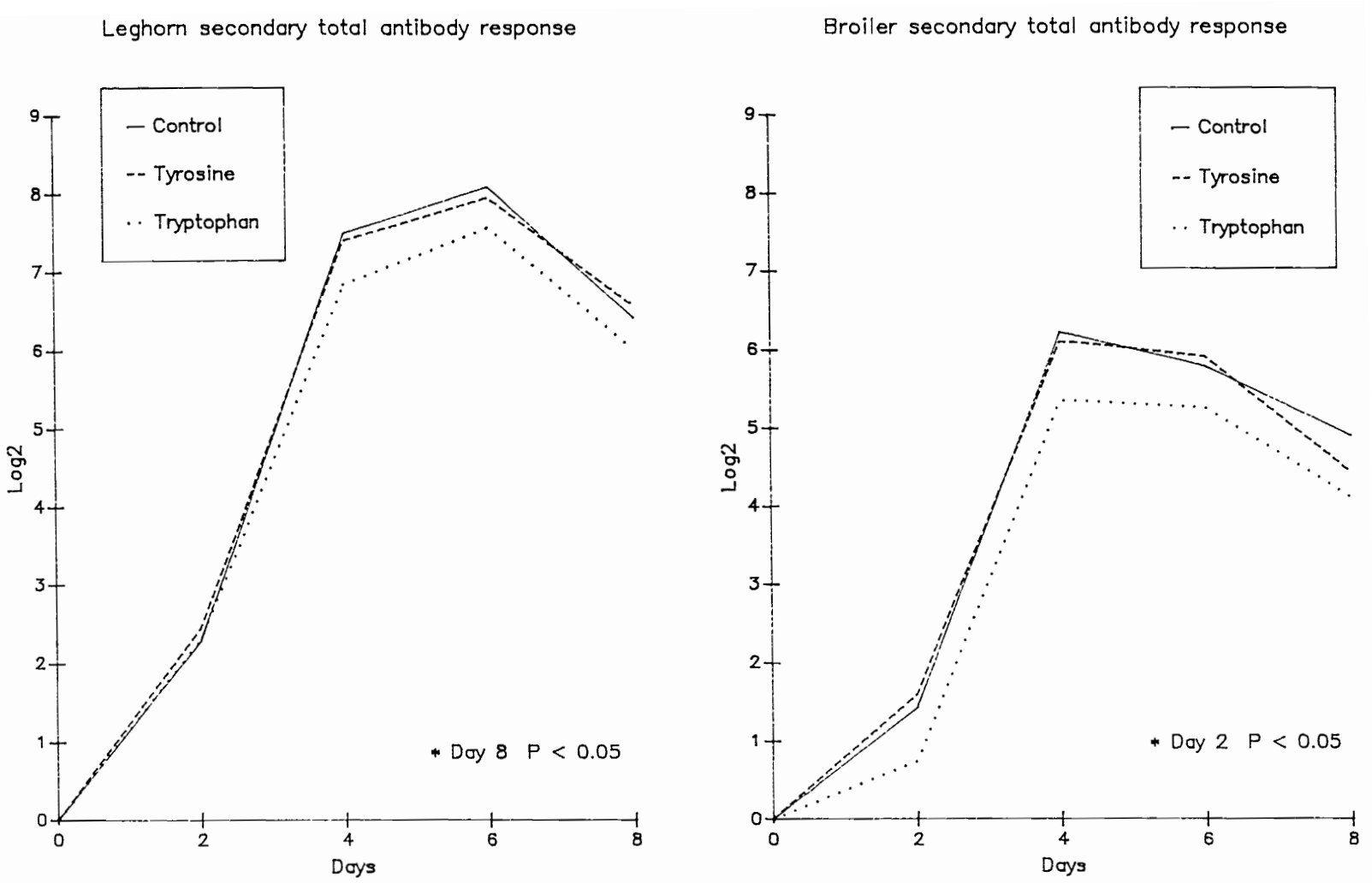


Figure 4. Secondary total antibody titers in Leghrons and broilers after pooling the two dietary addition levels of a given amino acid

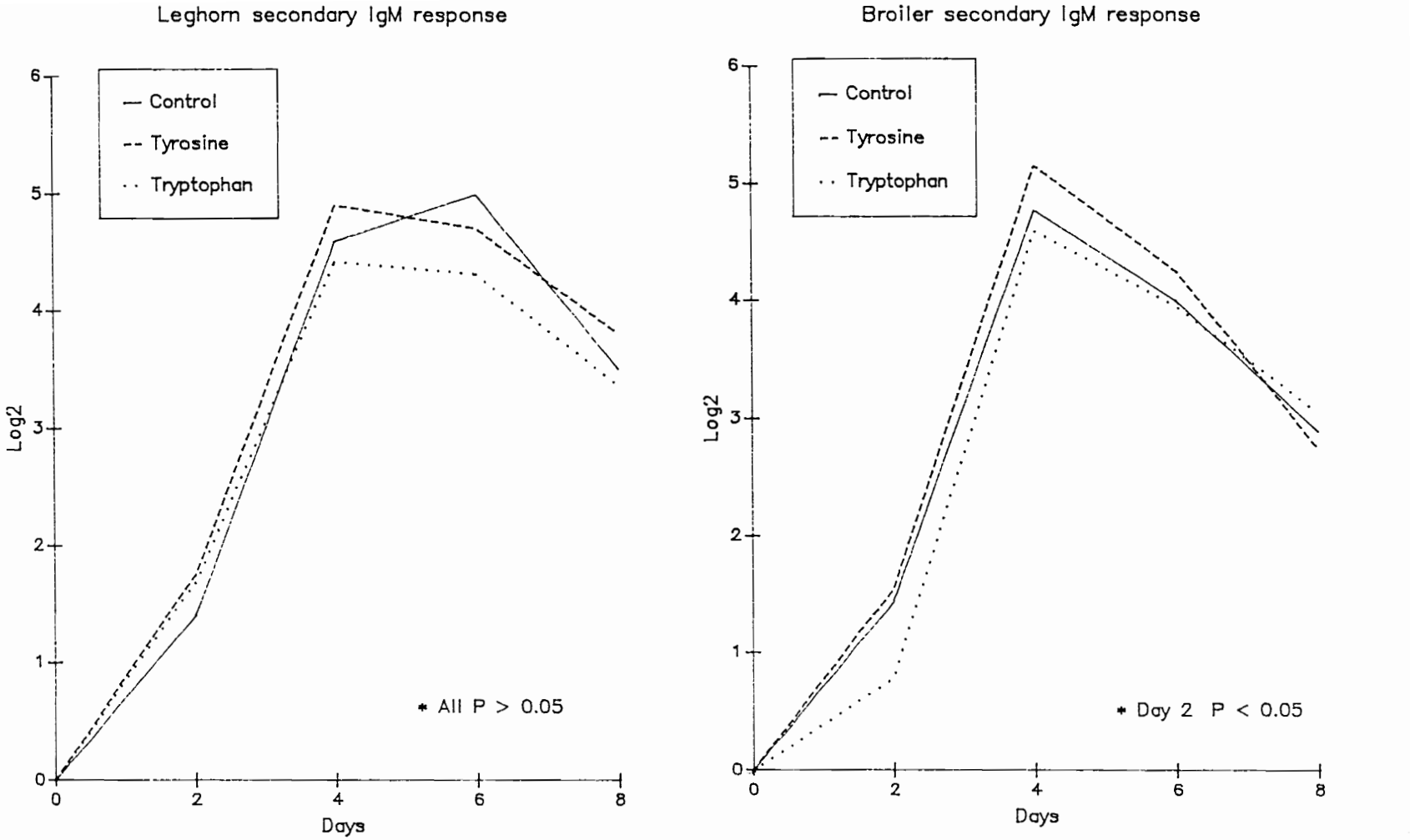


Figure 5. Secondary IgM titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

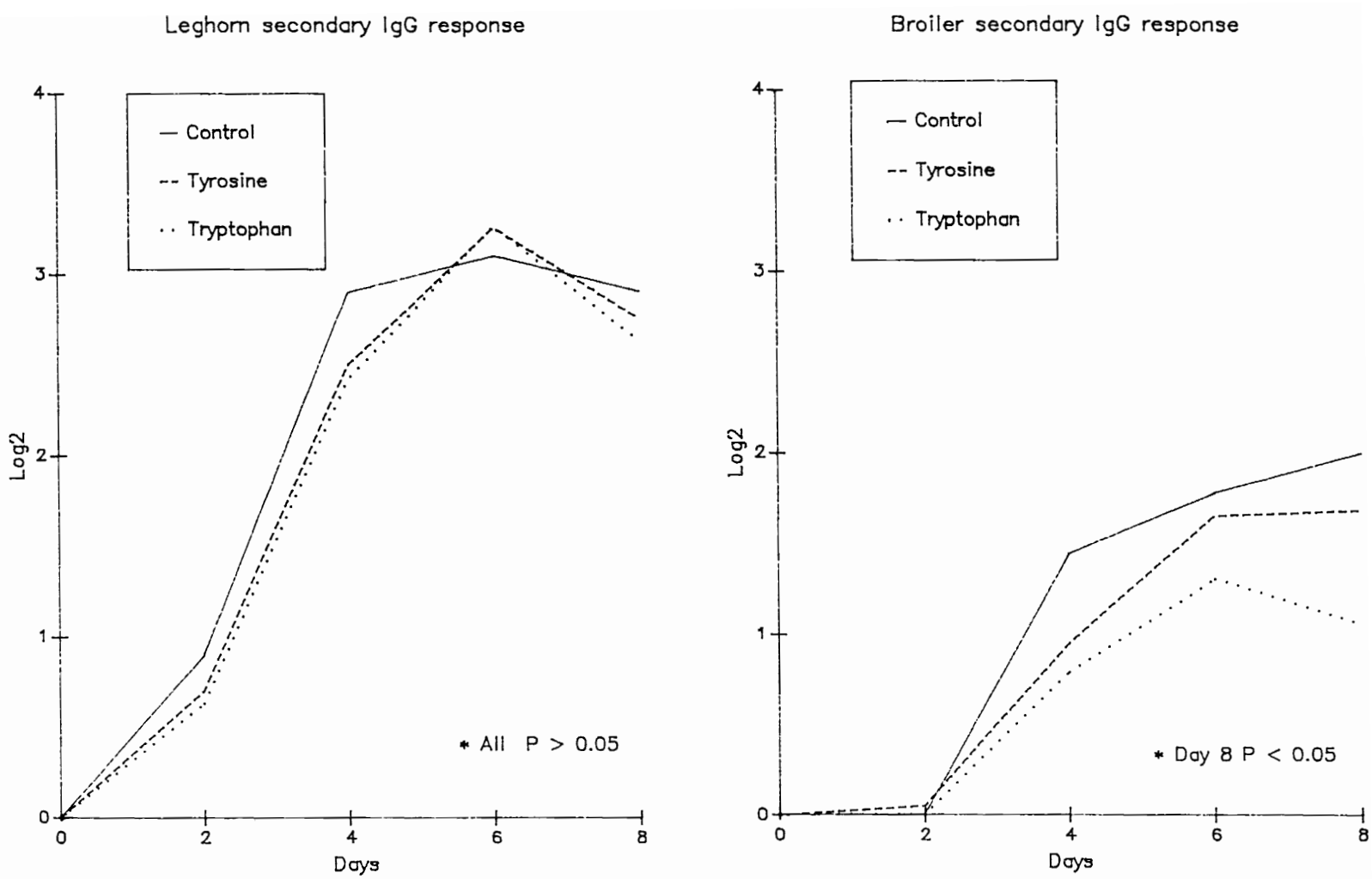


Figure 6. Secondary IgG titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

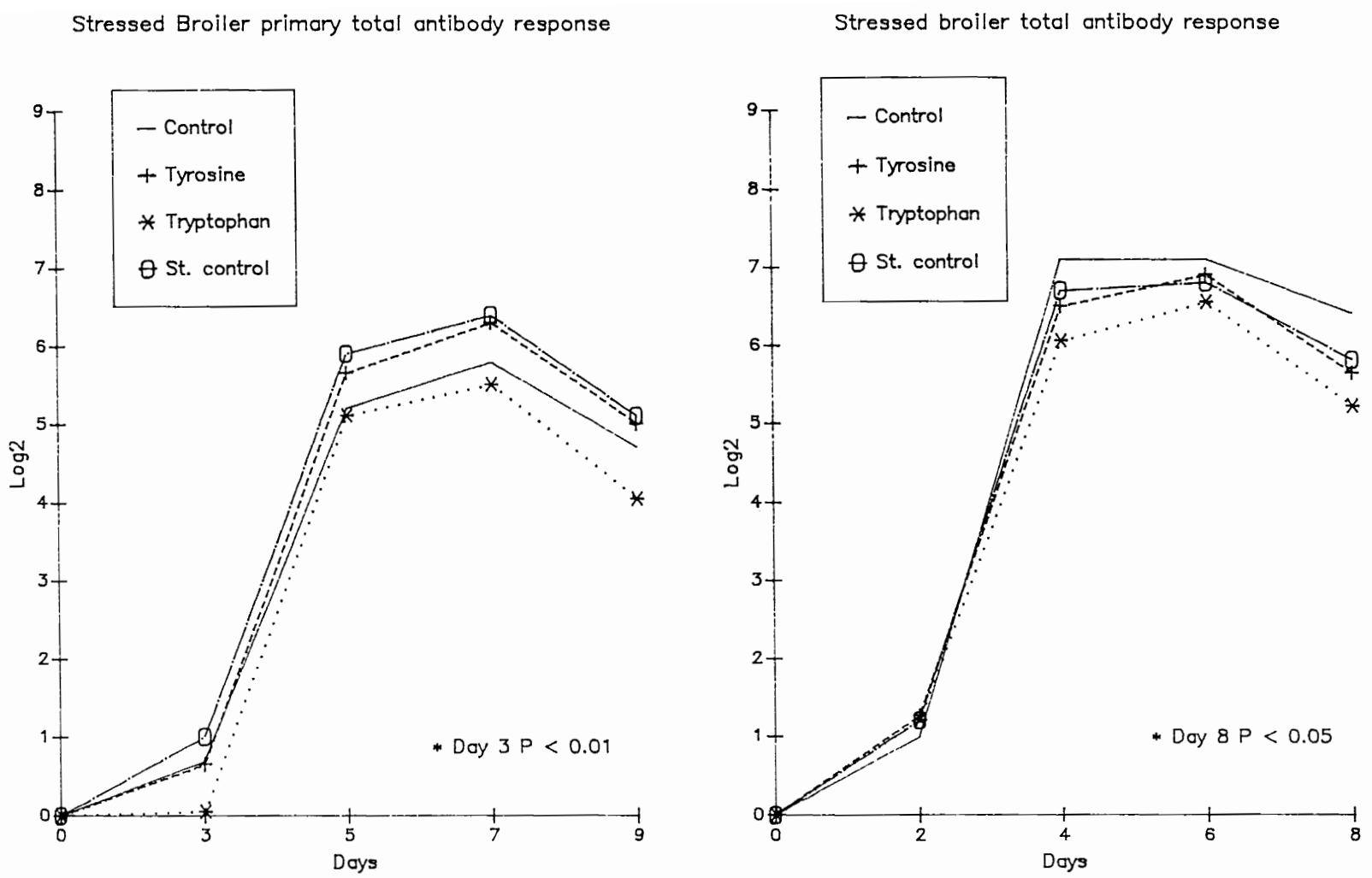


Figure 7. Primary and secondary total titers in Leghorns and broilers after pooling the two dietary addition levels of a given amino acid

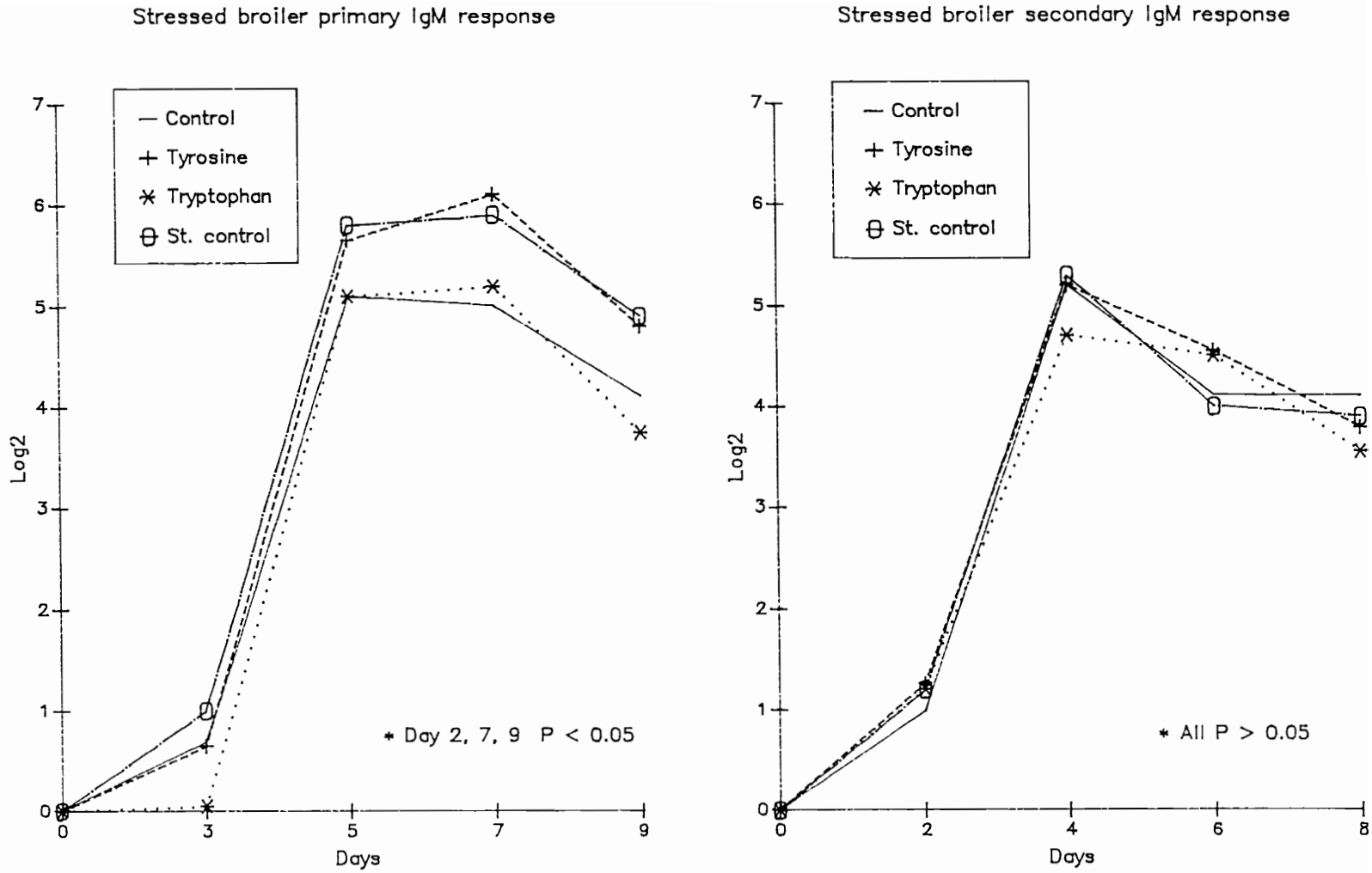


Figure 8. Primary and secondary IgM titers in stressed broilers after pooling the two dietary addition levels of a given amino acid

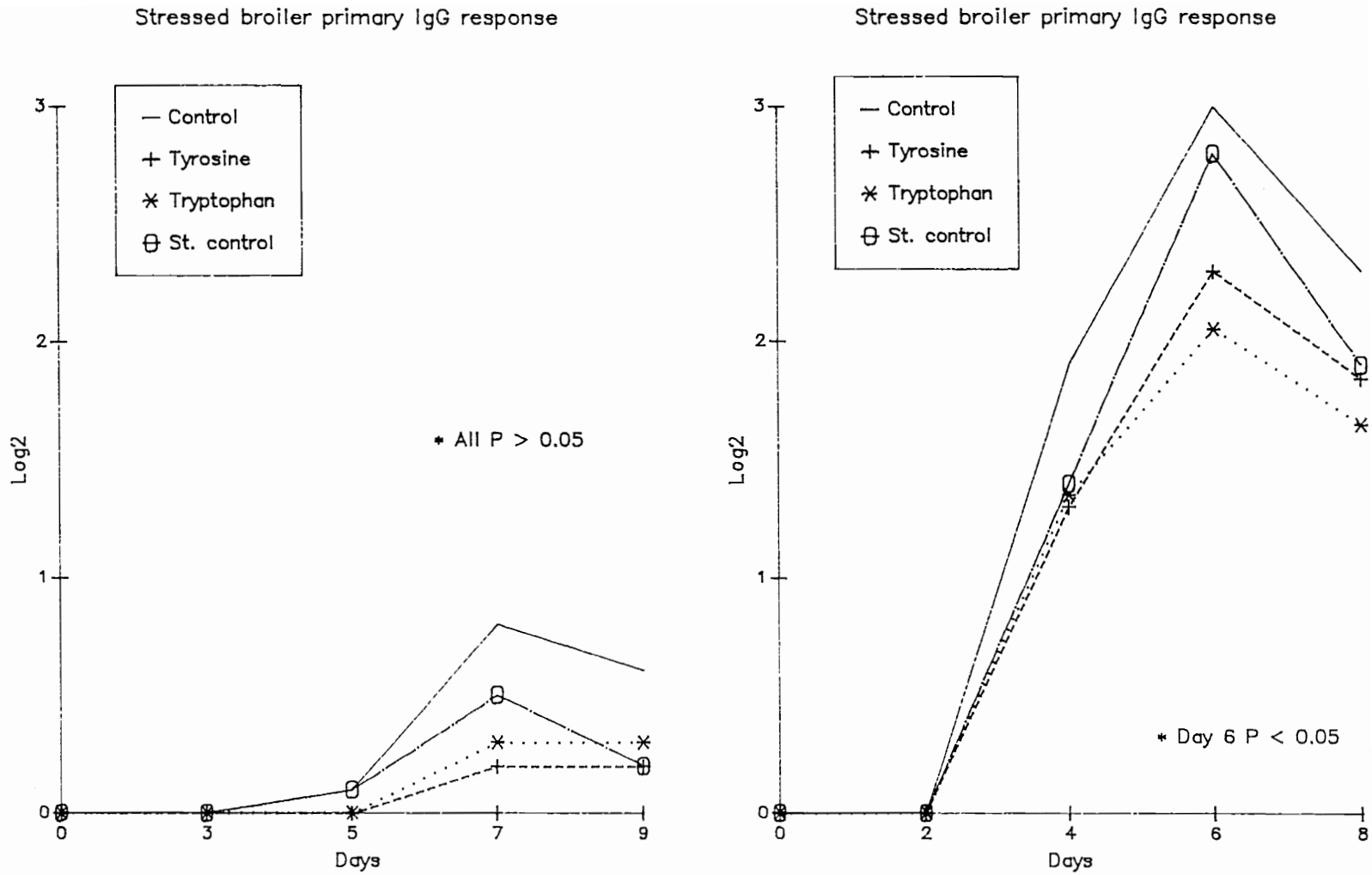


Figure 9. Primary and secondary IgG titers in stressed broilers after pooling the two dietary addition levels of a given amino acid

## *Appendix A. ASSAY PROCEDURES*

### Antibody Titers for Sheep Red Blood Cells

| Materials: | Chemicals: |
|---|---|
| Syringes (1cc, 60cc) | Dextrose |
| Needle (18 and 26 gouge) | Sodium citrate dihydrate |
| 96 well microtiter plates with "V" bottoms | Citric acid monohydrate |
| 25 $\mu$l dispenser | Sodium chloride |
| 25 $\mu$l diluter | 2-Mercaptoethanol |
| Incubator | |
| Centrifuge | |

*Collecting SRBC*

1. Using an 18 gauge needle and a syringe or vacutainer tube, collect blood from a sheep. Use an anticoagulant to prevent clotting. If blood is to be stored for a period of time, then everything must be sterilized.

2. Centrifuge the blood at 2000 *g* for 15 min and remove plasma. Wash the cells two times with saline and then a third time with Alsver's. Cells can be stored refrigerated for 2 weeks, 3-4 weeks if kept sterile.

* Preparation of Alsever's solution

| Chemicals | g/l | g/100 ml | g/200 ml |
|---|---|---|---|
| dextrose | 20.5 | 2.05 | 4.10 |
| sodium citrate dihydrate | 8.0 | 0.80 | 1.60 |
| citric acid monohydrate | 0.55 | 0.055 | 0.11 |
| NaCl | 4.2 | 0.42 | 0.84 |

pH = 6.1

Autoclave for 10 min.

3. Determine the hematocrit of the SRBC solution and use the following formula to dilute to the proper strength:

$$x = \frac{(Y \text{ ml of desired soln.}) \times (\% \text{ desired soln.})}{(\% \text{ stock soln.})}$$

X = ml of stock soln. needed to make Y ml of desired soln.
Y - X = ml of saline needed.

*Microagglutination test for total antibody titers*

1. Immunization and serum collection

   a. Inject birds with 1 ml of a 5% (or 0.1 ml of 0.5%) solution of SRBC's using a 26 gauge needle.

   b. At some period(s) of time after inoculation, usually 7 days, take 0.3-0.4 cc of blood per bird.
   DO NOT USE AN ANTICOAGULANT.

   c. Let the blood stand, usually 1-2 hr, until a clot forms. Then centrifuge the samples and collect the serum. If necessary, blood can be kept in the refrigerator overnight and the serum collected the next day.

   d. If measuring both IgG and IgM titers, it is necessary to heat inactivate the serum at $56^0$C for 1 hr. If only total titers are to be measured, then it is not necessary to heat inactivate the serum.

      * Preparation of 0.2M 2-mercaptoethanol (Used only for determining IgM)
         - 1.4 ml 2-mercaptoethanol + 98.6 ml saline
         - Make solution fresh daily.
         - Store 2-ME refrigerated and in the dark.

2. Procedure for total antibody titers

   a. Add 25 $\mu$l of saline to each well of microtiter plate. If also doing IgM titers, only add saline to the first well, do step 6, cover plates and incubate at 37 C for 30 min. Then add saline to the remaining wells.

   b. Add 25 $\mu$l of antiserum to the first well of each row.

   c. Mix the first well and then, using a 25 $\mu$l wand, transfer 25 $\mu$l to the next well, mix and continue procedure to the final well.

   d. Add 25 $\mu$l of antigen (2% SRBC) to each well, excluding the last well. Add saline to the last well to use as a control.

   e. Incubate at 37 C for 30 minutes.

   f. Read immediately. Values represent $\log_2$.

   NOTE: If measuring both IgM and IgG, need 30 ml of 2% SRBC per 30 blood samples.

3. Procedure for IgM titers

   a. Prepare a second set of microtiter plates by placing 25 $\mu$l of 0.2 M 2-ME in the first well.

   b. Place 25 $\mu$l of antiserum in the first well, cover with Saran wrap and incubate for 30 minutes at 37 C.

   c. Add 25 $\mu$l of saline to remaining wells.

d. Repeat steps c-f above.

e. Titers are read and recorded as ME-resistant (ME-R) titers. The ME-sensitive (ME-S) titer is the difference between the total titers and the ME-R titers.

* The ME-S Ig is associated with levels of IgM and ME-R Ig is IgG levels.

## Refferences

McCorkle, F. M., and B. Glick. 1980. The effect of aging on the immune competence in the chicken: antibody-mediated immunity. Poultry Sci. 59:669-672.

Wegman, T. G., and O. Smithies. 1966. A simple hemagglutination system requiring small amounts of red cells and antibodies. Transfusion 6:67-73.

Delhanty, J. J., and J. B. Solomon. 1966. The nature of the antibodies to goat erythrocytes in the developing chicken. Immunol. 11:103-113.

## Brain Catecholamine and Indoleamine Assay for EC-HPLC

*Materinals:*

Volumetric Flasks
- 1 1 Liter
- 1 250 ml
- 7 100 ml
- 1 25 ml
- 7 10 ml

Balances (0.00 mg and 0.000 g)
Millipore Filtering Apparatus
PH Meter
Stir Plate
Brinkman Polytron
Microfilters (Bioanalytical System)
Refrigerated Centrifuge
Ultralow Freezer(-70)

*Chemicals:*

70% Perchloric Acid
diNaEDTA
Sodium Hydroxide
Sodium Octyl Sulfate
Citric Acid
Acetronitrile
Triethanolamine
Norepinephrine Bitartrate (NE)
L-dopa (DOPA)
Dopamine (DA)
Serotonin (5HT)
5-Hydroxyindole Acetic Acid (5-HIAA)
HPLC Grade Water
Buffer Solution PH 4.0 and 7.0
Epinephrine (E)
3,4 Dihydroxybenzylamine (DHBA)

***Procedure:***

Plan how many samples you can run that day. Each sample takes about 20 minutes to run. In addition to samples plan to run a standard every 4 samples. Keep brains and all solution on ice.

1. Weight brain sections in 10 ml tubes and record.

2. Add brain buffer:

   a. 3ml for diencephalon, brain stem, and crebellum
   b. 6 ml for telencephalon

3. Add 30 ul of 10 ug/ml solution of DHBA in 3ml buffer and 60 ul in 6 ml buffer

4. Homogenize using Brinkman polytron.

5. Centrifuge ($0^0$C) at 11,000 *g* for 15 minutes.

6. Pour off supernatant into BAS filters and centrifuge 5 minutes at 3000 *g*.

7. Freeze samples immediately and keep until ready to inject.

8. Inject 100 ul of filtered supernatant into HPLC.

9. Calibrate HPLC by injecting 100 ul of mixed standard

*HPLC Moble Phase for Brain Catecholamine and Indolamine Assay*

1. 150 mg diNaOH
2. 350 mg NaOH
3. 19.2 g Citric acid
4. 225 mg Sodium octyl sulfate (variable)
5. 85 ml Acetonitrile
6. 1.8 ml Triethylamine
7. Bring up to 1 liter with HPLC grade water
8. Set PH to 3.1 using NaOH or citric acid
9. Filter and degas by passing through degaser before it enters HPLC

* Sodium octyl sulfate increases retention time; acetonitrile decreasesretention time; triethylamine helps decrease "tail-in" of last peaks.

*Brain Buffer (for 250 mls)*

1. 37.5 mg diNa EDTA
2. 1.075 mls of 70% perchloric acid (0.5M)
3. Bring to 250 mls with HPLC grade water and refrigerate

*Standards:*

All standards are made with 0.1M $HClO_4$ (8.6 ml of 70% $HClO_4$/liter)

1. Preparation of Stock solutions (50 mg/100 mls)

   a. NE - 94.36 mg per 100 mls of 0.1M $HClO_4$
   b. E - 90.97 mg per 100 mls of 0.1M $HClO_4$
   c. DOPA - 50.00 mg per 100 mls of 0.1M $HClO_4$
   d. DA - 61.91 mg per 100 mls of 0.1M $HClO_4$
   e. 5HT - 110.56 mg per 100 mls 0.1M $HClO_4$
   f. 5HIAA - 50.0 mg per 100 mls 0.1M $HClO_4$
   g. DHBA - 80 mg per 100 mls of 0.1M $HClO_4$

2. Preparation of working stock solution (10 $\mu$g/ml)

   a. For all the above standards, add 200 ul of the stock sulution to a 10 ml volumetric flask.
   b. Bring to 10 ml using 0.1M $HClO_4$

3. Preparation of mixed standard for analysis

   a. For all standard, except DHBA, add 125 $\mu$l of the working stock solution to a 25 ml volumetric flask.
   b. Bring to 25 mls using the brain buffer. Make the mixed standard daily.
   c. Refrigerate between uses.

*HPLC Settings and Specifications:*

Column specifications - Biophase ODS 5 $\mu$m; 250 x 4.6 mm (Bioanalitical Syrtems - Part # MF6017)

Set pump at 1.5 ml/min

Set detector at 600 mV potential and 10 nA/V or 20 nA/V

*Sample Calculation ($\mu$g/g)*

$$\frac{\dfrac{\text{pk ht of X}}{\text{pk ht of DHBA unknown}}}{\dfrac{\text{pk ht of Y}}{\text{pk ht of DHBA known}}} \times \text{std. conc. x dil. factor}$$

$$\text{Brain Part Weight}$$

pk ht = Peak height

X = catecholamine or indoleamine of interest from sample run

DHBA unknown = peak height of DHBA from the sample run

Y = catecholamine of indoleamine of interest from calibration run

DHBA known = peak height of DHBA from calibration run

std. conc. = concentration of the standard used for calibration

NOTE: The dilution factor takes into account the amount injected into the HPLC verses the amount of extract. For example if 100 $\mu$l are injected and one had 3 mls of extract, the dilution factor would be 3000 $\mu$l/100 $\mu$l or 30.

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

## *Appendix B FEED FORMULATION OF STARTER, GROWER, AND SUPPLEMENTED DIET*

Appendix B table. Feed formulation ingredient (%) for starter and grower diet

| Ingredients | Starter | Grower |
|---|---|---|
| Corn | 64.70 | 68.60 |
| Soybean meal | 24.00 | 20.00 |
| Fish meal | 1.00 | - |
| Meat and bone meal | 6.50 | 7.60 |
| Limestone | 0.55 | 0.55 |
| Stabilized Fat | 2.50 | 2.50 |
| DL-methionine | 0.20 | 0.20 |
| Iodized salt | 0.25 | 0.25 |
| Vitamin premix[1] | 0.25 | 0.25 |
| Trace mineral premix[2] | 0.05 | 0.05 |

* Supplemented diet was formulated by adding 0.5% or 1% of L-tyrosine or L-tryptophan into grower diet.

1 Vitamin Premix supplied (per kilogram feed):
vitamin A, 2200 IU; vitamin $D_3$, 1100 IU; Vitamin E, 0.55 IU; choline chloride, 125 mg; menadione sodium bisulfite, 0.88 mg; calcium D-pantothenate, 1.65 mg; vitamin $B_{12}$, 2.75 $\mu$g; selenium, 0.1 mg; riboflavin, 1.65 mg; folic acid, 0.138 mg; niacin, 16.5 mg.

2 Trace mineral premix supplied (per kilogram feed) manganese (min.) and zinc, 60 mg; iron, 20 mg; copper, 2.5 mg; iodine, 1 mg; cobalt, 0.225 mg and calcium (min.), 120 mg to (max.) 160 mg.

*Appendix C*

## ANALYSIS OF VARIANCE TABLES

Appendix C Table 1. Analysis of variance for primary antibody titers in Leghorns fed 0.1% tyrosine or tryptophan supplemental diets at different days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 1.267 | 0.98 | 0.3873 |
| | | Error | 27 | 17.400 | | |
| | | Total | 29 | 18.667 | | |
| | 5 | Trmt | 2 | 2.067 | 1.04 | 0.3682 |
| | | Error | 27 | 26.900 | | |
| | | Total | 29 | 28.967 | | |
| | 7 | Trmt | 2 | 3.267 | 0.89 | 0.4213 |
| | | Error | 27 | 49.400 | | |
| | | Total | 29 | 52.667 | | |
| | 9 | Trmt | 2 | 2.600 | 1.34 | 0.2788 |
| | | Error | 27 | 26.200 | | |
| | | Total | 29 | 28.800 | | |
| IgG | 3 | Trmt | 2 | 0 | . | . |
| | | Error | 27 | 0 | | |
| | | Total | 29 | 0 | | |
| | 5 | Trmt | 2 | 0.467 | 2.10 | 0.1420 |
| | | Error | 27 | 3.000 | | |
| | | Total | 29 | 3.467 | | |
| | 7 | Trmt | 2 | 2.600 | 1.24 | 0.3040 |
| | | Error | 27 | 28.200 | | |
| | | Total | 29 | 30.800 | | |
| | 9 | Trmt | 2 | 0.600 | 0.46 | 0.6376 |
| | | Error | 27 | 17.700 | | |
| | | Total | 29 | 18.300 | | |
| IgM | 3 | Trmt | 2 | 1.267 | 0.98 | 0.3873 |
| | | Error | 27 | 17.400 | | |
| | | Total | 29 | 18.667 | | |
| | 5 | Trmt | 2 | 0.600 | 0.30 | 0.7424 |
| | | Error | 27 | 26.900 | | |
| | | Total | 29 | 27.500 | | |
| | 7 | Trmt | 2 | 5.067 | 0.81 | 0.4552 |
| | | Error | 27 | 84.400 | | |
| | | Total | 29 | 89.467 | | |
| | 9 | Trmt | 2 | 0.800 | 0.35 | 0.7066 |
| | | Error | 27 | 30.700 | | |
| | | Total | 29 | 31.500 | | |

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Appendix C Table 1. continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 0.5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 4.267 | 2.29 | 0.1201 |
| | | Error | 27 | 25.100 | | |
| | | Total | 29 | 29.367 | | |
| | 5 | Trmt | 2 | 8.267 | 1.39 | 0.2668 |
| | | Error | 27 | 80.400 | | |
| | | Total | 29 | 88.667 | | |
| | 7 | Trmt | 2 | 1.400 | 0.38 | 0.6878 |
| | | Error | 27 | 49.800 | | |
| | | Total | 29 | 51.200 | | |
| | 9 | Trmt | 2 | 7.467 | 2.29 | 0.1205 |
| | | Error | 27 | 44.000 | | |
| | | Total | 29 | 51.467 | | |
| IgG | 3 | Trmt | 2 | 0 | . | . |
| | | Error | 27 | 0 | | |
| | | Total | 29 | 0 | | |
| | 5 | Trmt | 2 | 0.200 | 1.08 | 0.3538 |
| | | Error | 27 | 2.500 | | |
| | | Total | 29 | 2.700 | | |
| | 7 | Trmt | 2 | 1.400 | 0.92 | 0.4116 |
| | | Error | 27 | 20.600 | | |
| | | Total | 29 | 22.000 | | |
| | 9 | Trmt | 2 | 0.267 | 0.22 | 0.8075 |
| | | Error | 27 | 16.700 | | |
| | | Total | 29 | 16.967 | | |
| IgM | 3 | Trmt | 2 | 4.267 | 2.29 | 0.1201 |
| | | Error | 27 | 25.100 | | |
| | | Total | 29 | 29.367 | | |
| | 5 | Trmt | 2 | 10.466 | 1.59 | 0.2225 |
| | | Error | 27 | 88.900 | | |
| | | Total | 29 | 99.367 | | |
| | 7 | Trmt | 2 | 4.200 | 1.16 | 0.3295 |
| | | Error | 27 | 49.000 | | |
| | | Total | 29 | 53.200 | | |
| | 9 | Trmt | 2 | 8.267 | 3.29 | 0.0526 |
| | | Error | 44 | 44.300 | | |
| | | Total | 48 | 48.000 | | |

Appendix C Table 2. Analysis of variance for the secondary antibody titers in leghorns fed 0.1% tyrosine and tryptophan supplemental diets at different days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 1.667 | 0.80 | 0.4606 |
| | | Error | 27 | 28.200 | | |
| | | Total | 29 | 29.867 | | |
| | 5 | Trmt | 2 | 4.666 | 0.17 | 0.8440 |
| | | Error | 27 | 36.900 | | |
| | | Total | 29 | 37.367 | | |
| | 7 | Trmt | 2 | 2.400 | 2.19 | 0.1315 |
| | | Error | 27 | 14.800 | | |
| | | Total | 29 | 17.200 | | |
| | 9 | Trmt | 2 | 3.800 | 2.95 | 0.0695 |
| | | Error | 27 | 17.400 | | |
| | | Total | 29 | 21.200 | | |
| IgG | 3 | Trmt | 2 | 0.600 | 0.48 | 0.6244 |
| | | Error | 27 | 16.900 | | |
| | | Total | 29 | 17.500 | | |
| | 5 | Trmt | 2 | 2.467 | 0.60 | 0.5571 |
| | | Error | 27 | 55.700 | | |
| | | Total | 29 | 58.167 | | |
| | 7 | Trmt | 2 | 0.800 | 0.30 | 0.7457 |
| | | Error | 27 | 36.400 | | |
| | | Total | 29 | 37.200 | | |
| | 9 | Trmt | 2 | 8.467 | 5.36 | 0.0111 |
| | | Error | 27 | 21.400 | | |
| | | Total | 29 | 29.867 | | |
| IgM | 3 | Trmt | 2 | 0.267 | 0.10 | 0.9029 |
| | | Error | 27 | 35.100 | | |
| | | Total | 29 | 35.367 | | |
| | 5 | Trmt | 2 | 0.800 | 0.22 | 0.8015 |
| | | Error | 27 | 48.400 | | |
| | | Total | 29 | 49.200 | | |
| | 7 | Trmt | 2 | 0.800 | 0.28 | 0.7613 |
| | | Error | 27 | 39.200 | | |
| | | Total | 29 | 40.000 | | |
| | 9 | Trmt | 2 | 14.066 | 6.21 | 0.0061 |
| | | Error | 27 | 30.600 | | |
| | | Total | 29 | 44.667 | | |

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Appenxix C Table 2 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 0.5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 0.467 | 0.16 | 0.8517 |
| | | Error | 27 | 39.000 | | |
| | | Total | 29 | 39.467 | | |
| | 5 | Trmt | 2 | 0.200 | 0.05 | 0.9485 |
| | | Error | 27 | 51.000 | | |
| | | Total | 29 | 51.200 | | |
| | 7 | Trmt | 2 | 0.267 | 0.12 | 0.8911 |
| | | Error | 27 | 31.100 | | |
| | | Total | 29 | 31.367 | | |
| | 9 | Trmt | 2 | 0.473 | 0.12 | 0.8834 |
| | | Error | 26 | 49.389 | | |
| | | Total | 28 | 49.862 | | |
| IgG | 3 | Trmt | 2 | 3.467 | 3.35 | 0.0429 |
| | | Error | 27 | 13.200 | | |
| | | Total | 29 | 16.667 | | |
| | 5 | Trmt | 2 | 3.467 | 1.38 | 0.2686 |
| | | Error | 27 | 33.900 | | |
| | | Total | 29 | 37.367 | | |
| | 7 | Trmt | 2 | 3.467 | 1.40 | 0.2646 |
| | | Error | 27 | 33.500 | | |
| | | Total | 29 | 36.967 | | |
| | 9 | Trmt | 2 | 8.267 | 3.58 | 0.0419 |
| | | Error | 27 | 31.200 | | |
| | | Total | 29 | 39.467 | | |
| IgM | 3 | Trmt | 2 | 4.067 | 1.21 | 0.3141 |
| | | Error | 27 | 45.400 | | |
| | | Total | 29 | 49.467 | | |
| | 5 | Trmt | 2 | 2.467 | 0.52 | 0.5988 |
| | | Error | 27 | 63.700 | | |
| | | Total | 29 | 66.167 | | |
| | 7 | Trmt | 2 | 5.600 | 1.27 | 0.2975 |
| | | Error | 27 | 59.600 | | |
| | | Total | 29 | 65.200 | | |
| | 9 | Trmt | 2 | 13.186 | 3.17 | 0.0586 |
| | | Error | 26 | 54.056 | | |
| | | Total | 28 | 67.241 | | |

Appendix C Table 3. Analysis of variance for primary antibody titers in broilers fed 0.1% tyrosine or tryptophan supplemental diets at days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 4.267 | 1.22 | 0.3102 |
| | | Error | 27 | 47.100 | | |
| | | Total | 29 | 51.367 | | |
| | 5 | Trmt | 2 | 2.067 | 0.74 | 0.4874 |
| | | Error | 27 | 37.800 | | |
| | | Total | 29 | 39.867 | | |
| | 7 | Trmt | 2 | 1.267 | 0.71 | 0.5022 |
| | | Error | 27 | 24.200 | | |
| | | Total | 29 | 25.467 | | |
| | 9 | Trmt | 2 | 1.267 | 0.72 | 0.4951 |
| | | Error | 27 | 23.700 | | |
| | | Total | 29 | 24.967 | | |
| | 11 | Trmt | 2 | 0.800 | 0.51 | 0.6065 |
| | | Error | 27 | 21.200 | | |
| | | Total | 2 | 22.000 | | |
| IgG | 3 | Trmt | 2 | 0 | . | . |
| | | Error | 27 | 0 | | |
| | | Total | 29 | 0 | | |
| | 5 | Trmt | 2 | 0 | . | . |
| | | Error | 27 | 0 | | |
| | | Total | 29 | 0 | | |
| | 7 | Trmt | 2 | 1.400 | 0.52 | 0.5983 |
| | | Error | 27 | 36.100 | | |
| | | Total | 29 | 37.500 | | |
| | 9 | Trmt | 2 | 0.600 | 0.24 | 0.7907 |
| | | Error | 27 | 34.200 | | |
| | | Total | 29 | 34.800 | | |
| | 11 | Trmt | 2 | 0.867 | 0.81 | 0.4567 |
| | | Error | 27 | 14.500 | | |
| | | Total | 29 | 15.367 | | |
| IgM | 3 | Trmt | 2 | 4.267 | 1.22 | 0.3102 |
| | | Error | 27 | 47.100 | | |
| | | Total | 29 | 51.367 | | |
| | 5 | Trmt | 2 | 2.067 | 0.74 | 0.4874 |
| | | Error | 27 | 37.800 | | |
| | | Total | 29 | 39.867 | | |
| | 7 | Trmt | 2 | 2.467 | 1.17 | 0.3261 |
| | | Error | 27 | 28.500 | | |
| | | Total | 29 | 30.967 | | |
| | 9 | Trmt | 2 | 0.467 | 0.16 | 0.8513 |
| | | Error | 27 | 38.900 | | |
| | | Total | 29 | 39.367 | | |
| | 11 | Trmt | 2 | 3.267 | 1.57 | 0.2266 |
| | | Error | 27 | 28.100 | | |
| | | Total | 29 | 31.367 | | |

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Appendix C Table 3 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 0.5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 4.467 | 1.70 | 0.2012 |
| | | Error | 27 | 3.700 | | |
| | | Total | 29 | 4.167 | | |
| | 5 | Trmt | 2 | 0.600 | 0.18 | 0.8349 |
| | | Error | 27 | 44.600 | | |
| | | Total | 29 | 45.200 | | |
| | 6 | Trmt | 2 | 2.726 | 0.76 | 0.4785 |
| | | Error | 26 | 46.722 | | |
| | | Total | 28 | 49.448 | | |
| | 7 | Trmt | 2 | 9.030 | 2.19 | 0.1318 |
| | | Error | 26 | 53.522 | | |
| | | Total | 28 | 62.552 | | |
| | 9 | Trmt | 2 | 5.130 | 1.35 | 0.2770 |
| | | Error | 26 | 49.422 | | |
| | | Total | 28 | 54.552 | | |
| IgG | 3 | Trmt | 2 | 0 | 0 | 1.0000 |
| | | Error | 27 | 19.200 | | |
| | | Total | 29 | 19.200 | | |
| | 5 | Trmt | 2 | 1.067 | 1.13 | 0.3394 |
| | | Error | 27 | 12.800 | | |
| | | Total | 29 | 13.867 | | |
| | 6 | Trmt | 2 | 0.341 | 0.18 | 0.8378 |
| | | Error | 27 | 24.900 | | |
| | | Total | 29 | 25.241 | | |
| | 7 | Trmt | 2 | 0.718 | 0.25 | 0.7814 |
| | | Error | 26 | 37.489 | | |
| | | Total | 28 | 38.207 | | |
| | 9 | Trmt | 2 | 0.293 | 0.53 | 0.5938 |
| | | Error | 26 | 7.156 | | |
| | | Total | 28 | 7.448 | | |
| IgM | 3 | Trmt | 2 | 0.467 | 0.33 | 0.7194 |
| | | Error | 27 | 18.900 | | |
| | | Total | 29 | 19.367 | | |
| | 5 | Trmt | 2 | 2.466 | 0.56 | 0.5753 |
| | | Error | 27 | 59.000 | | |
| | | Total | 29 | 61.467 | | |
| | 6 | Trmt | 2 | 1.139 | 0.31 | 0.7358 |
| | | Error | 27 | 47.689 | | |
| | | Total | 29 | 48.828 | | |
| | 7 | Trmt | 2 | 11.252 | 2.74 | 0.0829 |
| | | Error | 26 | 53.300 | | |
| | | Total | 28 | 64.552 | | |
| | 9 | Trmt | 2 | 7.203 | 2.25 | 0.1252 |
| | | Error | 26 | 41.556 | | |
| | | Total | 28 | 48.758 | | |

Appendix C Table 4. Analysis of variance for the secondary antibody titers in broilers fed 0.1% tyrosine and tryptophan supplemental diets at days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | 5% SRBC Injection | | | |
| Total | 3 | Trmt | 2 | 8.467 | 0.92 | 0.4125 |
| | | Error | 27 | 124.900 | | |
| | | Total | 29 | 133.367 | | |
| | 5 | Trmt | 2 | 1.400 | 0.19 | 0.8279 |
| | | Error | 27 | 99.400 | | |
| | | Total | 29 | 100.800 | | |
| | 7 | Trmt | 2 | 2.600 | 0.47 | 0.6282 |
| | | Error | 27 | 74.200 | | |
| | | Total | 29 | 76.800 | | |
| | 9 | Trmt | 2 | 6.467 | 0.82 | 0.4529 |
| | | Error | 27 | 107.000 | | |
| | | Total | 29 | 113.467 | | |
| IgG | 3 | Trmt | 2 | 0.200 | 0.05 | 0.9473 |
| | | Error | 27 | 49.800 | | |
| | | Total | 29 | 50.000 | | |
| | 5 | Trmt | 2 | 0.067 | 0.01 | 0.9867 |
| | | Error | 27 | 67.400 | | |
| | | Total | 29 | 67.467 | | |
| | 7 | Trmt | 2 | 6.179 | 2.00 | 0.1568 |
| | | Error | 27 | 38.678 | | |
| | | Total | 29 | 44.857 | | |
| | 9 | Trmt | 2 | 0.467 | 0.11 | 0.8978 |
| | | Error | 27 | 58.200 | | |
| | | Total | 29 | 58.667 | | |
| IgM | 3 | Trmt | 2 | 10.067 | 1.67 | 0.2068 |
| | | Error | 27 | 81.300 | | |
| | | Total | 29 | 91.367 | | |
| | 5 | Trmt | 2 | 6.067 | 4.85 | 0.0159 |
| | | Error | 27 | 16.900 | | |
| | | Total | 29 | 22.967 | | |
| | 7 | Trmt | 2 | 1.003 | 0.34 | 0.7139 |
| | | Error | 27 | 36.711 | | |
| | | Total | 29 | 37.714 | | |
| | 9 | Trmt | 2 | 10.067 | 5.35 | 0.0110 |
| | | Error | 27 | 25.400 | | |
| | | Total | 29 | 35.467 | | |

Appendix C Table 5. Analysis of variance for fold differences of wattle thickness in response to PHA injection in Leghorns and broilers fed 0.1% tyrosine or tryptophan supplemental diets

| Parameter | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|
| | | Leghorns | | | |
| Fold differences | Trmt | 2 | 3.838 | 2.18 | 0.1223 |
| | Error | 27 | 50.163 | | |
| | Total | 29 | 54.001 | | |
| | | Broilers | | | |
| | Trmt | 2 | 0.141 | 0.21 | 0.8082 |
| | Error | 50 | 16.448 | | |
| | Total | 52 | 16.588 | | |

Appendix C Table 6. Analysis of variance for relative body weight changes in Leghorns fed 0.1% tyrosine and tryptophan supplemental diets at days after *E. coli* challenge

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Weight change | 1 | Trmt | 2 | 0.001 | 0.24 | 0.7874 |
| | | Error | 33 | 0.074 | | |
| | | Total | 35 | 0.075 | | |
| | 2 | Trmt | 2 | 0.002 | 0.90 | 0.4173 |
| | | Error | 33 | 0.036 | | |
| | | Total | 35 | 0.038 | | |
| | 3 | Trmt | 2 | 0.005 | 2.50 | 0.0977 |
| | | Error | 33 | 0.032 | | |
| | | Total | 35 | 0.036 | | |
| | 4 | Trmt | 2 | 0.002 | 0.81 | 0.4527 |
| | | Error | 33 | 0.049 | | |
| | | Total | 35 | 0.051 | | |

Appendix C Table 7. Analysis of variance for transformed scores of lesion of *E. coli* challenge in Leghorns fed 0.1% tyrosine or tryptophan supplemental diets

| Parameter | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|
| Total | Trmt | 2 | 0.097 | 0.11 | 0.8955 |
| | Error | 24 | 10.533 | | |
| | Total | 26 | 10.630 | | |

Appendix C Table 8. Analysis of variance for primary and secondary antibody titers in Leghorns fed 0.5% or 1% tyrosine and tryptophan supplemental diets at days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 4 | 4.170 | 1.36 | 0.2641 |
| | | Error | 44 | 33.789 | | |
| | | Total | 48 | 37.960 | | |
| | 5 | Trmt | 4 | 3.285 | 0.94 | 0.4492 |
| | | Error | 44 | 38.389 | | |
| | | Total | 48 | 41.673 | | |
| | 7 | Trmt | 4 | 6.508 | 1.28 | 0.2921 |
| | | Error | 44 | 55.900 | | |
| | | Total | 48 | 62.408 | | |
| | 9 | Trmt | 4 | 3.875 | 0.95 | 0.4419 |
| | | Error | 44 | 44.656 | | |
| | | Total | 48 | 48.531 | | |
| | 11 | Trmt | 4 | 3.042 | 0.68 | 0.6122 |
| | | Error | 44 | 49.489 | | |
| | | Total | 48 | 52.530 | | |
| IgG | 3 | Trmt | 4 | 0 | . | . |
| | | Error | 44 | 0 | | |
| | | Total | 48 | 0 | | |
| | 5 | Trmt | 4 | 1.739 | 0.99 | 0.4230 |
| | | Error | 44 | 19.322 | | |
| | | Total | 48 | 21.061 | | |
| | 7 | Trmt | 4 | 2.076 | 1.33 | 0.2743 |
| | | Error | 44 | 17.189 | | |
| | | Total | 48 | 19.265 | | |
| | 9 | Trmt | 4 | 2.813 | 1.39 | 0.2536 |
| | | Error | 44 | 22.289 | | |
| | | Total | 48 | 25.102 | | |
| | 11 | Trmt | 4 | 5.276 | 2.28 | 0.0762 |
| | | Error | 44 | 25.500 | | |
| | | Total | 48 | 30.776 | | |
| IgM | 3 | Trmt | 4 | 4.170 | 1.36 | 0.2641 |
| | | Error | 44 | 33.78 | | |
| | | Total | 48 | 37.959 | | |
| | 5 | Trmt | 4 | 7.672 | 2.44 | 0.0634 |
| | | Error | 44 | 35.022 | | |
| | | Total | 48 | 42.061 | | |
| | 7 | Trmt | 4 | 12.244 | 1.62 | 0.1873 |
| | | Error | 44 | 69.756 | | |
| | | Total | 48 | 80.000 | | |

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Appendix C Table 8 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | 9 | Trmt | 4 | 4.965 | 1.13 | 0.3544 |
| | | Error | 44 | 48.300 | | |
| | | Total | 48 | 53.265 | | |
| | 11 | Trmt | 4 | 8.800 | 2.91 | 0.0323 |
| | | Error | 44 | 33.322 | | |
| | | Total | 48 | 42.122 | | |
| | | Secondary antibody response | | | | |
| Total | 2 | Trmt | 4 | 0.432 | 0.21 | 0.9331 |
| | | Error | 44 | 22.956 | | |
| | | Total | 48 | 23.388 | | |
| | 4 | Trmt | 4 | 7.759 | 1.47 | 0.2286 |
| | | Error | 44 | 58.200 | | |
| | | Total | 48 | 65.959 | | |
| | 6 | Trmt | 6 | 4.794 | 1.20 | 0.3238 |
| | | Error | 44 | 43.900 | | |
| | | Total | 48 | 48.694 | | |
| | 8 | Trmt | 4 | 6.353 | 1.45 | 0.2324 |
| | | Error | 44 | 48.056 | | |
| | | Total | 48 | 54.408 | | |
| IgG | 2 | Trmt | 4 | 1.078 | 1.33 | 0.2743 |
| | | Error | 44 | 8.922 | | |
| | | Total | 48 | 10.000 | | |
| | 4 | Trmt | 4 | 2.467 | 1.54 | 0.2081 |
| | | Error | 44 | 17.656 | | |
| | | Total | 48 | 20.122 | | |
| | 6 | Trmt | 4 | 1.531 | 1.12 | 0.3582 |
| | | Error | 44 | 15.000 | | |
| | | Total | 48 | 16.531 | | |
| | 8 | Trmt | 4 | 1.662 | 1.32 | 0.2788 |
| | | Error | 44 | 13.889 | | |
| | | Total | 48 | 15.551 | | |
| IgM | 2 | Trmt | 4 | 1.046 | 0.41 | 0.8002 |
| | | Error | 44 | 28.056 | | |
| | | Total | 48 | 29.102 | | |
| | 4 | Trmt | 4 | 6.246 | 1.30 | 0.2848 |
| | | Error | 44 | 52.856 | | |
| | | Total | 48 | 59.102 | | |
| | 6 | Trmt | 4 | 3.533 | 0.88 | 0.4830 |
| | | Error | 44 | 44.100 | | |
| | | Total | 48 | 47.633 | | |
| | 8 | Trmt | 4 | 3.700 | 0.92 | 0.4616 |
| | | Error | 44 | 44.300 | | |
| | | Total | 48 | 48.000 | | |

Appendix C Table 9. Analysis of variance for primary and secondary antibody titers in broilers fed 0.5% or 1% tyrosine and tryptophan supplemental diets at days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 4 | 1.000 | 0.59 | 0.6701 |
| | | Error | 45 | 19.000 | | |
| | | Total | 49 | 20.000 | | |
| | 5 | Trmt | 4 | 4.666 | 0.62 | 0.6502 |
| | | Error | 45 | 83.400 | | |
| | | Total | 49 | 88.000 | | |
| | 7 | Trmt | 4 | 11.320 | 1.73 | 0.1594 |
| | | Error | 45 | 73.500 | | |
| | | Total | 49 | 84.820 | | |
| | 9 | Trmt | 4 | 11.000 | 1.90 | 0.1263 |
| | | Error | 45 | 65.000 | | |
| | | Total | 49 | 76.000 | | |
| IgG | 3 | Trmt | 4 | 0.120 | 0.75 | 0.5632 |
| | | Error | 45 | 1.800 | | |
| | | Total | 49 | 1.920 | | |
| | 5 | Trmt | 4 | 0.080 | | 0.4175 |
| | | Error | 45 | 0.900 | | |
| | | Total | 49 | 0.980 | | |
| | 7 | Trmt | 4 | 2.520 | 2.25 | 0.0785 |
| | | Error | 45 | 12.600 | | |
| | | Total | 49 | 15.120 | | |
| | 9 | Trmt | 4 | 1.520 | 1.41 | 0.2450 |
| | | Error | 45 | 12.100 | | |
| | | Total | 49 | 13.620 | | |
| IgM | 3 | Trmt | 4 | 0.720 | 0.55 | 0.7019 |
| | | Error | 45 | 14.800 | | |
| | | Total | 49 | 15.520 | | |
| | 5 | Trmt | 4 | 5.680 | 0.82 | 0.5178 |
| | | Error | 45 | 77.700 | | |
| | | Total | 49 | 83.380 | | |
| | 7 | Trmt | 4 | 11.480 | 1.80 | 0.1462 |
| | | Error | 45 | 71.900 | | |
| | | Total | 49 | 83.380 | | |
| | 9 | Trmt | 4 | 10.120 | 2.32 | 0.0715 |
| | | Error | 45 | 49.100 | | |
| | | Total | 49 | 59.220 | | |

(Continued on next page)

Appendix C Table 9 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Secondary antibody response | | | |
| Total | 2 | Trmt | 4 | 7.971 | 4.26 | 0.0053 |
| | | Error | 44 | 20.560 | | |
| | | Total | 48 | 28.531 | | |
| | 4 | Trmt | 4 | 12.587 | 1.90 | 0.1269 |
| | | Error | 44 | 72.760 | | |
| | | Total | 48 | 85.347 | | |
| | 6 | Trmt | 6 | 11.055 | 3.52 | 0.0142 |
| | | Error | 44 | 34.578 | | |
| | | Total | 48 | 45.633 | | |
| | 8 | Trmt | 4 | 5.496 | 1.29 | 0.2884 |
| | | Error | 43 | 40.909 | | |
| | | Total | 47 | 49.979 | | |
| IgG | 2 | Trmt | 4 | 0.080 | 0.97 | 0.4321 |
| | | Error | 44 | 0.900 | | |
| | | Total | 48 | 0.980 | | |
| | 4 | Trmt | 4 | 3.560 | 1.08 | 0.3794 |
| | | Error | 44 | 36.359 | | |
| | | Total | 48 | 39.918 | | |
| | 6 | Trmt | 4 | 3.248 | 0.97 | 0.4354 |
| | | Error | 44 | 36.957 | | |
| | | Total | 48 | 40.204 | | |
| | 8 | Trmt | 4 | 9.070 | 2.38 | 0.0661 |
| | | Error | 43 | 40.909 | | |
| | | Total | 47 | 49.979 | | |
| IgM | 2 | Trmt | 4 | 7.100 | 3.14 | 0.0234 |
| | | Error | 44 | 24.860 | | |
| | | Total | 48 | 31.959 | | |
| | 4 | Trmt | 4 | 6.062 | 1.28 | 0.2909 |
| | | Error | 44 | 51.938 | | |
| | | Total | 48 | 58.000 | | |
| | 6 | Trmt | 4 | 4.628 | 1.64 | 0.1813 |
| | | Error | 44 | 31.045 | | |
| | | Total | 48 | 35.673 | | |
| | 8 | Trmt | 4 | 2.665 | 0.60 | 0.6652 |
| | | Error | 43 | 47.814 | | |
| | | Total | 47 | 50.479 | | |

Appendix C Table 10. Analysis of variance for primary and secondary antibody titers in broilers fed 0.5% or 1% tyrosine and trytpophan supplemental diets at days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 5 | 9.483 | 18.62 | 0.0001 |
| | | Error | 54 | 5.500 | | |
| | | Total | 59 | 14.983 | | |
| | 5 | Trmt | 5 | 8.933 | 2.11 | 0.0786 |
| | | Error | 54 | 45.800 | | |
| | | Total | 59 | 54.733 | | |
| | 7 | Trmt | 5 | 12.333 | 1.63 | 0.1672 |
| | | Error | 54 | 81.600 | | |
| | | Total | 59 | 93.933 | | |
| | 9 | Trmt | 5 | 13.950 | 1.48 | 0.2112 |
| | | Error | 54 | 101.700 | | |
| | | Total | 59 | 115.650 | | |
| IgG | 3 | Trmt | 5 | 0 | . | . |
| | | Error | 54 | 0 | | |
| | | Total | 59 | 0 | | |
| | 5 | Trmt | 5 | 0.133 | 0.80 | 0.5546 |
| | | Error | 54 | 1.800 | | |
| | | Total | 59 | 1.933 | | |
| | 7 | Trmt | 5 | 3.083 | 1.58 | 0.1819 |
| | | Error | 54 | 21.100 | | |
| | | Total | 59 | 24.183 | | |
| | 9 | Trmt | 5 | 2.200 | 1.29 | 0.2813 |
| | | Error | 54 | 18.400 | | |
| | | Total | 59 | 20.600 | | |
| IgM | 3 | Trmt | 5 | 9.483 | 18.62 | 0.0001 |
| | | Error | 54 | 5.500 | | |
| | | Total | 59 | 14.983 | | |
| | 5 | Trmt | 5 | 8.600 | 2.12 | 0.0769 |
| | | Error | 54 | 43.800 | | |
| | | Total | 59 | 52.400 | | |
| | 7 | Trmt | 5 | 14.283 | 2.33 | 0.0551 |
| | | Error | 54 | 66.300 | | |
| | | Total | 59 | 80.583 | | |
| | 9 | Trmt | 5 | 18.150 | 2.29 | 0.0582 |
| | | Error | 54 | 85.500 | | |
| | | Total | 59 | 103.650 | | |

(continued on next page)

Appendix C Table 10

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Secondary antibody response | | | |
| Total | 2 | Trmt | 5 | 1.314 | 0.88 | 0.5038 |
| | | Error | 52 | 15.600 | | |
| | | Total | 57 | 16.914 | | |
| | 4 | Trmt | 5 | 10.505 | 1.24 | 0.3032 |
| | | Error | 52 | 87.978 | | |
| | | Total | 57 | 98.483 | | |
| | 6 | Trmt | 5 | 5.384 | 0.71 | 0.6202 |
| | | Error | 50 | 76.044 | | |
| | | Total | 55 | 81.429 | | |
| | 8 | Trmt | 5 | 9.885 | 1.96 | 0.1002 |
| | | Error | 51 | 51.378 | | |
| | | Total | 56 | 61.263 | | |
| IgG | 2 | Trmt | 5 | 0 | . | . |
| | | Error | 52 | 0 | | |
| | | Total | 57 | 0 | | |
| | 4 | Trmt | 5 | 2.468 | 0.64 | 0.6728 |
| | | Error | 51 | 39.567 | | |
| | | Total | 56 | 42.035 | | |
| | 6 | Trmt | 5 | 7.898 | 2.10 | 0.0812 |
| | | Error | 50 | 37.656 | | |
| | | Total | 55 | 45.554 | | |
| | 8 | Trmt | 5 | 3.033 | 0.62 | 0.6846 |
| | | Error | 51 | 49.844 | | |
| | | Total | 56 | 52.877 | | |
| IgM | 2 | Trmt | 5 | 1.314 | 0.88 | 0.5038 |
| | | Error | 52 | 15.600 | | |
| | | Total | 57 | 16.914 | | |
| | 4 | Trmt | 5 | 7.686 | 1.07 | 0.3870 |
| | | Error | 51 | 73.156 | | |
| | | Total | 56 | 80.842 | | |
| | 6 | Trmt | 5 | 3.536 | 0.52 | 0.7576 |
| | | Error | 50 | 67.589 | | |
| | | Total | 55 | 71.125 | | |
| | 8 | Trmt | 5 | 2.368 | 0.35 | 0.8820 |
| | | Error | 51 | 69.667 | | |
| | | Total | 56 | 72.035 | | |

Appendix C Table 11. Analysis of variance for 5-HIAA, 5-HT levels and their retios in the brain stem of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | 2 | Trmt | 4 | 890671.1 | 8.60 | 0.0003 |
| | | Error | 20 | 517900.4 | | |
| | | Total | 24 | 1408571.6 | | |
| | 4 | Trmt | 4 | 496964.6 | 1.26 | 0.3182 |
| | | Error | 20 | 1971192 | | |
| | | Total | 24 | 2468156.8 | | |
| | 6 | Trmt | 4 | 704718.1 | 4.42 | 0.0108 |
| | | Error | 19 | 757583.1 | | |
| | | Total | 23 | 1462301.2 | | |
| | 8 | Trmt | 4 | 185912.5 | 1.96 | 0.1397 |
| | | Error | 20 | 474253.8 | | |
| | | Total | 24 | 660166.4 | | |
| | 10 | Trmt | 4 | 151537.7 | 1.01 | 0.4258 |
| | | Error | 19 | 711002.4 | | |
| | | Total | 23 | 862540.1 | | |
| 5-HIAA | 2 | Trmt | 4 | 244694.4 | 21.13 | 0.0001 |
| | | Error | 20 | 57888.4 | | |
| | | Total | 24 | 302582.8 | | |
| | 4 | Trmt | 4 | 282506.6 | 6.60 | 0.0015 |
| | | Error | 20 | 204121.9 | | |
| | | Total | 24 | 496528.5 | | |
| | 6 | Trmt | 4 | 138163.0 | 14.48 | 0.0001 |
| | | Error | 19 | 45311.5 | | |
| | | Total | 23 | 183474.5 | | |
| | 8 | Trmt | 4 | 13061.8 | 0.70 | 0.5980 |
| | | Error | 20 | 92676.8 | | |
| | | Total | 24 | 105738.6 | | |
| | 10 | Trmt | 4 | 11647.4 | 0.71 | 0.5966 |
| | | Error | 19 | 78187.3 | | |
| | | Total | 23 | 89834.7 | | |
| 5-HIAA/5-HT | 2 | Trmt | 4 | 0.031 | 1.21 | 0.3381 |
| | | Error | 20 | 0.128 | | |
| | | Total | 24 | 0.159 | | |
| | 4 | Trmt | 4 | 0.074 | 7.87 | 0.0006 |
| | | Error | 20 | 0.047 | | |
| | | Total | 24 | 0.121 | | |
| | 6 | Trmt | 4 | 0.029 | 3.77 | 0.0200 |
| | | Error | 19 | 0.036 | | |
| | | Total | 23 | 0.064 | | |
| | 8 | Trmt | 4 | 0.064 | 1.12 | 0.3762 |
| | | Error | 20 | 0.287 | | |
| | | Total | 24 | 0.351 | | |
| | 10 | Trmt | 4 | 0.016 | 0.77 | 0.5602 |
| | | Error | 19 | 0.010 | | |
| | | Total | 23 | 0.116 | | |

Appendix C Table 11. Analysis of variance for 5-HIAA, 5-HT levels and their retios in the brain stem of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | Control | Trmt | 4 | 115398.1 | 0.93 | 0.4660 |
| | | Error | 20 | 619851.1 | | |
| | | Total | 24 | 735249.2 | | |
| | 0.5% Tyr | Trmt | 4 | 587838.0 | 2.00 | 0.1361 |
| | | Error | 19 | 1398984.6 | | |
| | | Total | 23 | 1986822.6 | | |
| | 1% Tyr | Trmt | 4 | 260198.6 | 1.85 | 0.1581 |
| | | Error | 20 | 701685.1 | | |
| | | Total | 24 | 961883.6 | | |
| | 0.5% Trp | Trmt | 4 | 522112.3 | 2.86 | 0.0521 |
| | | Error | 19 | 867870.4 | | |
| | | Total | 23 | 1389982.7 | | |
| | 1% Trp | Trmt | 4 | 631950.9 | 3.75 | 0.0197 |
| | | Error | 20 | 843526.6 | | |
| | | Total | 24 | 1475477.5 | | |
| 5-HIAA | Control | Trmt | 4 | 14207.1 | 1.17 | 0.3549 |
| | | Error | 20 | 60856.8 | | |
| | | Total | 24 | 75063.9 | | |
| | 0.5% Tyr | Trmt | 4 | 38217.5 | 1.95 | 0.1433 |
| | | Error | 19 | 93070.1 | | |
| | | Total | 23 | 131287.6 | | |
| | 1% Tyr | Trmt | 4 | 41860.2 | 4.80 | 0.0071 |
| | | Error | 20 | 43618.1 | | |
| | | Total | 24 | 85478.3 | | |
| | 0.5% Trp | Trmt | 4 | 90243.8 | 2.84 | 0.0532 |
| | | Error | 19 | 151035.8 | | |
| | | Total | 23 | 241279.6 | | |
| | 1% Trp | Trmt | 4 | 289108.3 | 10.36 | 0.0001 |
| | | Error | 20 | 139493.9 | | |
| | | Total | 24 | 428602.1 | | |
| 5-HIAA/5-HT | Control | Trmt | 4 | 0.0294 | 1.71 | 0.1873 |
| | | Error | 20 | 0.0861 | | |
| | | Total | 24 | 0.1155 | | |
| | 0.5% Tyr | Trmt | 4 | 0.0764 | 1.43 | 0.2612 |
| | | Error | 19 | 0.2531 | | |
| | | Total | 23 | 0.3296 | | |
| | 1% Tyr | Trmt | 4 | 0.0441 | 4.01 | 0.0151 |
| | | Error | 20 | 0.0550 | | |
| | | Total | 24 | 0.0991 | | |
| | 0.5% Trp | Trmt | 4 | 0.0318 | 0.98 | 0.4420 |
| | | Error | 19 | 0.1543 | | |
| | | Total | 23 | 0.1861 | | |
| | 1% Trp | Trmt | 4 | 0.0545 | 5.56 | 0.0036 |
| | | Error | 20 | 0.0491 | | |
| | | Total | 24 | 0.1036 | | |

Appendix C Table 12. Analysis of variance for 5-HIAA, 5-HT levels and their ratios in the diencephalon of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan at selected days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | 2 | Trmt | 4 | 278537.1 | 0.83 | 0.5206 |
| | | Error | 18 | 1501364.4 | | |
| | | Total | 22 | 1779899.5 | | |
| | 4 | Trmt | 4 | 374988.0 | 4.08 | 0.0140 |
| | | Error | 20 | 459117.2 | | |
| | | Total | 24 | 834105.2 | | |
| | 6 | Trmt | 4 | 722594.6 | 8.32 | 0.0005 |
| | | Error | 19 | 412364.2 | | |
| | | Total | 23 | 1134958.8 | | |
| | 8 | Trmt | 4 | 44635.7 | 0.44 | 0.7798 |
| | | Error | 20 | 509863.0 | | |
| | | Total | 24 | 554498.6 | | |
| | 10 | Trmt | 4 | 20567.5 | 0.20 | 0.9341 |
| | | Error | 19 | 4483388.6 | | |
| | | Total | 23 | 503956.0 | | |
| 5-HIAA | 2 | Trmt | 4 | 178497.3 | 3.82 | 0.0193 |
| | | Error | 19 | 222240.5 | | |
| | | Total | 23 | 400737.8 | | |
| | 4 | Trmt | 4 | 99403.8 | 4.54 | 0.0090 |
| | | Error | 20 | 109359.5 | | |
| | | Total | 24 | 208763.3 | | |
| | 6 | Trmt | 4 | 122830.8 | 2.97 | 0.0460 |
| | | Error | 19 | 196243.0 | | |
| | | Total | 23 | 319073.8 | | |
| | 8 | Trmt | 4 | 12103.0 | 1.42 | 0.2626 |
| | | Error | 20 | 42513.1 | | |
| | | Total | 24 | 54616.2 | | |
| | 10 | Trmt | 4 | 11894.7 | 0.94 | 0.4619 |
| | | Error | 19 | 60067.8 | | |
| | | Total | 23 | 71962.1 | | |
| 5-HIAAA/5-HT | 2 | Trmt | 4 | 0.0919 | 4.98 | 0.0070 |
| | | Error | 18 | 0.0831 | | |
| | | Total | 22 | 0.1750 | | |
| | 4 | Trmt | 4 | 0.0521 | 6.20 | 0.0021 |
| | | Error | 20 | 0.0420 | | |
| | | Total | 24 | 0.0941.7 | | |
| | 6 | Trmt | 4 | 0.0181 | 1.14 | 0.3695 |
| | | Error | 19 | 0.0757 | | |
| | | Total | 23 | 0.0938.5 | | |
| | 8 | Trmt | 4 | 0.0202 | 2.44 | 0.0803 |
| | | Error | 20 | 0.04132 | | |
| | | Total | 24 | 0.0614 | | |
| | 10 | Trmt | 4 | 0.0128 | 0.62 | 0.6537 |
| | | Error | 19 | 0.0977 | | |
| | | Total | 23 | 0.1104 | | |

Appendix C Table 12. Analysis of variance for 5-HIAA, 5-HT levels and their ratios in the diencephalon of male Leghorns between days within each dietay treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | Control | Trmt | 4 | 465229.2 | 2.61 | 0.0684 |
| | | Error | 19 | 847564.2 | | |
| | | Total | 23 | 1312793.4 | | |
| | 0.5% Tyr | Trmt | 4 | 453089.2 | 3.08 | 0.0398 |
| | | Error | 20 | 736320.9 | | |
| | | Total | 24 | 1189410.0 | | |
| | 1% Tyr | Trmt | 4 | 495136.5 | 3.82 | 0.0216 |
| | | Error | 17 | 551332.1 | | |
| | | Total | 21 | 1046468.7 | | |
| | 0.5% Trp | Trmt | 4 | 1360496.1 | 8.27 | 0.0005 |
| | | Error | 19 | 781061.4 | | |
| | | Total | 23 | 2141557.5 | | |
| | 1% Trp | Trmt | 4 | 1381726.3 | 15.52 | 0.0001 |
| | | Error | 19 | 422894.0 | | |
| | | Total | 23 | 1804620.3 | | |
| 5-HIAA | Control | Trmt | 4 | 15507.4 | 0.67 | 0.6224 |
| | | Error | 19 | 110355.5 | | |
| | | Total | 23 | 125862.9 | | |
| | 0.5% Tyr | Trmt | 4 | 28511.1 | 1.60 | 0.2136 |
| | | Error | 20 | 89171.5 | | |
| | | Total | 24 | 117682.6 | | |
| | 1% Tyr | Trmt | 4 | 42063.5 | 2.82 | 0.0578 |
| | | Error | 17 | 63291.4 | | |
| | | Total | 21 | 105354.9 | | |
| | 0.5% Trp | Trmt | 4 | 108653.9 | 3.89 | 0.0171 |
| | | Error | 20 | 139735.9 | | |
| | | Total | 24 | 248389.8 | | |
| | 1% Trp | Trmt | 4 | 1381726.3 | 15.52 | 0.0001 |
| | | Error | 19 | 422894.0 | | |
| | | Total | 23 | 1804620 | | |
| 5-HIAAA/5-HT | Control | Trmt | 4 | 0.0188 | 0.97 | 0.4459 |
| | | Error | 19 | 0.0918 | | |
| | | Total | 23 | 0.1105 | | |
| | 0.5% Tyr | Trmt | 4 | 0.0863 | 6.63 | 0.0014 |
| | | Error | 20 | 0.0651 | | |
| | | Total | 24 | 0.1515 | | |
| | 1% Tyr | Trmt | 4 | 0.0781 | 6.91 | 0.0017 |
| | | Error | 17 | 0.0481 | | |
| | | Total | 21 | 0.1262.5 | | |
| | 0.5% Trp | Trmt | 4 | 0.0357 | 3.20 | 0.0363 |
| | | Error | 19 | 0.0531 | | |
| | | Total | 23 | 0.0888 | | |
| | 1% Trp | Trmt | 4 | 0.0373 | 1.99 | 0.1363 |
| | | Error | 19 | 0.0888 | | |
| | | Total | 23 | 0.1261 | | |

Appendix C Table 13. Analysis of variance for 5-HIAA, 5-HT levels and their ratios in the telencephalon of male Leghorns fed with 0.5% or 1% supplemental tyrosine and trytophan

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | 2 | Trmt | 4 | 236496.5 | 3.20 | 0.0347 |
| | | Error | 20 | 369227.2 | | |
| | | Total | 24 | 605723.7 | | |
| | 4 | Trmt | 4 | 456852.0 | 2.48 | 0.0768 |
| | | Error | 20 | 920670.7 | | |
| | | Total | 24 | 1377522.7 | | |
| | 6 | Trmt | 4 | 60021.1 | 0.91 | 0.4772 |
| | | Error | 20 | 329863.4 | | |
| | | Total | 24 | 389884.6 | | |
| | 8 | Trmt | 4 | 148952.5 | 0.61 | 0.6611 |
| | | Error | 20 | 1223613.2 | | |
| | | Total | 24 | 1372565.7 | | |
| | 10 | Trmt | 4 | 70089.3 | 0.35 | 0.8381 |
| | | Error | 20 | 989646.5 | | |
| | | Total | 24 | 1059735.8 | | |
| 5-HIAA | 2 | Trmt | 4 | 27661.2 | 7.84 | 0.0006 |
| | | Error | 20 | 17649.4 | | |
| | | Total | 24 | 45310.6 | | |
| | 4 | Trmt | 4 | 15012.8 | 1.57 | 0.2202 |
| | | Error | 20 | 47725.4 | | |
| | | Total | 24 | 62738.2 | | |
| | 6 | Trmt | 4 | 7415.5 | 5.89 | 0.0027 |
| | | Error | 20 | 6290.5 | | |
| | | Total | 24 | 13706.0 | | |
| | 8 | Trmt | 4 | 800.9 | 0.82 | 0.5317 |
| | | Error | 17 | 4165.2 | | |
| | | Total | 21 | 4966.0 | | |
| | 10 | Trmt | 4 | 78.8 | 0.11 | 0.9793 |
| | | Error | 20 | 3736.4 | | |
| | | Total | 24 | 3815.2 | | |
| 5-HIAA/5-HT | 2 | Trmt | 4 | 0.008 | 3.33 | 0.0304 |
| | | Error | 20 | 0.012 | | |
| | | Total | 24 | 0.020 | | |
| | 4 | Trmt | 4 | 0.013 | 0.93 | 0.4645 |
| | | Error | 20 | 0.070 | | |
| | | Total | 24 | 0.083 | | |
| | 6 | Trmt | 4 | 0.0062 | 4.43 | 0.0100 |
| | | Error | 20 | 0.070 | | |
| | | Total | 24 | 0.0132 | | |
| | 8 | Trmt | 4 | 0.0014 | 4.25 | 0.0145 |
| | | Error | 17 | 0.014 | | |
| | | Total | 21 | 0.0029 | | |
| | 10 | Trmt | 4 | 0.0013 | 0.16 | 0.9579 |
| | | Error | 20 | 0.0408 | | |
| | | Total | 24 | 0.0420 | | |

Appendix C Table 13. Analysis of variance for 5-HIAA, 5-HT levels and their ratios in the telencephalon of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | Control | Trmt | 4 | 1208259.6 | 6.24 | 0.0018 |
| | | Error | 21 | 1016370.4 | | |
| | | Total | 25 | 2224630.0 | | |
| | 0.5% Tyr | Trmt | 4 | 1214819.5 | 9.65 | 0.0002 |
| | | Error | 20 | 629237.8 | | |
| | | Total | 24 | 1844057.3 | | |
| | 1% Tyr | Trmt | 4 | 733622.3 | 5.00 | 0.0059 |
| | | Error | 20 | 733731.8 | | |
| | | Total | 24 | 1467354.1 | | |
| | 0.5% Trp | Trmt | 4 | 1533589.8 | 12.63 | 0.0001 |
| | | Error | 20 | 606985.8 | | |
| | | Total | 24 | 2140575.6 | | |
| | 1% Trp | Trmt | 4 | 2244030.0 | 7.83 | 0.0006 |
| | | Error | 20 | 1433273.6 | | |
| | | Total | 24 | 3677303.6 | | |
| 5-HIAA | Control | Trmt | 4 | 9056.8 | 2.25 | 0.1001 |
| | | Error | 20 | 20146.8 | | |
| | | Total | 24 | 29203.7 | | |
| | 0.5% Tyr | Trmt | 4 | 2409.9 | 1.84 | 0.0533 |
| | | Error | 19 | 4035.8 | | |
| | | Total | 23 | 6445.7 | | |
| | 1% Tyr | Trmt | 4 | 6712.5 | 1.13 | 0.3710 |
| | | Error | 20 | 29723.4 | | |
| | | Total | 24 | 36436.0 | | |
| | 0.5% Trp | Trmt | 4 | 18055.4 | 5.63 | 0.0033 |
| | | Error | 20 | 16042.3 | | |
| | | Total | 24 | 34097.7 | | |
| | 1% Trp | Trmt | 4 | 35936.6 | 10.26 | 0.0001 |
| | | Error | 19 | 16643.0 | | |
| | | Total | 23 | 52579.6 | | |
| 5-HIAA/5-HT | Control | Trmt | 4 | 0.0025 | 1.07 | 0.3967 |
| | | Error | 20 | 0.0118 | | |
| | | Total | 24 | 0.0143 | | |
| | 0.5% Tyr | Trmt | 4 | 0.0034 | 1.98 | 0.1381 |
| | | Error | 19 | 0.0082 | | |
| | | Total | 23 | 0.0116 | | |
| | 1% Tyr | Trmt | 4 | 0.0010 | 0.86 | 0.5038 |
| | | Error | 20 | 0.0580 | | |
| | | Total | 24 | 0.0680 | | |
| | 0.5% Trp | Trmt | 4 | 0.0177 | 2.42 | 0.0826 |
| | | Error | 20 | 0.0367 | | |
| | | Total | 24 | 0.0545 | | |
| | 1% Trp | Trmt | 4 | 0.0164 | 4.39 | 0.0111 |
| | | Error | 19 | 0.0177 | | |
| | | Total | 23 | 0.0341 | | |

Appendix C Table 14. Analysis of variance for 5-HIAA, 5-HT levels, and 5-HIAA/5-HT ratios in the cerebellum of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | 2 | Trmt | 4 | 25176.2 | 2.15 | 0.1162 |
| | | Error | 18 | 52662.9 | | |
| | | Total | 22 | 77839.1 | | |
| | 4 | Trmt | 4 | 90670.4 | 1.01 | 0.4318 |
| | | Error | 17 | 383097.1 | | |
| | | Total | 21 | 473767.6 | | |
| | 6 | Trmt | 4 | 126825.6 | 2.54 | 0.0737 |
| | | Error | 19 | 237276.3 | | |
| | | Total | 23 | 364101.9 | | |
| | 8 | Trmt | 4 | 158874.9 | 1.83 | 0.1645 |
| | | Error | 19 | 412054.2 | | |
| | | Total | 23 | 570929.1 | | |
| | 10 | Trmt | 4 | 41361.9 | 1.16 | 0.3577 |
| | | Error | 19 | 168782.7 | | |
| | | Total | 23 | 210144.6 | | |
| 5-HIAA | 2 | Trmt | 4 | 5522.3 | 5.21 | 0.0114 |
| | | Error | 12 | 3177.6 | | |
| | | Total | 16 | 8700.0 | | |
| | 4 | Trmt | 4 | 1021.3 | 1.04 | 0.4236 |
| | | Error | 13 | 3189.9 | | |
| | | Total | 17 | 4211.2 | | |
| | 6 | Trmt | 4 | 1354.9 | 0.91 | 0.4765 |
| | | Error | 20 | 7434.5 | | |
| | | Total | 24 | 8789.4 | | |
| | 8 | Trmt | 4 | 5550.1 | 1.00 | 0.4350 |
| | | Error | 18 | 25071.5 | | |
| | | Total | 22 | 30621.6 | | |
| | 10 | Trmt | 4 | 961.1 | 0.80 | 0.5393 |
| | | Error | 19 | 5697.6 | | |
| | | Total | 23 | 6658.7 | | |
| 5-HIAAA/5-HT | 2 | Trmt | 4 | 0.0477 | 4.73 | 0.0160 |
| | | Error | 12 | 0.0302 | | |
| | | Total | 16 | 0.0779 | | |
| | 4 | Trmt | 4 | 0.0100 | 0.87 | 0.5056 |
| | | Error | 13 | 0.0371 | | |
| | | Total | 17 | 0.0470 | | |
| | 6 | Trmt | 4 | 0.0280 | 3.44 | 0.0281 |
| | | Error | 19 | 0.0386 | | |
| | | Total | 23 | 0.0665 | | |
| | 8 | Trmt | 4 | 0.0191 | 0.12 | 0.9751 |
| | | Error | 18 | 0.7403 | | |
| | | Total | 22 | 0.7594 | | |
| | 10 | Trmt | 4 | 0.0086 | 0.92 | 0.4743 |
| | | Error | 19 | 0.0446 | | |
| | | Total | 23 | 0.0533 | | |

Appendix C Table 14. Analysis of variance for 5-HIAA, 5-HT levels, and 5-HIAA/5-HT ratios in the cerebellum of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| 5-HT | Control | Trmt | 4 | 147561.8 | 3.56 | 0.0238 |
| | | Error | 20 | 207094.9 | | |
| | | Total | 24 | 354656.7 | | |
| | 0.5% Tyr | Trmt | 4 | 82214.9 | 1.12 | 0.3762 |
| | | Error | 19 | 348526.8 | | |
| | | Total | 23 | 430741.7 | | |
| | 1% Tyr | Trmt | 4 | 26452.5 | 1.11 | 0.3874 |
| | | Error | 16 | 95613.7 | | |
| | | Total | 20 | 122066.2 | | |
| | 0.5% Trp | Trmt | 4 | 68253.5 | 2.12 | 0.1177 |
| | | Error | 19 | 152627.2 | | |
| | | Total | 23 | 220979.7 | | |
| | 1% Trp | Trmt | 4 | 39798.6 | 0.14 | 0.8074 |
| | | Error | 18 | 449911.8 | | |
| | | Total | 22 | 489710.4 | | |
| 5-HIAA | Control | Trmt | 4 | 4657.6 | 1.14 | 0.3737 |
| | | Error | 15 | 15265.9 | | |
| | | Total | 19 | 19923.5 | | |
| | 0.5% Tyr | Trmt | 4 | 1066.0 | 1.59 | 0.2288 |
| | | Error | 15 | 2517.8 | | |
| | | Total | 19 | 3583.8 | | |
| | 1% Tyr | Trmt | 4 | 4126.8 | 1.91 | 0.1586 |
| | | Error | 16 | 8662.0 | | |
| | | Total | 20 | 12788.8 | | |
| | 0.5% Trp | Trmt | 4 | 11399.1 | 6.04 | 0.0026 |
| | | Error | 19 | 8957.3 | | |
| | | Total | 23 | 20356.4 | | |
| | 1% Trp | Trmt | 4 | 8289.9 | 3.92 | 0.0197 |
| | | Error | 17 | 8999.2 | | |
| | | Total | 21 | 17289.1 | | |
| 5-HIAAA/5-HT | Control | Trmt | 4 | 0.1777 | 1.14 | 0.3752 |
| | | Error | 15 | 0.5844 | | |
| | | Total | 19 | 0.7621 | | |
| | 0.5% Tyr | Trmt | 4 | 0.0984 | 5.63 | 0.0057 |
| | | Error | 15 | 0.0655 | | |
| | | Total | 19 | 0.1639 | | |
| | 1% Tyr | Trmt | 4 | 0.1163 | 6.97 | 0.0022 |
| | | Error | 15 | 0.0626 | | |
| | | Total | 19 | 0.1789 | | |
| | 0.5% Trp | Trmt | 4 | 0.0831 | 3.60 | 0.0241 |
| | | Error | 19 | 0.1098 | | |
| | | Total | 23 | 0.1929 | | |
| | 1% Trp | Trmt | 4 | 0.0737 | 4.57 | 0.0109 |
| | | Error | 17 | 0.0686 | | |
| | | Total | 21 | 0.1423 | | |

Appendix C Table 15. Analysis of variance for L-dopa and catecholamine levels in the brain stem of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| L-Dopa | 2 | Trmt | 4 | 4.170 | 1.36 | 0.2641 |
| | | Error | 44 | 33.789 | | |
| | | Total | 48 | 37.960 | | |
| | 4 | Trmt | 4 | 3.285 | 0.94 | 0.4492 |
| | | Error | 44 | 38.389 | | |
| | | Total | 48 | 41.673 | | |
| | 6 | Trmt | 4 | 3.285 | 0.94 | 0.4492 |
| | | Error | 44 | 38.389 | | |
| | | Total | 48 | 41.673 | | |
| | 8 | Trmt | 4 | 6.508 | 1.28 | 0.2921 |
| | | Error | 44 | 55.900 | | |
| | | Total | 48 | 62.408 | | |
| | 10 | Trmt | 4 | 3.875 | 0.95 | 0.4419 |
| | | Error | 44 | 44.656 | | |
| | | Total | 48 | 48.531 | | |
| Dopamine | 2 | Trmt | 4 | 0 | . | . |
| | | Error | 44 | 0 | | |
| | | Total | 48 | 0 | | |
| | 4 | Trmt | 4 | 1.739 | 0.99 | 0.4230 |
| | | Error | 44 | 19.322 | | |
| | | Total | 48 | 21.061 | | |
| | 6 | Trmt | 4 | 1.739 | 0.99 | 0.4230 |
| | | Error | 44 | 19.322 | | |
| | | Total | 48 | 21.061 | | |
| | 8 | Trmt | 4 | 2.076 | 1.33 | 0.2743 |
| | | Error | 44 | 17.189 | | |
| | | Total | 48 | 19.265 | | |
| | 10 | Trmt | 4 | 2.813 | 1.39 | 0.2536 |
| | | Error | 44 | 22.289 | | |
| | | Total | 48 | 25.102 | | |
| Norepinephine | 2 | Trmt | 4 | 4.170 | 1.36 | 0.2641 |
| | | Error | 44 | 33.789 | | |
| | | Total | 48 | 37.959 | | |
| | 4 | Trmt | 4 | 7.672 | 2.44 | 0.0634 |
| | | Error | 44 | 35.022 | | |
| | | Total | 48 | 42.061 | | |
| | 6 | Trmt | 4 | 12.244 | 1.62 | 0.1873 |
| | | Error | 44 | 69.756 | | |
| | | Total | 48 | 80.000 | | |
| | 8 | Trmt | 4 | 4.965 | 1.13 | 0.3544 |
| | | Error | 44 | 48.300 | | |
| | | Total | 48 | 53.265 | | |
| | 10 | Trmt | 4 | 8.800 | 2.91 | 0.0323 |
| | | Error | 44 | 33.322 | | |
| | | Total | 48 | 42.122 | | |

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Appendix C Table 15 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Epinephine | 2 | Trmt | 4 | 3.285 | 0.94 | 0.4492 |
| | | Error | 44 | 38.389 | | |
| | | Total | 48 | 41.673 | | |
| | 4 | Trmt | 4 | 3.285 | 0.94 | 0.4492 |
| | | Error | 44 | 38.389 | | |
| | | Total | 48 | 41.673 | | |
| | 6 | Trmt | 4 | 6.508 | 1.28 | 0.2921 |
| | | Error | 44 | 55.900 | | |
| | | Total | 48 | 62.408 | | |
| | 8 | Trmt | 4 | 3.875 | 0.95 | 0.4419 |
| | | Error | 44 | 44.656 | | |
| | | Total | 48 | 48.531 | | |
| | 10 | Trmt | 4 | 3.042 | 0.68 | 0.6122 |
| | | Error | 44 | 49.489 | | |
| | | Total | 48 | 52.530 | | |

Appendix C Table 15. Analysis of variance for L-dopa and catecholamine levels in the brain stem of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| L-Dopa | Control | Trmt | 4 | 248295.3 | 4.11 | 0.0154 |
| | | Error | 18 | 271910.5 | | |
| | | Total | 22 | 520205.9 | | |
| | 0.5% Tyr | Trmt | 4 | 300627.4 | 9.22 | 0.0003 |
| | | Error | 19 | 154898.3 | | |
| | | Total | 23 | 455525.7 | | |
| | 1% Tyr | Trmt | 4 | 270769.4 | 12.41 | 0.0001 |
| | | Error | 19 | 103631.8 | | |
| | | Total | 23 | 374401.2 | | |
| | 0.5% Trp | Trmt | 4 | 455097.5 | 14.57 | 0.0001 |
| | | Error | 18 | 140576.0 | | |
| | | Total | 22 | 595673.5 | | |
| | 1% Trp | Trmt | 4 | 324535.2 | 7.30 | 0.0011 |
| | | Error | 18 | 199987.8 | | |
| | | Total | 22 | 524522.9 | | |
| Dopamine | Control | Trmt | 4 | 6744.6 | 3.13 | 0.0375 |
| | | Error | 20 | 10771.1 | | |
| | | Total | 24 | 175157 | | |
| | 0.5% Tyr | Trmt | 4 | 34310.8 | 0.91 | 0.4788 |
| | | Error | 19 | 179366.6 | | |
| | | Total | 23 | 213677.4 | | |
| | 1% Tyr | Trmt | 4 | 2049.0 | 1.32 | 0.2964 |
| | | Error | 20 | 7756.1 | | |
| | | Total | 24 | 9805.0 | | |
| | 0.5% Trp | Trmt | 4 | 6094.9 | 2.84 | 0.0530 |
| | | Error | 19 | 10193.1 | | |
| | | Total | 23 | 16288.0 | | |
| | 1% Trp | Trmt | 4 | 11688.8 | 4.34 | 0.0109 |
| | | Error | 20 | 13473.7 | | |
| | | Total | 24 | 25162.5 | | |
| Norepinephrine | Control | Trmt | 4 | 423802.3 | 3.17 | 0.0361 |
| | | Error | 20 | 669351.3 | | |
| | | Total | 24 | 1093153.6 | | |
| | 0.5% Tyr | Trmt | 4 | 165912.5 | 3.34 | 0.0314 |
| | | Error | 19 | 236113.8 | | |
| | | Total | 23 | 402026.3 | | |
| | 1% Tyr | Trmt | 4 | 294941.9 | 6.10 | 0.0022 |
| | | Error | 20 | 241809.1 | | |
| | | Total | 24 | 536751.0 | | |
| | 0.5% Trp | Trmt | 4 | 808477.8 | 12.42 | 0.0001 |
| | | Error | 19 | 309183.8 | | |
| | | Total | 23 | 1117661.6 | | |
| | 1% Trp | Trmt | 4 | 1413043.9 | 17.69 | 0.0001 |
| | | Error | 19 | 379453.5 | | |
| | | Total | 23 | 1792497.4 | | |

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Appendix C Table 15 continued

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Epinephine | Control | Trmt | 4 | 2563.1 | 1.30 | 0.3087 |
| | | Error | 17 | 8365.7 | | |
| | | Total | 21 | 10928.8 | | |
| | 0.5% Tyr | Trmt | 4 | 13366.8 | 5.47 | 0.0051 |
| | | Error | 17 | 10388.1 | | |
| | | Total | 21 | 23754.9 | | |
| | 1% Tyr | Trmt | 4 | 8534.3 | 1.03 | 0.4199 |
| | | Error | 17 | 35190.1 | | |
| | | Total | 21 | 43724.3 | | |
| | 0.5% Trp | Trmt | 4 | 115639.7 | 3.47 | 0.0303 |
| | | Error | 17 | 141773.4 | | |
| | | Total | 21 | 257413.1 | | |
| | 1% Trp | Trmt | 4 | 85691.2 | 1.12 | 0.3799 |
| | | Error | 18 | 345659.6 | | |
| | | Total | 22 | 431350.7 | | |

Appendix C Table 16. Analysis of variance for L-dopa and catecholamine levels in the diencephalon of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan at selected days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| L-Dopa | 2 | Trmt | 4 | 177557.6 | 2.16 | 0.1176 |
| | | Error | 17 | 349421.3 | | |
| | | Total | 21 | 526978.9 | | |
| | 4 | Trmt | 4 | 38320.3 | 12.13 | 0.0001 |
| | | Error | 19 | 15008.7 | | |
| | | Total | 23 | 53328.0 | | |
| | 6 | Trmt | 4 | 30087.5 | 2.03 | 0.1310 |
| | | Error | 19 | 70441.9 | | |
| | | Total | 23 | 100529.4 | | |
| | 8 | Trmt | 4 | 68596.3 | 0.68 | 0.6168 |
| | | Error | 16 | 404414.7 | | |
| | | Total | 20 | 473011.0 | | |
| | 10 | Trmt | 4 | 202195.5 | 2.10 | 0.1212 |
| | | Error | 19 | 458058.5 | | |
| | | Total | 23 | 660254.0 | | |
| Dopamine | 2 | Trmt | 4 | 180607.2 | 1.98 | 0.1390 |
| | | Error | 19 | 433910.1 | | |
| | | Total | 23 | 614517.3 | | |
| | 4 | Trmt | 4 | 310982.1 | 15.98 | 0.0001 |
| | | Error | 20 | 97305.9 | | |
| | | Total | 24 | 408288.0 | | |
| | 6 | Trmt | 4 | 147896.3 | 9.70 | 0.0002 |
| | | Error | 19 | 72429.2 | | |
| | | Total | 23 | 220325.5 | | |
| | 8 | Trmt | 4 | 7098.3 | 1.02 | 0.4213 |
| | | Error | 20 | 34824.6 | | |
| | | Total | 24 | 41922.9 | | |
| | 10 | Trmt | 4 | 5409.6 | 0.57 | 0.6855 |
| | | Error | 19 | 44846.8 | | |
| | | Total | 23 | 50256.3 | | |
| Norepinephine | 2 | Trmt | 4 | 33958.2 | 0.55 | 0.7004 |
| | | Error | 19 | 292598.5 | | |
| | | Total | 23 | 326556.8 | | |
| | 4 | Trmt | 4 | 20104.2 | 0.24 | 0.9121 |
| | | Error | 20 | 418173.4 | | |
| | | Total | 24 | 437277.7 | | |
| | 6 | Trmt | 4 | 37651.1 | 0.52 | 0.7188 |
| | | Error | 19 | 340775.4 | | |
| | | Total | 23 | 378426.5 | | |

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Appendix C Table 16 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | 8 | Trmt | 4 | 214490.5 | 0.67 | 0.6232 |
| | | Error | 20 | 1610926.2 | | |
| | | Total | 24 | 1825416.6 | | |
| | 10 | Trmt | 4 | 407561.6 | 1.60 | 0.2153 |
| | | Error | 19 | 1210152.4 | | |
| | | Total | 23 | 1617714.0 | | |
| Epinephine | 2 | Trmt | 4 | 60597.2 | 4.16 | 0.0272 |
| | | Error | 11 | 40077.9 | | |
| | | Total | 15 | 100675.1 | | |
| | 4 | Trmt | 4 | 4502.7 | 0.77 | 0.5563 |
| | | Error | 20 | 29170.9 | | |
| | | Total | 24 | 33673.7 | | |
| | 6 | Trmt | 4 | 8432.2 | 1.65 | 0.2057 |
| | | Error | 18 | 23018.1 | | |
| | | Total | 22 | 31451.3 | | |
| | 8 | Trmt | 4 | 67496.2 | 0.54 | 0.7055 |
| | | Error | 20 | 620622.7 | | |
| | | Total | 24 | 688119.0 | | |
| | 10 | Trmt | 4 | 177701.2 | 1.60 | 0.2210 |
| | | Error | 17 | 473326.5 | | |
| | | Total | 21 | 651027.7 | | |

Appendix C Table 16. Analysis of variance for L-dopa and catecholamine levels in the diencephalon of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| L-Dopa | Control | Trmt | 4 | 1751536.8 | 14.96 | 0.0001 |
| | | Error | 18 | 526934.7 | | |
| | | Total | 22 | 2278471.5 | | |
| | 0.5% Tyr | Trmt | 4 | 2686415.3 | 41.86 | 0.0001 |
| | | Error | 19 | 304867.7 | | |
| | | Total | 23 | 2991283.0 | | |
| | 1% Tyr | Trmt | 4 | 1094714.7 | 25.40 | 0.0001 |
| | | Error | 17 | 183195.5 | | |
| | | Total | 21 | 1277910.2 | | |
| | 0.5% Trp | Trmt | 4 | 1272061.5 | 25.66 | 0.0001 |
| | | Error | 17 | 210702.1 | | |
| | | Total | 21 | 1482763.6 | | |
| | 1% Trp | Trmt | 4 | 1568619.0 | 95.01 | 0.0001 |
| | | Error | 17 | 70165.9 | | |
| | | Total | 21 | 1638784.9 | | |
| Dopamine | Control | Trmt | 4 | 66775.5 | 1.01 | 0.4286 |
| | | Error | 19 | 315053.1 | | |
| | | Total | 23 | 381828.6 | | |
| | 0.5% Tyr | Trmt | 4 | 30903.1 | 1.93 | 0.1453 |
| | | Error | 20 | 80198.7 | | |
| | | Total | 24 | 111101.8 | | |
| | 1% Tyr | Trmt | 4 | 26331.5 | 3.07 | 0.0449 |
| | | Error | 17 | 36435.5 | | |
| | | Total | 21 | 62767.0 | | |
| | 0.5% Trp | Trmt | 4 | 129590.9 | 4.99 | 0.0059 |
| | | Error | 20 | 129787.1 | | |
| | | Total | 24 | 259378.0 | | |
| | 1% Trp | Trmt | 4 | 380775.2 | 14.07 | 0.0001 |
| | | Error | 19 | 128517.8 | | |
| | | Total | 23 | 509303.0 | | |
| Norepinephine | Control | Trmt | 4 | 1473160.9 | 9.25 | 0.0003 |
| | | Error | 19 | 756775.7 | | |
| | | Total | 23 | 2229936.6 | | |
| | 0.5% Tyr | Trmt | 4 | 2918723.6 | 12.05 | 0.0001 |
| | | Error | 20 | 1210664.1 | | |
| | | Total | 24 | 4129387.7 | | |
| | 1% Tyr | Trmt | 4 | 1353047.3 | 5.40 | 0.0054 |
| | | Error | 17 | 1063967.9 | | |
| | | Total | 21 | 2417015.3 | | |

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Appendix C Table 16.

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | 0.5% Trp | Trmt | 4 | 696605.0 | 6.76 | 0.0013 |
| | | Error | 20 | 515361.9 | | |
| | | Total | 24 | 1211966.8 | | |
| | 1% Trp | Trmt | 4 | 1624071.5 | 23.62 | 0.0001 |
| | | Error | 19 | 326652.5 | | |
| | | Total | 23 | 1950724.2 | | |
| Epinephine | Control | Trmt | 4 | 106866.8 | 6.02 | 0.0033 |
| | | Error | 17 | 75461.4 | | |
| | | Total | 21 | 182328.2 | | |
| | 0.5% Tyr | Trmt | 4 | 354651.8 | 5.01 | 0.0063 |
| | | Error | 19 | 336439.4 | | |
| | | Total | 23 | 691091.2 | | |
| | 1% Tyr | Trmt | 4 | 224147.8 | 1.35 | 0.2937 |
| | | Error | 17 | 708075.1 | | |
| | | Total | 21 | 932222.9 | | |
| | 0.5% Trp | Trmt | 4 | 30325.0 | 2.38 | 0.0920 |
| | | Error | 17 | 54048.0 | | |
| | | Total | 21 | 84373.0 | | |
| | 1% Trp | Trmt | 4 | 41625.7 | 7.87 | 0.0015 |
| | | Error | 14 | 18511.8 | | |
| | | Total | 18 | 60137.4 | | |

Appendix C Table 17. Analysis of variance for L-dopa and catecholamine levels in the telencephalon of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Dopamine | 2 | Trmt | 4 | 63615.1 | 1.54 | 0.2300 |
| | | Error | 20 | 207116.8 | | |
| | | Total | 24 | 270731.9 | | |
| | 4 | Trmt | 4 | 447633.9 | 4.70 | 0.0078 |
| | | Error | 20 | 476622.7 | | |
| | | Total | 24 | 924256.0 | | |
| | 6 | Trmt | 4 | 14258.0 | 0.13 | 0.9683 |
| | | Error | 20 | 534979.9 | | |
| | | Total | 24 | 549238.0 | | |
| | 8 | Trmt | 4 | 240086.0 | 2.59 | 0.0678 |
| | | Error | 20 | 463332.1 | | |
| | | Total | 24 | 703418.1 | | |
| | 10 | Trmt | 4 | 40377.5 | 1.79 | 0.1696 |
| | | Error | 20 | 112523.2 | | |
| | | Total | 24 | 152900.6 | | |
| Norepinephine | 2 | Trmt | 4 | 6197.7 | 1.33 | 0.3087 |
| | | Error | 14 | 16366.7 | | |
| | | Total | 18 | 22564.4 | | |
| | 4 | Trmt | 4 | 15163.1 | 0.52 | 0.7200 |
| | | Error | 20 | 144967.2 | | |
| | | Total | 24 | 160130.3 | | |
| | 6 | Trmt | 4 | 24214.3 | 2.36 | 0.0877 |
| | | Error | 20 | 51218.0 | | |
| | | Total | 24 | 75432.3 | | |
| | 8 | Trmt | 4 | 9641.4 | 0.60 | 0.6678 |
| | | Error | 20 | 80512.7 | | |
| | | Total | 24 | 90154.1 | | |
| | 10 | Trmt | 4 | 618.0 | 0.09 | 0.9860 |
| | | Error | 20 | 36249.0 | | |
| | | Total | 24 | 36867.1 | | |

Appendix C Table 17. Analysis of variance for L-Dopa and catecholamine levels in the telencephalon of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Dopamine | Control | Trmt | 4 | 36533.3 | 0.66 | 0.6278 |
| | | Error | 21 | 291412.1 | | |
| | | Total | 25 | 327945.5 | | |
| | 0.5% Tyr | Trmt | 4 | 99439.8 | 1.90 | 0.1493 |
| | | Error | 20 | 261279.9 | | |
| | | Total | 24 | 360719.7 | | |
| | 1% Tyr | Trmt | 4 | 76525.2 | 1.22 | 0.3334 |
| | | Error | 20 | 313451.1 | | |
| | | Total | 24 | 389976.4 | | |
| | 0.5% Trp | Trmt | 4 | 199994.8 | 2.29 | 0.0951 |
| | | Error | 20 | 436059.2 | | |
| | | Total | 24 | 636053.9 | | |
| | 1% Trp | Trmt | 4 | 535110.0 | 5.31 | 0.0044 |
| | | Error | 20 | 503528.6 | | |
| | | Total | 24 | 1038638.6 | | |
| Norepinephine | Control | Trmt | 4 | 27596.6 | 3.69 | 0.0209 |
| | | Error | 20 | 37426.9 | | |
| | | Total | 24 | 65023.5 | | |
| | 0.5% Tyr | Trmt | 4 | 99439.8 | 1.90 | 0.1493 |
| | | Error | 20 | 261279.9 | | |
| | | Total | 24 | 360719.7 | | |
| | 1% Tyr | Trmt | 4 | 16040.5 | 0.77 | 0.5601 |
| | | Error | 20 | 104769.3 | | |
| | | Total | 24 | 120809.8 | | |
| | 0.5% Trp | Trmt | 4 | 13317.5 | 0.92 | 0.4754 |
| | | Error | 18 | 65344.0 | | |
| | | Total | 22 | 78661.5 | | |
| | 1% Trp | Trmt | 4 | 9670.4 | 1.10 | 0.3905 |
| | | Error | 16 | 35184.3 | | |
| | | Total | 20 | 44854.6 | | |

Appendix C Table 18. Analysis of variance for norepinephrine in the cerebellum of male Leghorns fed 0.5% or 1% supplemental tyrosine and tryptophan at selected days after SRBC injection

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Norepinephrine | 2 | Trmt | 4 | 85647.6 | 2.60 | 0.0712 |
| | | Error | 18 | 148478.3 | | |
| | | Total | 22 | 234125.9 | | |
| | 4 | Trmt | 4 | 52797.8 | 2.79 | 0.0576 |
| | | Error | 18 | 85041.5 | | |
| | | Total | 22 | 137839.2 | | |
| | 6 | Trmt | 4 | 56663.6 | 1.02 | 0.4209 |
| | | Error | 20 | 277763.6 | | |
| | | Total | 24 | 334427.2 | | |
| | 8 | Trmt | 4 | 23282.2 | 1.10 | 0.3833 |
| | | Error | 19 | 100162.5 | | |
| | | Total | 23 | 123444.7 | | |
| | 10 | Trmt | 4 | 29623.1 | 0.60 | 0.6654 |
| | | Error | 19 | 233516.1 | | |
| | | Total | 23 | 263139.2 | | |

Appendix C Table 18. Analysis of variance for norepinephrine in the cerebellum of male Leghorns between days within each dietary treatment

| Parameter | Trmt | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| Norepinephrine | Control | Trmt | 4 | 157720.2 | 5.50 | 0.0037 |
| | | Error | 20 | 143425.2 | | |
| | | Total | 24 | 301145.4 | | |
| | 0.5% Tyr | Trmt | 4 | 4079.6 | 0.11 | 0.9792 |
| | | Error | 19 | 183632.9 | | |
| | | Total | 23 | 187712.5 | | |
| | 1% Tyr | Trmt | 4 | 66803.6 | 2.06 | 0.1289 |
| | | Error | 18 | 146014.3 | | |
| | | Total | 22 | 212817.9 | | |
| | 0.5% Trp | Trmt | 4 | 26585.1 | 0.71 | 0.5932 |
| | | Error | 19 | 177099.2 | | |
| | | Total | 23 | 203684.3 | | |
| | 1% Trp | Trmt | 4 | 85345.5 | 1.97 | 0.1422 |
| | | Error | 18 | 194790.4 | | |
| | | Total | 22 | 280135.9 | | |

Appendix C Table 19. Analysis of variance for average daily feed intake and body weight gain of Leghorns and broilers during the dietary supplementation period

| Parameter | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|
| | | Leghorns | | | |
| Feed intake | Trmt | 4 | 9.83 | 0.34 | 0.8420 |
| | Error | 10 | 71.36 | | |
| | Total | 14 | 81.19 | | |
| Body weight gain | Trmt | 4 | 147.66 | 0.39 | 0.8099 |
| | Error | 10 | 941.77 | | |
| | Total | 14 | 1089.43 | | |
| | | Broilers | | | |
| Feed intake | Trmt | 4 | 79.34 | 0.99 | 0.4898 |
| | Error | 5 | 100.20 | | |
| | Total | 9 | 179.54 | | |
| Body weight gain | Trmt | 4 | 18.68 | 0.40 | 0.8002 |
| | Error | 5 | 57.90 | | |
| | Total | 9 | 76.59 | | |

Appendix C Table 20. Analysis of variance for average daily feed intake and body weight gain of stressed broilers during the dietary supplementation period

| Parameter | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|
| | | Stressed broilers | | | |
| Feed intake | Trmt | 5 | 173.79 | 0.77 | 0.6023 |
| | Error | 6 | 269.76 | | |
| | Total | 9 | 430.55 | | |
| Body weight gain | Trmt | 5 | 18.682 | 0.40 | 0.8002 |
| | Error | 6 | 57.904 | | |
| | Total | 9 | 76.586 | | |

Appendix B Table 21. Analysis of variance for the titers of Leghorn primary and secondary antibody titer following SRBC injection by pooling the two addition levels with the same amino acid supplementation

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 2 | 4.083 | 2.77 | 0.0730 |
| | | Error | 46 | 33.876 | | |
| | | Total | 48 | 37.959 | | |
| | 5 | Trmt | 2 | 0.666 | 0.37 | 0.6905 |
| | | Error | 46 | 41.008 | | |
| | | Total | 48 | 41.673 | | |
| | 7 | Trmt | 2 | 2.0320 | 0.77 | 0.4671 |
| | | Error | 46 | 60.376 | | |
| | | Total | 48 | 62.408 | | |
| | 9 | Trmt | 2 | 3.796 | 1.95 | 0.1536 |
| | | Error | 46 | 44.734 | | |
| | | Total | 48 | 48.531 | | |
| | 11 | Trmt | 2 | 2.954 | 1.37 | 0.2641 |
| | | Error | 46 | 49.576 | | |
| | | Total | 48 | 52.531 | | |
| IgG | 3 | Trmt | 2 | 0 | . | . |
| | | Error | 46 | 0 | | |
| | | Total | 48 | 0 | | |
| | 5 | Trmt | 2 | 0.306 | 0.34 | 0.7142 |
| | | Error | 46 | 20.755 | | |
| | | Total | 48 | 21.061 | | |
| | 7 | Trmt | 2 | 0.615 | 0.76 | 0.4740 |
| | | Error | 46 | 18.650 | | |
| | | Total | 48 | 19.265 | | |
| | 9 | Trmt | 2 | 0.368 | 0.34 | 0.7121 |
| | | Error | 46 | 24.734 | | |
| | | Total | 48 | 25.102 | | |
| | 11 | Trmt | 2 | 2.954 | 2.44 | 0.0981 |
| | | Error | 46 | 24.821 | | |
| | | Total | 48 | 30.776 | | |
| IgM | 3 | Trmt | 2 | 4.083 | 2.77 | 0.0730 |
| | | Error | 46 | 33.876 | | |
| | | Total | 48 | 37.959 | | |
| | 5 | Trmt | 2 | 0.704 | 0.39 | 0.6821 |
| | | Error | 46 | 41.989 | | |
| | | Total | 48 | 42.694 | | |
| | 7 | Trmt | 2 | 2.874 | 0.86 | 0.4311 |
| | | Error | 44 | 77.126 | | |
| | | Total | 48 | 80.000 | | |
| | 9 | Trmt | 2 | 2.865 | 1.31 | 0.2803 |
| | | Error | 46 | 50.400 | | |
| | | Total | 48 | 53.265 | | |
| | 11 | Trmt | 2 | 5.515 | 3.46 | 0.0397 |
| | | Error | 48 | 42.122 | | |
| | | Total | 48 | 42.122 | | |

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Appendix C Table 21 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Secondary antibody response | | | |
| Total | 2 | Trmt | 2 | 0.232 | 0.23 | 0.7947 |
| | | Error | 46 | 23.155 | | |
| | | Total | 48 | 23.388 | | |
| | 4 | Trmt | 2 | 4.133 | 1.54 | 0.2258 |
| | | Error | 46 | 61.826 | | |
| | | Total | 48 | 65.959 | | |
| | 6 | Trmt | 2 | 2.212 | 1.09 | 0.3432 |
| | | Error | 46 | 46.482 | | |
| | | Total | 48 | 48.694 | | |
| | 8 | Trmt | 2 | 3.058 | 1.37 | 0.2643 |
| | | Error | 46 | 51.350 | | |
| | | Total | 48 | 54.408 | | |
| IgG | 2 | Trmt | 2 | 0.479 | 1.16 | 0.3234 |
| | | Error | 46 | 9.521 | | |
| | | Total | 48 | 10.000 | | |
| | 4 | Trmt | 2 | 1.591 | 1.97 | 0.1504 |
| | | Error | 46 | 28.122 | | |
| | | Total | 48 | 20.122 | | |
| | 6 | Trmt | 2 | 0.196 | 0.28 | 0.7596 |
| | | Error | 46 | 16.334 | | |
| | | Total | 48 | 16.531 | | |
| | 8 | Trmt | 2 | 0.480 | 0.73 | 0.4862 |
| | | Error | 46 | 15.071 | | |
| | | Total | 48 | 15.551 | | |
| IgM | 2 | Trmt | 2 | 0.847 | 0.69 | 0.5070 |
| | | Error | 46 | 28.255 | | |
| | | Total | 48 | 29.102 | | |
| | 4 | Trmt | 2 | 2.270 | 0.92 | 0.4062 |
| | | Error | 46 | 56.832 | | |
| | | Total | 48 | 59.102 | | |
| | 6 | Trmt | 2 | 3.327 | 1.73 | 0.1891 |
| | | Error | 46 | 44.305 | | |
| | | Total | 48 | 47.633 | | |
| | 8 | Trmt | 2 | 1.879 | 0.94 | 0.3991 |
| | | Error | 46 | 46.121 | | |
| | | Total | 48 | 48.000 | | |

Appendix B Table 22. Analysis of variance for the titers of the broiler primary and secondary antibody titers following SRBC injection by pooling the two levels with the same amino acid supplementation

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 2 | 0.500 | 0.60 | 0.5516 |
| | | Error | 47 | 19.500 | | |
| | | Total | 49 | 20.000 | | |
| | 5 | Trmt | 2 | 0.900 | 0.24 | 0.7854 |
| | | Error | 47 | 87.100 | | |
| | | Total | 49 | 88.000 | | |
| | 7 | Trmt | 2 | 11.270 | 3.60 | 0.0351 |
| | | Error | 47 | 73.550 | | |
| | | Total | 49 | 84.820 | | |
| | 9 | Trmt | 2 | 10.350 | 3.70 | 0.0321 |
| | | Error | 47 | 65.650 | | |
| | | Total | 49 | 76.000 | | |
| IgG | 3 | Trmt | 2 | 0.070 | 0.89 | 0.4178 |
| | | Error | 47 | 1.850 | | |
| | | Total | 49 | 1.920 | | |
| | 5 | Trmt | 2 | 0.030 | 0.74 | 0.4816 |
| | | Error | 47 | 0.950 | | |
| | | Total | 49 | 0.980 | | |
| | 7 | Trmt | 2 | 1.220 | 2.06 | 0.1385 |
| | | Error | 47 | 13.900 | | |
| | | Total | 49 | 15.120 | | |
| | 9 | Trmt | 2 | 1.470 | 2.84 | 0.0683 |
| | | Error | 47 | 12.150 | | |
| | | Total | 49 | 13.620 | | |
| IgM | 3 | Trmt | 2 | 0.270 | 0.42 | 0.6620 |
| | | Error | 47 | 15.250 | | |
| | | Total | 49 | 15.520 | | |
| | 5 | Trmt | 2 | 1.230 | 0.35 | 0.7053 |
| | | Error | 47 | 82.150 | | |
| | | Total | 49 | 83.380 | | |
| | 7 | Trmt | 2 | 9.630 | 3.07 | 0.0559 |
| | | Error | 47 | 73.750 | | |
| | | Total | 49 | 83.380 | | |
| | 9 | Trmt | 2 | 9.720 | 4.61 | 0.0148 |
| | | Error | 47 | 49.500 | | |
| | | Total | 49 | 59.220 | | |

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Appendix C Table 22 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Secondary antibody response | | | |
| Total | 2 | Trmt | 2 | 7.758 | 8.59 | 0.0007 |
| | | Error | 46 | 20.772 | | |
| | | Total | 48 | 28.531 | | |
| | 4 | Trmt | 2 | 7.441 | 2.20 | 0.1227 |
| | | Error | 46 | 77.906 | | |
| | | Total | 48 | 85.347 | | |
| | 6 | Trmt | 2 | 4.527 | 2.53 | 0.0904 |
| | | Error | 46 | 46.106 | | |
| | | Total | 48 | 45.633 | | |
| | 8 | Trmt | 2 | 3.929 | 1.87 | 0.1661 |
| | | Error | 45 | 47.320 | | |
| | | Total | 47 | 51.250 | | |
| IgG | 2 | Trmt | 2 | 0.0296 | 0.72 | 0.4939 |
| | | Error | 46 | 0.950 | | |
| | | Total | 48 | 0.980 | | |
| | 4 | Trmt | 2 | 2.996 | 1.87 | 0.1662 |
| | | Error | 46 | 36.922 | | |
| | | Total | 48 | 39.918 | | |
| | 6 | Trmt | 2 | 1.899 | 1.14 | 0.3287 |
| | | Error | 46 | 38.306 | | |
| | | Total | 48 | 40.204 | | |
| | 8 | Trmt | 2 | 6.924 | 3.62 | 0.0349 |
| | | Error | 45 | 43.055 | | |
| | | Total | 47 | 49.979 | | |
| IgM | 2 | Trmt | 2 | 7.037 | 6.49 | 0.0033 |
| | | Error | 46 | 24.922 | | |
| | | Total | 48 | 31.959 | | |
| | 4 | Trmt | 2 | 3.094 | 1.30 | 0.2834 |
| | | Error | 44 | 54.906 | | |
| | | Total | 48 | 58.000 | | |
| | 6 | Trmt | 2 | 0.973 | 0.65 | 0.5292 |
| | | Error | 46 | 34.700 | | |
| | | Total | 48 | 35.674 | | |
| | 8 | Trmt | 2 | 0.956 | 0.43 | 0.6504 |
| | | Error | 45 | 49.523 | | |
| | | Total | 47 | 50.479 | | |

Appendix B Table 23. Analysis of variance for the titers of the stressed broiler primary and secondary antibody response following SRBC injection by pooling the two addition levels with the same amino acid supplementation

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Primary antibody response | | | |
| Total | 3 | Trmt | 3 | 6.983 | 16.29 | 0.0001 |
| | | Error | 56 | 8.000 | | |
| | | Total | 59 | 14.983 | | |
| | 5 | Trmt | 3 | 5.883 | 2.25 | 0.0927 |
| | | Error | 56 | 48.850 | | |
| | | Total | 59 | 54.733 | | |
| | 7 | Trmt | 3 | 8.733 | 1.91 | 0.1379 |
| | | Error | 56 | 85.200 | | |
| | | Total | 59 | 93.933 | | |
| | 9 | Trmt | 3 | 11.700 | 2.10 | 0.1104 |
| | | Error | 56 | 103.950 | | |
| | | Total | 59 | 115.650 | | |
| IgG | 3 | Trmt | 3 | 0 | . | . |
| | | Error | 56 | 0 | | |
| | | Total | 59 | 0 | | |
| | 5 | Trmt | 3 | 0.133 | 1.38 | 0.2575 |
| | | Error | 56 | 1.800 | | |
| | | Total | 59 | 1.933 | | |
| | 7 | Trmt | 3 | 2.683 | 2.33 | 0.0842 |
| | | Error | 56 | 21.500 | | |
| | | Total | 59 | 24.183 | | |
| | 9 | Trmt | 3 | 1.200 | 1.15 | 0.3352 |
| | | Error | 56 | 19.400 | | |
| | | Total | 59 | 20.600 | | |
| IgM | 3 | Trmt | 3 | 6.983 | 16.29 | 0.0001 |
| | | Error | 56 | 8.000 | | |
| | | Total | 59 | 14.983 | | |
| | 5 | Trmt | 3 | 5.550 | 2.21 | 0.0968 |
| | | Error | 56 | 46.850 | | |
| | | Total | 59 | 52.400 | | |
| | 7 | Trmt | 3 | 12.683 | 3.49 | 0.0215 |
| | | Error | 56 | 67.900 | | |
| | | Total | 59 | 80.583 | | |
| | 9 | Trmt | 3 | 14.900 | 3.13 | 0.0326 |
| | | Error | 56 | 88.750 | | |
| | | Total | 59 | 103.650 | | |

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Appendix C Table 23 continued

| Parameter | Days | Source of variance | df | Sum of squares | F value | P |
|---|---|---|---|---|---|---|
| | | | Secondary antibody response | | | |
| Total | 2 | Trmt | 3 | 0.430 | 0.47 | 0.7051 |
| | | Error | 54 | 16.484 | | |
| | | Total | 57 | 16.914 | | |
| | 4 | Trmt | 3 | 7.807 | 1.55 | 0.2122 |
| | | Error | 54 | 90.676 | | |
| | | Total | 57 | 98.483 | | |
| | 6 | Trmt | 3 | 2.256 | 0.49 | 0.6880 |
| | | Error | 52 | 79.172 | | |
| | | Total | 55 | 81.429 | | |
| | 8 | Trmt | 3 | 9.864 | 3.39 | 0.0245 |
| | | Error | 53 | 51.399 | | |
| | | Total | 56 | 61.263 | | |
| IgG | 2 | Trmt | 3 | 0 | . | . |
| | | Error | 54 | 0 | | |
| | | Total | 57 | 0 | | |
| | 4 | Trmt | 3 | 2.269 | 1.01 | 0.3966 |
| | | Error | 53 | 39.766 | | |
| | | Total | 56 | 42.035 | | |
| | 6 | Trmt | 3 | 7.048 | 3.17 | 0.0317 |
| | | Error | 52 | 38.506 | | |
| | | Total | 55 | 45.554 | | |
| | 8 | Trmt | 3 | 2.912 | 1.03 | 0.3870 |
| | | Error | 53 | 49.965 | | |
| | | Total | 56 | 52.877 | | |
| IgM | 2 | Trmt | 3 | 0.430 | 0.47 | 0.7051 |
| | | Error | 54 | 16.484 | | |
| | | Total | 57 | 16.914 | | |
| | 4 | Trmt | 3 | 3.929 | 0.90 | 0.4462 |
| | | Error | 53 | 76.913 | | |
| | | Total | 56 | 80.842 | | |
| | 6 | Trmt | 3 | 2.792 | 0.71 | 0.5515 |
| | | Error | 52 | 68.333 | | |
| | | Total | 55 | 71.125 | | |
| | 8 | Trmt | 3 | 2.149 | 0.54 | 0.6548 |
| | | Error | 53 | 69.886 | | |
| | | Total | 56 | 72.035 | | |

## *VITA*

Jiangtao Zhu, son of Renshui Zhu and Xuchu Zhu, was born on July 5, 1962 in Fujian, P. R. of China. He attended elementary and high school at Reiken. Upon graduation in 1979, he enrolled at Fujian Agricultural College, Fuzhou, from which he received his Bachelor of Science in veterinary medicine in July, 1983. After graduation, he worked in the college for five years, then entered graduate school of Nanjing Agricultural University beginning a Master of Science in veterinary immunology in September, 1988. In August, 1989, he transferred his graduate studies to Virginia Polytechnic Institute and State University in Poultry Science.