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Original Article

A 13-week feeding toxicity study of L-threonine in rats with a recovery period of 5 weeks

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ABSTRACT — Rats were administered L-threonine in the diet at concentrations of 0 (basal diet control), 1.25%, 2.5%, or 5.0% for 13 weeks. Animals were sacrificed following the treatment period or after a 5-week recovery period (for animals receiving the control or 5.0% L-threonine diet). The mean achieved doses of L-threonine during the treatment period were 0, 811.5, 1615.3, and 3266.9 mg/kg body weight/day in males, and 0, 909.9, 1850.0, and 3673.3 mg/kg body weight/day in females. No toxicologically significant changes in general condition, body weight, food consumption, feed efficiency, water intake, ophthalmoscopy, urinalysis, hematology, blood chemistry or pathology were observed. Based on the results of the study, no-observed-adverse effect levels (NOAEL) of 3266.9 and 3673.3 mg/kg body weight/day can be established for male and female rats, respectively, under the present experimental conditions.

Key words: L-Threonine, Rats, Subchronic toxicity, Safety, Feed

INTRODUCTION

L-Threonine ((2S, 3R)-2-Amino-3-hydroxybutanoic acid; L-Thr) [CAS No. 72-19-5] is one of the essential amino acids with a hydroxy-ethyl side chain, which is classified as an uncharged polar amino acid. As an essential amino acid for humans and some livestock animals, it has been used as an additive in animal feed, medical products, food, and cosmetics (Kumagai, 2000). Supplementing 8.5% casein diet to nephritic rats with L-threonine and L-methionine has been reported to improve hyperlipidemia and proteinuria (Fujisawa *et al.*, 1995). In Japan, L-Thr is approved as a designated food additive by the Food Sanitation Law and is added to nutrition-supplement drinks in a small amount as an essential amino acid for reinforcement in combination with other essential amino acids. It is also used for sports drinks in combination with various kinds of amino acids (Ogasawara and Abe, 2008). It has also been reported that oral administration of L-Thr showed antispastic effect in patients with spinal spasticity (Growdon *et al.*, 1991; Lee and Patterson, 1993). For livestock animals, supplemental feeding of L-Thr to growing pigs has been reported to enhance immune response (Defa *et al.*, 1999).

The interperitoneal 50% lethal dose (LD_{50}), $LD_{99.9}$ and $LD_{99.99}$ of L-Thr for rats have been reported to be 3,098 mg/kg, 3,693 mg/kg and 3,931 mg/kg, respectively (Gullino *et al.*, 1956). For humans, no serious side effects were observed in daily doses of 2 to 4 g up to 12 months of repeated intake, except for slight gastrointestinal discomfort, headache, rhinorrhea, abdominal wind, astriction, and exanthema (Blin *et al.*, 1992; Growdon *et al.*, 1991; Lee and Patterson, 1993; Tandan *et al.*, 1996). However, available information regarding the toxicity of L-Thr is limited. Therefore, the oral subchronic toxicity of L-Thr was examined in rats in feed at 0%, 1.25%, 2.5%, or 5.0% for 13 weeks.

MATERIALS AND METHODS

Ethical considerations

The 13-week oral toxicity study was conducted in compliance with Good Laboratory Practices (Notification no 313, Ministry of Health, Labour and Welfare, Japan, March 31, 1982) (GLP).

Chemicals

L-Thr (100.3% purity, Lot no. O83175) was prepared

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by KYOWA HAKKO BIO CO., LTD. (Tokyo, Japan).

Animal and husbandry

Seventy-eight male and 78 female Crj:CD(SD) strain specific pathogen free rats (4 weeks of age) were purchased from Charles River Japan, Inc. (Tokyo, Japan), and allowed to acclimate for 2 weeks. Animals were maintained at 22°C, with a relative humidity of 55%, and food and water were provided *ad libitum*. At 6 weeks of age, 60 healthy males and 60 healthy females were selected for the study.

Test article administration

L-Thr was mixed with about 10-fold amount of basic food (CRF-1 (Lot. No.:930811, 930902, 931007, 931103, 931201, 940107); Oriental Yeast Co., Ltd. (Tokyo, Japan)), and then additional basic food was mixed to the specified concentration of L-Thr. Food was prepared more than once in 4 weeks.

Rats (12/sex/group) were administered L-Thr in the diet at concentrations of 0 (basal diet control), 1.25, 2.5, or 5.0% for 13 weeks. An additional 6 animals/sex/group were administered the basal diet or 5.0% L-Thr for 13 weeks, followed by the basal diet control for an additional 5 weeks (recovery period).

In-life assessments

Animals were observed for clinical signs and abnormal behavior twice daily during the treatment period, and once daily during the recovery period. Body weight was recorded twice weekly during the treatment and recovery periods. Three- or four-day cumulative food consumption was recorded twice weekly, and feed efficiency was calculated on a weekly basis. Water intake was recorded once weekly during the treatment and recovery periods. An ophthalmoscopic examination was conducted prior to test article administration (during the acclimation period), in Week 13 of the treatment period, and in Week 5 of the recovery period.

Urine samples were obtained in Weeks 5 and 13 of the treatment period, and in Week 5 of the recovery period. The following urinalysis parameters were assessed: pH, proteins, ketone body, glucose, occult blood, bilirubin, urobilinogen, color, sediment, volume, specific gravity (SG), sodium (Na), potassium (K), and chloride (Cl).

Blood samples were collected from the abdominal aorta under diethyl ether anesthesia into blood collection tubes (SB-41: Sysmex Corp., Hyogo, Japan) containing EDTA-2K. They were obtained following an overnight fast on the last day of the treatment period or the recovery period. Hematological parameters assessed included red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell count (WBC) (Coulter Counter T890, Beckman-Coulter Inc., Tokyo, Japan), reticulocyte ratio (Brecher's method) and differential white blood cell ratio (May-Giemsa microscopic method). In addition, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (Coagulometer ACL 100, Instrumentation Laboratory, Bedford, MA, USA) were determined on the plasma obtained by centrifuging the blood samples treated with 3.8 w/v% sodium citrate. Blood chemistry parameters assessed included alkaline phosphatase (ALP), total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium (Na), potassium (K), chloride, calcium, inorganic phosphorus (P), total protein, (Automatic Analyzer Monarch, Instrumentation Laboratory), albumin/globulin ratio (A/G ratio) (calculated from protein fraction ratios), and protein fractions (Densitometer CLINISCAN SA-V, Helena Laboratories, Inc., Saitama, Japan). In addition, the following parameters were determined on the plasma obtained from blood treated with heparin: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) (Automatic Analyzer Monarch, Instrumentation Laboratory).

Necropsy

After collection of blood samples, the animals were exsanguinated and subjected to necropsy. Animals were observed for external abnormalities, and organs and tissues of the cephalic, thoracic, and abdominal regions were examined for gross abnormalities. The following organs were weighed: brain, pituitary, thyroid glands (including parathyroid), salivary glands (submandibular and sublingual), thymus, heart, lung (including bronchus), liver, spleen, kidney, adrenal, testis, prostate, seminal vesicle, ovary, and uterus.

The following organs were prepared for histopathological examination: brain, spinal cord, sciatic nerves, thoracic aorta, heart, trachea, lung (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands (submandibular and sublingual), liver, pancreas, pituitary, thyroid glands (including parathyroids), adrenals, thymus, spleen, mesenteric lymph nodes, cervical lymph nodes, kidneys, urinary bladder, testes, epididymis, seminal vesicles, prostate, ovaries, uterus, vagina, mammary glands, skin, eyeballs, optical nerves, harderian glands, bone and bone marrow (sternum, femur), femoral muscle, and any

macroscopic lesions.

Statistics

Quantitative data were analyzed for homogeneity of variance by the Bartlett method. Data with homogeneous variance were subjected to an analysis of variance (ANOVA) with a Dunnett or Scheffe post-hoc test. Data with heterogeneous variance were analyzed by the Kruskal-Wallis rank test with a Dunnett or Scheffe post-hoc test. Statistical significance was designated at $p < 0.05$ or 0.01.

RESULTS

Based on food consumption and body weight data, the mean doses of L-Thr achieved during the treatment period were 0, 811.5, 1615.3, and 3266.9 mg/kg body weight/day in males, and 0, 909.9, 1850.0, and 3673.3 mg/kg body weight/day in females.

No overt signs of toxicity were observed in any animals administered the test diet. No mortality was observed during the treatment or recovery periods.

Data on body weight are presented in Table 1, data on food consumption are presented in Table 2, data on feed efficiency are presented in Table 3, data on L-Thr intake are presented in Table 4, and data on water intake are presented in Table 5.

Significantly lower body weight in males receiving 5.0% L-Thr was observed from day 14 to day 28 of treatment; however, there was no difference in the total body weight gain throughout the treatment and recovery period. Significant decrease in food consumption at 5.0% L-Thr was observed on days 3, 14, 17, and 52 of treatment in males, and on days 3, 42, and 52 of treatment in females; however, differences were not observed in mean food consumption during the treatment period. In addition, significant increase in food consumption in females receiving 5.0% L-Thr was observed on day 73 of treatment; however, it was thought to be an incidental change due to temporality. Transient decrease in food consumption in males receiving 2.5% L-Thr was observed on day 52 of treatment; however, it was thought to be an incidental change due to temporality. Significant decrease or a tendency toward decrease in feed efficiency in males and females receiving 5.0% L-Thr was observed in weeks 1 and 2 of treatment; however, mean feed efficiency during the treatment period was equivalent to the control. Transient decrease in water intake was observed in males receiving 5.0% L-Thr in Week 8 of treatment; however, it was thought to be an incidental change due to temporality.

No ophthalmological observations outside of incidental findings were noted (data not shown).

A summary of selected urinalysis results is provided in Table 6. In Week 5 of the treatment period, a significant increase in specific gravity was observed in females receiving 2.5% and 5.0% L-Thr, but these were not clear dose-dependent changes. In week 13 of the of the treatment period, there was a significant decrease in potassium in females receiving 5.0% L-Thr. In addition, a significant increase in specific gravity was observed in females receiving 2.5% L-Thr, but it was not a dose-dependent change.

The results of hematological and blood chemistry parameters are presented in Tables 7 and 8, respectively. No significant differences were observed in any animal receiving L-Thr at the end of the treatment period. At the end of the recovery period, a significant increase in LDH activity was observed in females receiving 5.0% L-Thr, and a significant increase in sodium in males receiving 5.0% L-Thr; however, these were not observed at the end of the treatment period, and therefore they were deemed to be incidental.

No remarkable findings were noted during gross pathological examination. Incidental findings at the end of the treatment and recovery periods are summarized in Table 9. The results of absolute and relative organ weights are presented in Tables 10 and 11, respectively. At necropsy in Week 13, a significant increase in the relative kidney weight was observed in females receiving 5.0% L-Thr compared to controls. At necropsy conducted following the 5-week recovery period, a significant decrease in the absolute thyroid weight was observed in females receiving 5.0% L-Thr, and significant decrease in the absolute ovary weight (unilateral) and significant increase in the relative ovary weight (unilateral) were observed in females receiving 5.0% L-Thr; however, these were thought to be incidental changes since they were only unilateral.

The results of histopathological findings are presented in Table 12. Histopathological changes observed at both the end of the treatment period and the recovery period were noted in the heart, stomach, cecum, liver, pancreas, spleen, kidney, testis, and eyeball; however, these were considered to be incidental based on the incidence of their occurrences and their pathological nature.

DISCUSSION

L-Thr is an essential amino acid which is added to food containing only a small amount and used in nutrition-supplement drinks together with other essential amino acids. It is also used for sports drinks in combination with var-

Table 1. Body weight (g) in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	18	12	12	18	18	12	12	18
0 days	199.4 ± 9.7	198.6 ± 9.4	198.3 ± 10.6	198.4 ± 9.2	155.8 ± 6.3	156.7 ± 6.7	156.8 ± 9.8	155.0 ± 8.7
3 days	226.8 ± 11.3	225.8 ± 10.4	224.8 ± 11.9	221.6 ± 11.2	170.9 ± 8.8	171.4 ± 10.0	170.6 ± 11.0	166.9 ± 10.2
7 days	254.5 ± 13.3	255.7 ± 11.0	252.8 ± 14.7	246.5 ± 11.9	181.8 ± 10.7	183.7 ± 10.8	181.8 ± 12.6	176.1 ± 11.1
10 days	282.1 ± 15.0	283.7 ± 13.0	278.6 ± 16.4	271.1 ± 13.8	194.7 ± 10.3	194.8 ± 11.5	194.0 ± 12.9	187.4 ± 12.5
14 days	310.5 ± 15.5	314.7 ± 14.6	307.6 ± 20.0	296.1 ± 15.1*	207.3 ± 9.9	207.5 ± 13.5	206.2 ± 15.2	198.4 ± 14.4
17 days	333.8 ± 17.7	339.4 ± 15.7	331.8 ± 22.2	317.7 ± 17.1*	218.9 ± 11.1	218.3 ± 12.7	215.4 ± 17.7	210.2 ± 15.0
21 days	355.4 ± 18.2	362.5 ± 16.6	353.0 ± 23.4	337.6 ± 20.8*	227.7 ± 12.1	227.2 ± 13.3	226.3 ± 19.0	219.3 ± 16.7
24 days	373.9 ± 21.2	383.0 ± 18.4	372.6 ± 26.2	356.1 ± 21.9*	236.4 ± 10.7	237.6 ± 16.6	234.7 ± 20.3	227.3 ± 16.9
28 days	391.9 ± 22.8	401.3 ± 20.0	385.9 ± 28.3	372.2 ± 24.3*	243.7 ± 10.5	245.8 ± 18.4	243.1 ± 20.3	232.9 ± 18.4
31 days	409.3 ± 25.2	418.4 ± 21.2	403.9 ± 33.3	388.2 ± 25.7	253.3 ± 13.8	251.3 ± 18.5	250.5 ± 22.5	238.5 ± 19.7
35 days	425.9 ± 27.6	435.8 ± 22.3	420.4 ± 35.3	406.4 ± 28.8	259.9 ± 14.8	259.3 ± 19.9	259.1 ± 21.6	245.8 ± 21.0
38 days	443.2 ± 28.9	451.8 ± 22.0	434.4 ± 35.1	421.8 ± 30.2	269.8 ± 14.6	267.1 ± 21.4	264.2 ± 23.0	252.9 ± 21.2
42 days	460.3 ± 29.0	473.3 ± 23.5	453.0 ± 36.8	437.8 ± 30.8	276.6 ± 15.2	273.3 ± 23.9	270.8 ± 26.3	258.3 ± 21.6
45 days	471.6 ± 30.6	485.6 ± 24.8	465.4 ± 38.9	449.1 ± 32.1	278.7 ± 11.9	278.0 ± 23.1	274.5 ± 26.4	262.4 ± 22.1
49 days	480.6 ± 32.7	497.6 ± 25.9	474.0 ± 40.4	458.9 ± 34.5	283.6 ± 14.3	280.0 ± 23.3	280.0 ± 27.0	266.1 ± 22.0
52 days	496.7 ± 33.8	508.6 ± 26.8	489.0 ± 41.3	472.2 ± 35.6	291.0 ± 17.9	285.4 ± 25.3	286.5 ± 28.0	270.6 ± 21.8
56 days	505.4 ± 33.2	520.3 ± 25.9	497.2 ± 42.1	482.2 ± 38.3	293.4 ± 17.3	290.4 ± 28.3	290.3 ± 28.1	273.7 ± 22.5
59 days	513.9 ± 35.2	529.3 ± 26.2	506.3 ± 43.6	491.1 ± 39.0	295.8 ± 17.5	295.1 ± 32.2	294.3 ± 28.2	277.9 ± 24.7
63 days	522.7 ± 37.1	539.6 ± 27.8	514.3 ± 43.3	497.7 ± 43.0	297.6 ± 19.3	297.3 ± 32.1	298.1 ± 29.3	279.8 ± 24.0
66 days	533.8 ± 38.2	549.9 ± 28.9	524.5 ± 45.0	509.2 ± 44.6	305.8 ± 18.8	302.7 ± 32.6	303.2 ± 29.8	287.5 ± 24.5
70 days	541.1 ± 39.2	561.0 ± 28.5	532.4 ± 48.8	515.3 ± 46.4	306.5 ± 19.5	304.8 ± 32.8	307.1 ± 29.8	287.2 ± 23.6
73 days	548.0 ± 39.2	571.3 ± 32.5	542.3 ± 49.3	524.1 ± 46.8	310.7 ± 20.5	308.5 ± 32.6	310.1 ± 32.0	292.9 ± 23.9
77 days	559.3 ± 41.6	577.3 ± 33.7	551.6 ± 48.7	532.9 ± 47.2	311.7 ± 23.4	310.9 ± 33.2	312.9 ± 33.2	295.5 ± 23.7
80 days	564.8 ± 42.1	583.1 ± 34.1	558.1 ± 49.7	540.0 ± 48.8	315.7 ± 23.1	315.8 ± 32.5	319.0 ± 32.2	297.8 ± 24.5
84 days	569.1 ± 43.7	588.2 ± 35.8	562.6 ± 49.4	544.4 ± 48.7	316.2 ± 23.0	315.1 ± 32.7	318.9 ± 33.4	298.4 ± 24.6
87 days	568.9 ± 44.7	587.0 ± 33.4	561.8 ± 51.8	544.2 ± 49.5	315.9 ± 24.0	313.5 ± 34.8	318.5 ± 34.5	297.7 ± 25.2
91 days	577.3 ± 46.3	597.2 ± 35.8	573.0 ± 52.5	554.6 ± 49.9	321.6 ± 25.5	318.9 ± 34.0	322.3 ± 33.9	300.9 ± 26.6
Gain 0-91 days	377.9 ± 45.8	398.6 ± 30.5	374.8 ± 47.4	356.1 ± 45.6	165.7 ± 23.4	162.3 ± 30.8	165.5 ± 26.5	145.9 ± 21.6
Number of animals	6			6	6			6
Recovery 1 day	556.3 ± 47.9			523.8 ± 49.2	325.5 ± 37.0			303.5 ± 34.8
Recovery 3 days	556.0 ± 46.4			527.7 ± 48.3	329.0 ± 33.0			308.3 ± 35.9
Recovery 7 days	564.3 ± 45.1			534.0 ± 48.2	332.3 ± 31.9			310.3 ± 31.5
Recovery 10 days	565.0 ± 42.5			539.2 ± 49.2	332.5 ± 32.1			311.5 ± 33.2
Recovery 14 days	569.3 ± 44.7			541.2 ± 45.4	331.2 ± 33.8			313.0 ± 37.1
Recovery 17 days	574.3 ± 44.6			546.8 ± 43.9	337.3 ± 39.1			316.2 ± 33.6
Recovery 21 days	579.7 ± 46.1			553.5 ± 46.6	339.5 ± 37.0			319.3 ± 33.5
Recovery 24 days	579.7 ± 45.3			557.2 ± 49.2	345.3 ± 39.6			322.8 ± 35.8
Recovery 28 days	581.3 ± 44.4			560.2 ± 49.7	347.8 ± 41.2			319.5 ± 35.1
Recovery 31 days	582.8 ± 46.9			559.7 ± 50.0	347.2 ± 40.3			321.5 ± 34.1
Recovery 35 days	584.8 ± 45.0			561.2 ± 50.2	351.8 ± 40.8			323.2 ± 34.8
Gain Recovery 1-35 days	28.5 ± 13.4			37.3 ± 8.7	26.3 ± 6.8			19.7 ± 7.9

Values are mean ± S.D.

* : $p < 0.05$: Significant difference from control

Table 2. Food consumption (g/rat/day) in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	18	12	12	18	18	12	12	18
0 days	22.1 ± 1.4	22.8 ± 2.9	21.7 ± 2.1	22.3 ± 1.7	16.5 ± 1.6	16.8 ± 1.5	16.8 ± 1.7	16.4 ± 1.5
3 days	23.0 ± 1.1	23.2 ± 2.0	22.0 ± 1.7	21.1 ± 1.4**	17.3 ± 1.8	17.3 ± 1.7	17.2 ± 1.4	15.6 ± 1.4**
7 days	23.5 ± 1.4	24.1 ± 1.4	23.5 ± 1.7	22.4 ± 1.3	16.9 ± 1.6	17.3 ± 1.2	17.2 ± 1.6	16.3 ± 1.1
10 days	25.1 ± 1.8	26.3 ± 2.7	25.4 ± 1.6	24.1 ± 1.7	17.7 ± 1.6	18.0 ± 1.9	18.7 ± 1.9	16.7 ± 1.9
14 days	26.3 ± 1.6	26.5 ± 1.5	25.9 ± 2.0	24.7 ± 1.6*	18.3 ± 1.4	18.1 ± 1.6	18.5 ± 1.9	17.2 ± 1.3
17 days	27.0 ± 1.9	27.7 ± 1.8	27.0 ± 2.0	25.3 ± 1.7*	19.0 ± 1.9	18.1 ± 1.6	18.6 ± 1.7	17.6 ± 1.5
21 days	26.8 ± 1.9	27.3 ± 1.5	26.8 ± 2.1	25.4 ± 1.9	18.4 ± 1.2	18.6 ± 1.9	18.7 ± 1.7	17.7 ± 1.5
24 days	27.0 ± 2.4	28.0 ± 1.7	26.9 ± 2.0	25.9 ± 1.7	19.0 ± 1.6	19.2 ± 2.3	19.0 ± 1.5	18.1 ± 1.5
28 days	27.3 ± 2.0	28.2 ± 1.5	26.4 ± 2.0	26.2 ± 1.9	19.0 ± 1.4	19.3 ± 2.1	19.0 ± 1.7	17.9 ± 1.5
31 days	28.5 ± 3.3	28.2 ± 2.4	27.1 ± 2.7	27.1 ± 2.1	20.0 ± 2.3	19.7 ± 2.8	19.6 ± 2.7	18.7 ± 2.1
35 days	28.2 ± 2.0	28.4 ± 2.5	27.5 ± 2.4	27.4 ± 2.4	19.7 ± 1.4	19.6 ± 2.2	19.7 ± 1.8	18.3 ± 1.7
38 days	29.2 ± 2.1	30.8 ± 2.3	29.0 ± 2.1	29.3 ± 2.2	21.6 ± 2.6	22.1 ± 3.2	22.7 ± 3.2	21.2 ± 2.6
42 days	28.3 ± 1.4	29.4 ± 1.8	27.5 ± 2.3	27.6 ± 1.9	19.9 ± 1.3	19.5 ± 2.7	19.6 ± 2.1	18.1 ± 1.6*
45 days	28.3 ± 1.8	29.6 ± 2.8	28.0 ± 2.0	27.9 ± 1.7	19.2 ± 1.1	19.5 ± 2.7	20.4 ± 2.8	19.1 ± 1.7
49 days	27.6 ± 1.9	28.7 ± 1.8	27.0 ± 2.1	26.9 ± 2.3	19.4 ± 1.8	19.0 ± 2.3	19.3 ± 1.9	18.0 ± 2.0
52 days	31.7 ± 3.5	28.6 ± 1.9	27.9 ± 1.9*	27.6 ± 2.1**	21.0 ± 2.2	19.4 ± 2.7	19.9 ± 1.9	18.3 ± 2.0**
56 days	27.4 ± 2.0	28.2 ± 2.0	26.8 ± 1.9	27.2 ± 2.3	19.1 ± 1.4	18.9 ± 2.7	19.0 ± 1.5	18.4 ± 1.4
59 days	27.2 ± 2.1	27.7 ± 1.8	26.4 ± 1.7	26.2 ± 2.3	18.3 ± 2.3	18.8 ± 3.1	18.6 ± 1.9	17.4 ± 1.6
63 days	27.2 ± 2.0	28.0 ± 1.6	26.6 ± 2.2	26.5 ± 2.7	18.3 ± 1.8	18.8 ± 2.7	18.8 ± 1.9	18.1 ± 1.2
66 days	27.9 ± 2.2	29.1 ± 2.4	27.2 ± 1.9	26.8 ± 2.5	19.1 ± 1.7	19.5 ± 2.7	19.9 ± 2.5	19.6 ± 1.5
70 days	27.1 ± 2.0	28.1 ± 2.3	26.5 ± 2.6	25.7 ± 2.7	18.4 ± 1.6	18.0 ± 2.4	19.1 ± 2.1	17.7 ± 1.5
73 days	26.7 ± 1.7	27.9 ± 2.0	26.9 ± 1.9	27.5 ± 2.1	17.8 ± 1.8	17.7 ± 2.4	19.0 ± 2.3	19.7 ± 2.1*
77 days	27.2 ± 2.2	27.8 ± 2.2	27.2 ± 2.2	26.7 ± 2.2	18.2 ± 1.5	18.0 ± 2.3	18.4 ± 1.8	18.1 ± 1.2
80 days	27.1 ± 1.8	28.5 ± 2.4	27.1 ± 1.8	27.5 ± 2.7	19.8 ± 2.3	19.1 ± 3.0	18.9 ± 2.1	17.9 ± 1.6
84 days	26.2 ± 1.7	26.8 ± 2.1	26.2 ± 2.3	25.9 ± 2.1	17.8 ± 1.7	17.8 ± 2.1	17.7 ± 1.9	17.4 ± 1.3
87 days	23.6 ± 1.8	22.5 ± 1.4	22.5 ± 2.2	22.7 ± 1.7	16.5 ± 2.0	15.2 ± 1.9	16.1 ± 1.8	16.1 ± 1.2
91 days	27.1 ± 2.4	27.2 ± 2.5	26.8 ± 2.3	26.0 ± 2.3	18.1 ± 1.4	18.1 ± 2.2	17.8 ± 1.6	17.2 ± 1.9
Average 0-91 days	27.0 ± 1.6	27.6 ± 1.7	26.5 ± 1.8	26.1 ± 1.7	18.7 ± 1.2	18.6 ± 2.0	18.9 ± 1.7	17.9 ± 1.2
Number of animals	6			6	6			6
Recovery 3 days	25.0 ± 0.9			26.5 ± 2.6	17.8 ± 2.0			19.3 ± 3.2
Recovery 7 days	27.0 ± 0.5			26.8 ± 2.9	18.2 ± 0.9			18.7 ± 1.2
Recovery 10 days	26.2 ± 1.3			27.8 ± 2.6	18.4 ± 1.6			19.0 ± 2.4
Recovery 14 days	26.7 ± 0.8			26.3 ± 2.9	19.1 ± 1.4			19.4 ± 2.6
Recovery 17 days	27.6 ± 1.4			28.9 ± 3.1	21.4 ± 1.9			20.8 ± 1.1
Recovery 21 days	27.3 ± 1.0			28.1 ± 2.2	19.8 ± 1.1			20.0 ± 1.7
Recovery 24 days	26.6 ± 1.1			29.4 ± 3.9	21.1 ± 2.3			21.3 ± 1.7
Recovery 28 days	26.0 ± 0.7			27.5 ± 3.0	19.6 ± 1.6			18.5 ± 1.2
Recovery 31 days	24.4 ± 1.3			25.7 ± 2.9	18.1 ± 2.9			17.8 ± 0.7
Recovery 35 days	25.7 ± 1.3			27.1 ± 2.6	20.4 ± 1.8			19.2 ± 1.8
Average Recovery 0-35 days	26.3 ± 0.7			27.4 ± 2.5	19.4 ± 1.4			19.4 ± 1.4

Values are mean ± S.D.

* : $p < 0.05$; ** : $p < 0.01$: Significant difference from control

Table 3. Feed efficiency (%) in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	18	12	12	18	18	12	12	18
1 week	33.8 ± 2.9	34.4 ± 1.9	34.1 ± 2.6	31.5 ± 1.8*	21.6 ± 3.4	22.1 ± 3.2	20.6 ± 3.3	18.7 ± 4*
2 weeks	31.0 ± 2.4	31.9 ± 1.4	30.4 ± 2.4	29.0 ± 3*	20.3 ± 3.3	18.8 ± 3.7	18.6 ± 2.6	18.5 ± 3.9
3 weeks	23.8 ± 2.3	24.9 ± 1.8	24.1 ± 2.5	23.3 ± 2.7	15.5 ± 4.1	15.3 ± 3.9	15.3 ± 3.6	16.9 ± 2.3
4 weeks	19.1 ± 2.8	19.7 ± 2.1	17.6 ± 5.5	18.9 ± 2.3	12.1 ± 4.4	13.5 ± 3.2	12.6 ± 3.2	10.8 ± 2.7
5 weeks	17.1 ± 3.0	17.3 ± 2.5	17.8 ± 4.5	17.9 ± 2.1	11.5 ± 5.0	9.6 ± 3.9	11.6 ± 2.1	9.9 ± 5.0
6 weeks	17.1 ± 2.8	17.9 ± 3.0	16.5 ± 3.0	15.8 ± 1.8	11.5 ± 4.9	9.4 ± 4.2	7.8 ± 4.1	9.2 ± 3.4
7 weeks	10.3 ± 3.6	11.9 ± 2.3	10.9 ± 1.7	10.9 ± 2.5	5.2 ± 4.6	5.2 ± 4.3	6.6 ± 3.5	5.9 ± 4.5
8 weeks	12.2 ± 3.0	11.4 ± 3.0	12.1 ± 1.3	12.0 ± 2.8	6.9 ± 4.1	7.5 ± 4.1	7.6 ± 2.6	6.0 ± 3.6
9 weeks	9.0 ± 2.5	9.8 ± 1.7	9.3 ± 2.7	8.2 ± 4.2	3.0 ± 3.8	4.9 ± 3.7	5.9 ± 2.5	4.7 ± 3.9
10 weeks	9.5 ± 1.7	10.8 ± 1.8	9.4 ± 4.0	9.4 ± 3.2	6.8 ± 2.9	5.6 ± 5.3	6.6 ± 2.3	5.6 ± 4.7
11 weeks	9.6 ± 3.2	8.2 ± 3.8	10.2 ± 2.1	9.3 ± 2.1	3.9 ± 4.9	5.0 ± 3.8	4.2 ± 3.5	6.4 ± 3.8
12 weeks	5.2 ± 2.3	5.5 ± 2.3	5.9 ± 1.0	6.1 ± 2.3	3.4 ± 4.1	3.2 ± 2.9	4.7 ± 2.5	2.3 ± 3.1
13 weeks	4.5 ± 3.1	5.0 ± 2.5	5.9 ± 2.5	5.8 ± 2.2	4.2 ± 3.7	3.1 ± 3.5	2.8 ± 2.9	2.0 ± 4.7
Average 1-13 weeks	15.5 ± 1.2	16.1 ± 0.6	15.7 ± 1.2	15.2 ± 1.2	9.7 ± 1.2	9.5 ± 0.9	9.6 ± 0.9	9.0 ± 0.9
Number of animals	6			6	6			6
Recovery 1 week	4.9 ± 3.6			6.6 ± 1.2	2.8 ± 2.9			5.1 ± 5.2
Recovery 2 weeks	2.7 ± 2.2			3.8 ± 3.9	-1.0 ± 2.7			1.6 ± 4.6
Recovery 3 weeks	5.3 ± 3.0			6.2 ± 3.5	5.8 ± 5.2			4.5 ± 4.6
Recovery 4 weeks	0.9 ± 3.7			3.2 ± 2.1	5.4 ± 6.8			0.1 ± 3.3
Recovery 5 weeks	1.9 ± 2.3			0.5 ± 1.9	2.9 ± 1.7			2.8 ± 1.9
Average Recovery 1-5 weeks	3.2 ± 1.5			4.1 ± 0.7	3.2 ± 0.8			2.8 ± 0.8

Values are mean ± S.D.
* : $p < 0.05$: Significant difference from control

Table 4. L-Thr intake in rats by dietary administration (mg/kg/day).

Sex	Male			Female		
Concentration(%)	1.25	2.5	5.0	1.25	2.5	5.0
Number of animals	12	12	18	12	12	18
1 week	1304.6 ± 64.2	2531.3 ± 93.5	4899.6 ± 174.9	1269.8 ± 69.2	2537.9 ± 137.6	4842.8 ± 212.4
2 weeks	1158.6 ± 55.2	2290.5 ± 57.9	4505.0 ± 201.6	1152.0 ± 60.0	2395.3 ± 140.5	4540.0 ± 210.5
3 weeks	1013.7 ± 30.7	2036.3 ± 78.0	3997.7 ± 121.5	1057.9 ± 61.9	2160.1 ± 47.7	4222.3 ± 103.8
4 weeks	919.9 ± 18.8	1805.1 ± 102.1	3671.7 ± 118.1	1015.8 ± 66.4	2029.0 ± 61.6	3981.0 ± 173.0
5 weeks	845.0 ± 35.0	1694.7 ± 67.4	3502.8 ± 127.8	971.7 ± 71.0	1954.2 ± 120.9	3867.0 ± 265.0
6 weeks	824.3 ± 23.1	1613.2 ± 77.5	3361.0 ± 144.3	966.1 ± 63.2	1979.4 ± 131.5	3854.5 ± 227.6
7 weeks	747.6 ± 22.2	1479.5 ± 58.1	3051.0 ± 106.3	866.4 ± 50.8	1795.6 ± 112.6	3530.3 ± 260.4
8 weeks	696.9 ± 27.5	1408.1 ± 67.4	2913.0 ± 112.8	835.4 ± 51.0	1702.9 ± 96.8	3409.0 ± 226.1
9 weeks	657.7 ± 14.2	1312.7 ± 50.4	2688.2 ± 121.0	797.2 ± 49.6	1593.6 ± 86.2	3222.7 ± 174.0
10 weeks	647.4 ± 26.5	1282.1 ± 51.5	2582.1 ± 126.5	773.9 ± 45.5	1609.6 ± 115.3	3271.6 ± 187.0
11 weeks	612.0 ± 19.3	1250.8 ± 65.5	2584.2 ± 131.2	725.2 ± 43.5	1508.8 ± 96.7	3235.0 ± 249.5
12 weeks	590.2 ± 22.5	1195.4 ± 46.5	2471.8 ± 126.3	733.8 ± 54.4	1447.1 ± 113.1	2976.7 ± 149.8
13 weeks	531.3 ± 25.4	1098.6 ± 38.1	2241.8 ± 89.2	663.7 ± 41.9	1336.3 ± 92.8	2799.9 ± 172.7
Average 1-13 weeks	811.5 ± 18.3	1615.3 ± 43.3	3266.9 ± 74.1	909.9 ± 40.1	1850.0 ± 77.1	3673.3 ± 140.9

Values are mean ± S.D.

Table 5. Water intake in rats following dietary administration of L-threonine (ml/rat/day).

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	18	12	12	18	18	12	12	18
0 weeks	30.5 ± 3.3	31.6 ± 3.0	34.8 ± 5.2*	31.3 ± 3.5	28.7 ± 6.1	29.7 ± 4.8	27.0 ± 4.6	29.8 ± 5.9
1 week	38.6 ± 5.1	39.0 ± 6.7	42.4 ± 11.7	37.3 ± 5.1	35.5 ± 12.5	32.3 ± 4.2	30.1 ± 5.7	33.3 ± 11.9
2 weeks	44.5 ± 9.7	43.5 ± 8.3	42.2 ± 6.9	39.3 ± 6.6	34.8 ± 9.0	38.3 ± 10.9	32.2 ± 6.6	34.3 ± 9.0
3 weeks	46.4 ± 11.6	48.3 ± 8.6	48.0 ± 13.5	43.6 ± 6.9	36.1 ± 7.5	35.4 ± 10.0	36.2 ± 9.3	42.9 ± 14.1
4 weeks	52.6 ± 14.6	45.1 ± 12.5	44.8 ± 12.5	45.1 ± 14.8	40.6 ± 13.5	39.8 ± 13.8	32.8 ± 8.8	37.4 ± 13.0
5 weeks	50.7 ± 13.2	49.3 ± 8.0	51.8 ± 17.1	48.3 ± 9.2	48.8 ± 14.0	44.1 ± 19.7	40.3 ± 7.0	39.8 ± 10.1
6 weeks	54.3 ± 16.5	54.4 ± 9.3	51.9 ± 13.6	48.5 ± 10.9	48.2 ± 14.6	44.8 ± 15.2	39.8 ± 8.8	43.7 ± 10.4
7 weeks	50.3 ± 12.8	46.8 ± 9.9	47.6 ± 12.7	43.9 ± 5.6	47.7 ± 14.0	45.6 ± 16.9	44.1 ± 11.9	40.5 ± 8.3
8 weeks	53.8 ± 12.5	46.2 ± 7.1	46.3 ± 12.2	43.7 ± 4.8*	39.1 ± 6.9	46.0 ± 17.9	36.4 ± 9.7	41.8 ± 7.6
9 weeks	50.1 ± 13.3	45.0 ± 7.7	49.1 ± 14.2	41.5 ± 7.9	37.9 ± 8.2	42.3 ± 15.1	37.9 ± 11.9	37.4 ± 7.5
10 weeks	53.9 ± 12.5	55.0 ± 16.0	51.3 ± 13.9	45.5 ± 7.1	45.4 ± 11.4	42.1 ± 8.5	35.9 ± 6.7	39.3 ± 12.9
11 weeks	54.1 ± 15.8	52.8 ± 13.3	51.3 ± 13.6	45.8 ± 7.0	39.2 ± 11.1	41.5 ± 12.5	36.0 ± 7.0	42.2 ± 8.3
12 weeks	52.7 ± 11.9	50.8 ± 14.9	48.7 ± 14.2	43.2 ± 6.6	43.1 ± 13.3	40.5 ± 10.4	36.5 ± 7.1	39.1 ± 7.4
13 weeks	53.6 ± 13.5	52.9 ± 10.5	51.7 ± 13.1	48.8 ± 10.0	41.3 ± 10.7	43.4 ± 11.2	40.5 ± 14.3	42.2 ± 10.6
Number of animals	6			6	6			6
Recovery 1 week	52.7 ± 8.6			50.3 ± 19.3	42.5 ± 9.4			50.2 ± 15.9
Recovery 2 weeks	54.2 ± 6.3			46.3 ± 10.1	48.5 ± 13.7			46.3 ± 6.0
Recovery 3 weeks	53.3 ± 7.7			47.8 ± 9.8	39.8 ± 10.4			38.2 ± 6.9
Recovery 4 weeks	49.0 ± 6.7			46.0 ± 7.9	48.3 ± 6.7			42.3 ± 5.2
Recovery 5 weeks	52.5 ± 10.3			51.2 ± 21.0	47.5 ± 8.8			46.7 ± 16.5

Values are mean ± S.D.

* : $p < 0.05$: Significant difference from control

Table 6. Selected urinalysis parameters in rats following dietary administration of L-threonine.

Sex	Concentration (%)	Number of animals	Urine Volume (mL/24 hr)	Specific Gravity (g/mL)	Na (mEq/24 hr)	K (mEq/24 hr)	Cl (mEq/24 hr)
At Week 5 of Treatment							
Male	0	12	13.7 ± 5.9	1.067 ± 0.019	1.52 ± 0.28	3.93 ± 0.66	2.13 ± 0.37
	1.25	12	12.3 ± 2.9	1.071 ± 0.013	1.58 ± 0.42	3.91 ± 0.54	2.17 ± 0.39
	2.5	12	13.5 ± 4.5	1.067 ± 0.011	1.51 ± 0.27	3.81 ± 0.76	2.19 ± 0.39
	5.0	12	12.4 ± 2.7	1.075 ± 0.014	1.57 ± 0.21	3.82 ± 0.64	2.12 ± 0.27
Female	0	12	6.7 ± 4.7	1.053 ± 0.017	0.61 ± 0.42	1.54 ± 0.89	0.86 ± 0.55
	1.25	12	7.6 ± 3.7	1.063 ± 0.014	0.76 ± 0.32	1.98 ± 0.78	1.08 ± 0.45
	2.5	12	6.4 ± 2.2	1.077 ± 0.013**	0.78 ± 0.30	2.09 ± 0.79	1.13 ± 0.43
	5.0	12	6.6 ± 2.0	1.068 ± 0.013*	0.67 ± 0.24	1.71 ± 0.41	0.92 ± 0.23
At Week 13 of Treatment							
Male	0	12	19.2 ± 9.0	1.055 ± 0.018	1.76 ± 0.37	3.86 ± 0.73	2.22 ± 0.48
	1.25	12	16.1 ± 5.2	1.059 ± 0.016	1.55 ± 0.37	3.61 ± 0.48	2.04 ± 0.40
	2.5	12	15.9 ± 5.0	1.056 ± 0.011	1.54 ± 0.40	3.37 ± 0.52	1.99 ± 0.35
	5.0	12	13.9 ± 4.7	1.067 ± 0.015	1.43 ± 0.34	3.50 ± 069	1.88 ± 0.41
Female	0	12	9.5 ± 3.3	1.051 ± 0.010	0.91 ± 0.30	2.07 ± 0.56	1.17 ± 0.37
	1.25	12	11.4 ± 3.2	1.050 ± 0.007	1.05 ± 0.35	2.37 ± 0.58	1.38 ± 0.43
	2.5	12	8.8 ± 3.0	1.060 ± 0.009*	0.99 ± 0.29	2.13 ± 0.46	1.27 ± 0.35
	5.0	12	8.0 ± 4.5	1.052 ± 0.010	0.65 ± 0.28	1.46 ± 0.63*	0.83 ± 0.31
At Week 5 of Recovery							
Male	0	6	19.2 ± 6.4	1.051 ± 0.015	2.03 ± 0.31	4.00 ± 0.31	2.42 ± 0.17
	5.0	6	14.4 ± 5.3	1.061 ± 0.011	1.80 ± 0.64	3.77 ± 1.50	2.33 ± 0.84
Female	0	6	10.4 ± 3.3	1.054 ± 0.015	1.14 ± 0.20	2.31 ± 0.41	1.42 ± 0.24
	5.0	6	12.4 ± 4.6	1.051 ± 0.018	1.05 ± 0.09	2.36 ± 0.26	1.39 ± 0.10

Values are mean ± S.D.

* : $p < 0.05$; ** : $p < 0.01$: Significant difference from control

Table 7. Hematological parameters in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
Red blood cell count ($x10^4/mm^3$)	857.5 ± 40.5	831.8 ± 52.2	838.8 ± 49.7	859.0 ± 37.4	776.4 ± 27.4	782.9 ± 21.9	778.5 ± 36.9	778.8 ± 34.9
Hemoglobin (g/dL)	15.5 ± 0.4	15.4 ± 0.5	15.6 ± 0.6	15.5 ± 0.5	15.0 ± 0.6	15.3 ± 0.4	14.9 ± 0.7	15.5 ± 0.7
Hematocrit (%)	46.1 ± 2.1	45.2 ± 2.1	46.1 ± 2.6	46.3 ± 1.8	43.3 ± 1.8	44.3 ± 1.1	42.8 ± 1.9	44.0 ± 1.9
Mean corpuscular volume (μ^3)	53.7 ± 2.0	54.3 ± 1.8	55.1 ± 1.6	54.0 ± 1.2	55.6 ± 2.0	56.3 ± 1.6	55.1 ± 1.8	56.6 ± 1.7
Mean corpuscular hemoglobin (pg)	18.1 ± 0.8	18.6 ± 0.9	18.6 ± 0.7	18.0 ± 0.8	19.3 ± 0.8	19.5 ± 0.6	19.1 ± 0.8	19.8 ± 0.7
Mean corpuscular hemoglobin concentration (%)	33.7 ± 1.0	34.2 ± 0.9	33.9 ± 1.1	33.4 ± 1.1	34.8 ± 0.5	34.7 ± 0.7	34.6 ± 0.5	35.1 ± 0.6
Reticulocyte ratio (%)	16.6 ± 3.8	19.9 ± 4.6	18.5 ± 4.1	19.6 ± 3.5	18.7 ± 3.7	17.7 ± 3.4	18.0 ± 3.3	15.9 ± 4.2
Platelelt count ($x10^4/mm^3$)	114.6 ± 12.6	112.0 ± 10.3	117.0 ± 9.6	114.8 ± 11.2	106.9 ± 9.1	115.2 ± 13.6	108.3 ± 11.5	113.9 ± 15.3
Prothrombin time (sec)	11.6 ± 0.3	11.7 ± 0.4	11.7 ± 0.5	11.6 ± 0.2	11.2 ± 0.4	11.2 ± 0.4	11.1 ± 0.3	11.5 ± 0.4
Activated partial thromboplastin time (sec)	15.0 ± 1.1	15.5 ± 1.1	15.7 ± 1.1	16.1 ± 1.2	13.3 ± 0.6	13.2 ± 0.7	13.3 ± 0.9	13.6 ± 0.6
Fibrinogen (mg/dL)	266.1 ± 33.8	279.6 ± 30.3	272.8 ± 26.8	258.3 ± 19.2	172.3 ± 18.6	181.2 ± 23.9	190.8 ± 47.7	181.5 ± 23.9
White blood cell count ($x10^2/mm^3$)	94.0 ± 27.3	95.5 ± 25.7	106.1 ± 18.4	91.7 ± 18.1	51.5 ± 19.3	56.9 ± 15.6	62.7 ± 20.1	58.5 ± 20.5
Lymphocytes (%)	86.0 ± 5.0	84.0 ± 4.4	82.4 ± 9.1	83.0 ± 6.0	84.9 ± 8.5	82.6 ± 9.2	85.5 ± 4.9	87.6 ± 5.9
Stab (%)	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
Seg. (%)	13.1 ± 5.2	15.3 ± 4.4	16.8 ± 9.1	16.5 ± 6.2	14.1 ± 8.0	16.5 ± 8.8	13.2 ± 5.1	11.8 ± 5.7
Eosinophils (%)	0.8 ± 0.6	0.6 ± 0.6	0.8 ± 0.5	0.5 ± 0.7	0.9 ± 0.9	0.8 ± 0.9	1.1 ± 0.8	0.5 ± 0.7
Basophils (%)	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Monocytes (%)	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.1
Others (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
At Week 5 of Recovery								
Number of animals	6			6	6			6
Red blood cell count ($x10^4/mm^3$)	878.5 ± 21.4			848.0 ± 42.9	767.2 ± 40.2			773.5 ± 28.2
Hemoglobin (g/dL)	16.3 ± 0.2			15.9 ± 0.5	15.4 ± 0.7			15.8 ± 0.6
Hematocrit (%)	47.3 ± 0.8			46.2 ± 1.9	44.0 ± 1.7			44.7 ± 1.5
Mean corpuscular volume (μ^3)	53.7 ± 0.9			54.3 ± 1.0	57.4 ± 1.4			57.6 ± 2.4
Mean corpuscular hemoglobin (pg)	18.6 ± 0.5			18.7 ± 0.5	20.1 ± 0.5			20.4 ± 1.0
Mean corpuscular hemoglobin concentration (%)	34.6 ± 0.8			34.5 ± 0.6	35.0 ± 0.3			35.4 ± 0.3
Reticulocyte ratio (%)	16.0 ± 3.1			15.0 ± 4.7	19.3 ± 3.1			16.7 ± 3.6
Platelelt count ($x10^4/mm^3$)	120.3 ± 16.2			114.2 ± 13.7	108.9 ± 11.6			118.4 ± 6.5
Prothrombin time (sec)	12.3 ± 0.5			12.2 ± 0.8	11.9 ± 0.3			11.6 ± 0.3
Activated partial thromboplastin time (sec)	17.0 ± 1.2			16.8 ± 1.3	13.8 ± 1.0			14.0 ± 0.9
Fibrinogen (mg/dL)	269.2 ± 31.4			275.0 ± 50.6	177.3 ± 35.0			183.0 ± 28.3
White blood cell count ($x10^2/mm^3$)	81.7 ± 24.6			114.0 ± 46.6	74.8 ± 25.0			52.8 ± 17.8
Lymphocytes (%)	75.5 ± 6.4			81.3 ± 6.3	83.8 ± 7.4			85.8 ± 3.0
Stab (%)	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0
Seg. (%)	23.8 ± 6.4			18.3 ± 6.3	14.8 ± 7.0			13.2 ± 3.4
Eosinophils (%)	0.6 ± 0.7			0.3 ± 0.4	1.3 ± 0.7			1.0 ± 0.8
Basophils (%)	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0
Monocytes (%)	0.2 ± 0.3			0.2 ± 0.4	0.1 ± 0.2			0.1 ± 0.2
Others (%)	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0

Values are mean ± S.D.

Table 8. Blood chemistry parameters in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
AST (IU/L)	50.6 ± 6.8	51.2 ± 14.0	47.4 ± 9.7	45.8 ± 10.6	62.2 ± 19.3	61.3 ± 17.1	54.7 ± 11.0	52.8 ± 7.1
ALT (IU/L)	36.3 ± 5.7	35.6 ± 8.0	35.5 ± 6.0	36.5 ± 4.7	37.7 ± 9.9	40.3 ± 18.4	36.5 ± 12.1	33.3 ± 4.4
LDH (IU/L)	36.4 ± 10.7	27.8 ± 8.0	33.2 ± 10.9	35.2 ± 12.2	28.9 ± 11.2	25.8 ± 13.2	25.2 ± 12.5	22.8 ± 7.3
ALP (IU/L)	196.6 ± 35.0	195.2 ± 42.9	202.5 ± 29.7	179.8 ± 28.2	95.7 ± 29.2	108.7 ± 32.2	113.1 ± 31.0	116.3 ± 30.7
Total cholesterol (mg/dL)	83.4 ± 21.0	90.4 ± 21.4	101.8 ± 19.8	90.5 ± 13.1	96.7 ± 13.8	93.0 ± 17.3	101.8 ± 18.4	94.2 ± 26.1
Triglyceride (mg/dL)	87.4 ± 37.7	102.1 ± 40.9	76.6 ± 25.5	86.8 ± 39.7	45.0 ± 6.5	47.8 ± 18.5	53.4 ± 24.5	41.8 ± 8.6
Phospholipid (mg/dL)	129.8 ± 27.2	141.3 ± 24.9	147.8 ± 23.4	140.4 ± 19.3	186.2 ± 25.0	175.0 ± 27.4	193.4 ± 28.9	179.8 ± 42.9
Total bilirubin (mg/dL)	0.12 ± 0.02	0.12 ± 0.03	0.13 ± 0.03	0.12 ± 0.03	0.11 ± 0.01	0.11 ± 0.03	0.11 ± 0.01	0.11 ± 0.02
Glucose (mg/dL)	130.8 ± 12.6	128.8 ± 14.5	128.4 ± 20.9	131.3 ± 10.7	131.0 ± 11.8	122.9 ± 11.8	131.3 ± 10.6	127.8 ± 10.6
Blood urea nitrogen (mg/dL)	14.8 ± 0.8	14.8 ± 1.7	14.4 ± 1.3	15.3 ± 1.4	15.6 ± 1.5	15.5 ± 1.6	16.0 ± 2.8	15.5 ± 1.6
Creatinine (mg/dL)	0.58 ± 0.04	0.59 ± 0.04	0.59 ± 0.05	0.60 ± 0.03	0.57 ± 0.07	0.61 ± 0.09	0.61 ± 0.04	0.62 ± 0.06
Na (mmol/L)	145.4 ± 1.4	145.9 ± 1.4	146.2 ± 1.2	145.3 ± 1.1	144.5 ± 0.9	144.6 ± 1	144.3 ± 1.3	144.8 ± 1
K (mmol/L)	4.7 ± 0.3	4.7 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.3 ± 0.3	4.7 ± 0.4
Chloride (mmol/L)	116.4 ± 1.5	115.8 ± 1.7	116.3 ± 1.2	115.8 ± 1.4	117.6 ± 1.2	117.9 ± 1.6	116.8 ± 1.1	117.3 ± 1
Calcium (mg/dL)	9.5 ± 0.3	9.6 ± 0.3	9.5 ± 0.2	9.4 ± 0.3	9.6 ± 0.3	9.5 ± 0.4	9.7 ± 0.3	9.4 ± 0.3
P (mg/dL)	7.3 ± 0.8	7.5 ± 1	7.4 ± 1	7.6 ± 0.9	5.7 ± 1	6.2 ± 1.3	6.4 ± 1.2	6.3 ± 0.8
Total protein (g/dL)	6.8 ± 0.4	6.9 ± 0.2	6.9 ± 0.3	6.9 ± 0.3	7.1 ± 0.3	7.1 ± 0.4	7.3 ± 0.5	7.2 ± 0.5
A/G ratio	0.80 ± 0.05	0.75 ± 0.07	0.77 ± 0.08	0.78 ± 0.07	1.07 ± 0.10	1.00 ± 0.11	1.02 ± 0.14	0.96 ± 0.10
Albumin (%)	44.4 ± 1.5	42.8 ± 2.4	43.5 ± 2.6	43.6 ± 2.3	51.5 ± 2.3	49.8 ± 2.6	50.3 ± 3.4	48.9 ± 2.6
Globulin(%)								
α1-globulin (%)	20.0 ± 1.8	21.7 ± 1.9	21.1 ± 2.4	21.6 ± 2.2	17.2 ± 2.3	16.8 ± 2.3	17.7 ± 1.9	17.6 ± 1.0
α2-globulin (%)	10.9 ± 0.7	10.8 ± 0.8	10.8 ± 0.7	10.4 ± 1.8	8.6 ± 0.8	8.9 ± 0.9	9.0 ± 1.1	9.2 ± 0.7
β-globulin (%)	18.1 ± 1.1	18.3 ± 1.6	17.8 ± 1.2	18.1 ± 1.5	15.5 ± 1.3	16.1 ± 1.5	15.6 ± 1.9	16.4 ± 1.5
γ-globulin (%)	6.5 ± 1.0	6.4 ± 1.1	6.7 ± 0.6	6.4 ± 1.0	7.2 ± 1.0	8.4 ± 2.0	7.3 ± 1.7	7.9 ± 1.3
At Week 5 of Recovery								
Number of animals	6			6	6			6
AST (IU/L)	40.2 ± 14.8			41.2 ± 7.1	54.3 ± 5.2			55.8 ± 13.7
ALT (IU/L)	38.0 ± 4.6			35.5 ± 3.1	33.7 ± 6.5			45.8 ± 28.1
LDH (IU/L)	37.3 ± 12.6			35.3 ± 10.7	15.3 ± 2.8			20.5 ± 5.8*
ALP (IU/L)	128.2 ± 36.5			137.5 ± 23.0	65.2 ± 11.5			71.2 ± 14.9
Total cholesterol (mg/dL)	84.7 ± 24.6			81.2 ± 15.9	92.0 ± 22.6			92.7 ± 19.6
Triglyceride (mg/dL)	110.3 ± 54.6			74.5 ± 16.6	45.5 ± 20.9			40.2 ± 7.7
Phospholipid (mg/dL)	135.3 ± 28.5			125.8 ± 16.9	184.2 ± 42.0			187.0 ± 40.6
Total bilirubin (mg/dL)	0.14 ± 0.03			0.11 ± 0.01	0.12 ± 0.02			0.12 ± 0.01
Glucose (mg/dL)	125.8 ± 9.0			129.8 ± 9.9	114.5 ± 4.4			108.5 ± 5.2
Blood urea nitrogen (mg/dL)	14.0 ± 0.9			14.5 ± 0.5	12.7 ± 1.2			13.7 ± 1.8
Creatinine (mg/dL)	0.63 ± 0.05			0.61 ± 0.02	0.63 ± 0.05			0.65 ± 0.05
Na (mmol/L)	142.7 ± 1.0			144.2 ± 0.8*	142.5 ± 0.8			142.2 ± 1.7
K (mmol/L)	4.7 ± 0.2			4.8 ± 0.3	4.5 ± 0.4			4.6 ± 0.1
Chloride (mmol/L)	115.8 ± 2.2			114.7 ± 1.9	117.7 ± 1.2			117.5 ± 1.8
Calcium (mg/dL)	9.2 ± 0.2			9.1 ± 0.1	9.2 ± 0.3			9.4 ± 0.2
P (mg/dL)	6.7 ± 0.2			6.6 ± 0.5	5.8 ± 0.6			6.3 ± 0.2
Total protein (g/dL)	7.0 ± 0.3			6.8 ± 0.3	7.2 ± 0.3			7.4 ± 0.5
A/G ratio	0.77 ± 0.07			0.74 ± 0.08	0.96 ± 0.10			0.98 ± 0.22
Albumin (%)	43.4 ± 2.1			42.5 ± 2.5	48.7 ± 2.4			48.9 ± 5.0
Globulin(%)								
α1-globulin (%)	22.7 ± 1.7			23.1 ± 1.4	18.0 ± 1.4			18.5 ± 2.8
α2-globulin (%)	9.7 ± 0.5			10.1 ± 0.7	8.7 ± 1.0			8.5 ± 0.5
β-globulin (%)	17.9 ± 1.1			18.0 ± 1.1	16.8 ± 0.4			16.5 ± 1.3
γ-globulin (%)	6.3 ± 0.9			6.3 ± 0.5	7.8 ± 1.0			7.6 ± 1.1

Values are mean ± S.D.

* : $p < 0.05$: Significant difference from control.

Table 9. Number of gross pathological findings in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
Spleen								
Focal elevation	1	0	0	0	0	0	0	0
Stomach								
Dark red spot in glandular stomach	0	1	0	0	1	0	0	1
White nodule	0	0	0	1	0	0	0	0
Other tissues or organs								
Malocclusion	0	0	1	0	0	0	0	0
Fracture of incisors	0	0	0	0	0	0	0	1
At Week 5 of Recovery								
Number of animals	6			6	6			6
Liver								
Diaphragmatic hernia	1			0	0			0
Stomach								
Dark red spot in glandular stomach	1			0	0			0
Kidney								
Cyst	0			0	1			0

ious kinds of amino acids, but its hazard information is insufficient. In this study, L-Thr was given to rats by dietary administration for 13 weeks to evaluate its toxicity.

Following dietary administration of L-Thr in rats for 13 weeks, no toxicologically significant differences in clinical signs, water intake, ophthalmoscopic observations, hematology, blood chemistry, or gross examination were observed in rats fed diets containing 5.0% L-Thr compared to the basal diet control.

In body weight, food consumption and feed efficiency, significant decrease in body weight from day 14 to day 28 of treatment, significant decrease in food consumption on days 3, 14, 17, and 52 of treatment in males and significant decrease in food consumption on days 3, 42, and 52 of treatment in females were observed in the 5.0% L-Thr group compared to the basal diet control. Furthermore, significant decrease or a tendency toward decrease in feed efficiency in weeks 1 and 2 of treatment in males and females were observed in the 5.0% L-Thr group compared to the basal diet control. However, there were thought to be no toxicologically significant differences since they were slight and extremely short term or temporary variations, and there were no significant differences in body weight gain, mean food consumption or feed efficiency during the treatment period.

A decrease in potassium excretion in females receiving 5.0% L-Thr was observed in Week 13 of the treatment period. This finding is considered to be of no special significance because the urine volume was a little less in comparison with the control group, and since a decrease in potassium excretion was observed in individual rats with small urine volume.

An increase in the relative weight of the kidney was observed in females receiving 5.0% L-Thr; however, it was not toxicologically significant since there was no significant difference in the absolute weight of the kidney, there was no suggestion of kidney damage in gross pathology, histopathology, urinalysis or blood chemistry, and the change of organ weight was slight.

In conclusion, 13-week feeding toxicity of L-Thr was evaluated in this study. No adverse effects were observed in rats following dietary administration of L-Thr at concentrations of up to 5.0% for 13 weeks. The results of the subchronic oral toxicity study in rats support a no-observed-adverse-effect level (NOAEL) of 3266.9 mg/kg body weight/day in males and 3673.3 mg/kg body weight/day in females, the highest doses tested.

Conflict of interest---- The authors declare that there is no conflict of interest.

Table 10. Absolute organ weight in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
Body weight on the day of necropsy (g)	559.8 ± 42.6	566.8 ± 35.0	542.9 ± 51.7	541.3 ± 43.9	299.5 ± 18.1	299.3 ± 31.6	304.3 ± 34.7	280.8 ± 23.2
Brain (g)	2.18 ± 0.09	2.22 ± 0.08	2.18 ± 0.07	2.15 ± 0.06	1.98 ± 0.06	2.03 ± 0.06	1.98 ± 0.07	1.98 ± 0.08
Pituitary (mg)	13.7 ± 1.0	13.9 ± 2.3	14.1 ± 2.1	14.3 ± 1.8	15.6 ± 3.3	15.1 ± 3.0	16.1 ± 2.6	15.3 ± 2.6
Thyroid gland (R)(mg)	11.9 ± 2.7	12.5 ± 1.5	12.1 ± 2.1	12.1 ± 2.0	8.2 ± 1.7	8.5 ± 2.0	8.4 ± 2.1	8.3 ± 1.8
Thyroid gland (L)(mg)	11.7 ± 2.4	12.8 ± 1.3	10.9 ± 2.0	11.5 ± 1.8	8.3 ± 1.4	7.6 ± 1.6	7.7 ± 1.4	7.8 ± 1.3
Salivary gland (R)(mg)	406 ± 32	406 ± 41	410 ± 56	382 ± 25	261 ± 22	264 ± 30	249 ± 19	246 ± 22
Salivary gland (L)(mg)	408 ± 41	408 ± 39	406 ± 54	377 ± 27	257 ± 26	265 ± 32	242 ± 14	243 ± 25
Thymus (mg)	293 ± 55	268 ± 59	276 ± 63	301 ± 70	265 ± 63	220 ± 40	245 ± 51	215 ± 38
Heart (g)	1.58 ± 0.14	1.57 ± 0.11	1.53 ± 0.17	1.53 ± 0.13	0.95 ± 0.05	0.94 ± 0.09	0.96 ± 0.12	0.93 ± 0.08
Lung (g)	1.57 ± 0.11	1.56 ± 0.11	1.54 ± 0.13	1.52 ± 0.10	1.16 ± 0.05	1.14 ± 0.08	1.12 ± 0.08	1.08 ± 0.08
Liver (g)	14.77 ± 1.49	15.37 ± 1.34	14.60 ± 1.91	14.89 ± 1.50	7.35 ± 0.40	6.92 ± 0.82	7.35 ± 0.91	6.85 ± 0.80
Spleen (g)	0.83 ± 0.10	0.85 ± 0.14	0.87 ± 0.19	0.80 ± 0.10	0.54 ± 0.05	0.50 ± 0.10	0.52 ± 0.08	0.48 ± 0.05
Kidney (R)(g)	1.67 ± 0.11	1.67 ± 0.15	1.64 ± 0.14	1.68 ± 0.12	0.97 ± 0.06	0.93 ± 0.07	0.99 ± 0.07	0.98 ± 0.09
Kidney (L)(g)	1.68 ± 0.13	1.69 ± 0.15	1.67 ± 0.16	1.72 ± 0.13	0.96 ± 0.07	0.92 ± 0.06	0.98 ± 0.08	0.99 ± 0.09
Adrenal (R)(mg)	32 ± 5	32 ± 5	30 ± 5	30 ± 2	35 ± 4	37 ± 5	37 ± 6	33 ± 6
Adrenal (L)(mg)	34 ± 5	34 ± 5	32 ± 5	30 ± 3	39 ± 4	40 ± 7	39 ± 6	36 ± 6
Testis (R)(g)	1.81 ± 0.15	1.79 ± 0.15	1.69 ± 0.11	1.73 ± 0.08	NA	NA	NA	NA
Testis (L)(g)	1.81 ± 0.17	1.78 ± 0.18	1.66 ± 0.12	1.71 ± 0.06	NA	NA	NA	NA
Seminal vesicles (g)	1.55 ± 0.16	1.54 ± 0.20	1.45 ± 0.23	1.49 ± 0.17	NA	NA	NA	NA
Prostate (g)	1.33 ± 0.15	1.41 ± 0.24	1.33 ± 0.20	1.33 ± 0.17	NA	NA	NA	NA
Ovary (R)(mg)	NA	NA	NA	NA	44.1 ± 7.4	38.4 ± 6.9	42.3 ± 9.8	39.2 ± 9.1
Ovary (L)(mg)	NA	NA	NA	NA	43.4 ± 7.2	39.1 ± 7.2	37.0 ± 10.0	41.0 ± 9.3
Uterus (mg)	NA	NA	NA	NA	617 ± 142	558 ± 128	588 ± 127	560 ± 107
At Week 5 of Recovery								
Number of animals	6			6	6			6
Body weight on the day of necropsy (g)	559.5 ± 43.6			535.7 ± 50.3	330.3 ± 41.8			303.8 ± 32.5
Brain (g)	2.21 ± 0.13			2.16 ± 0.09	2.00 ± 0.11			1.98 ± 0.09
Pituitary (mg)	12.5 ± 0.6			12.4 ± 1.8	17.7 ± 3.4			17.2 ± 3.4
Thyroid gland (R)(mg)	11.7 ± 1.7			11.6 ± 2.7	9.9 ± 1.9			8.0 ± 1.4
Thyroid gland (L)(mg)	11.7 ± 2.1			11.7 ± 1.5	9.1 ± 1.3			6.8 ± 1.4*
Salivary gland (R)(mg)	410 ± 35			417 ± 71	259 ± 22			257 ± 14
Salivary gland (L)(mg)	405 ± 51			410 ± 71	248 ± 19			256 ± 15
Thymus (mg)	165 ± 32			216 ± 70	189 ± 33			176 ± 33
Heart (g)	1.51 ± 0.07			1.57 ± 0.19	1.00 ± 0.04			0.99 ± 0.04
Lung (g)	1.60 ± 0.06			1.57 ± 0.20	1.16 ± 0.08			1.14 ± 0.08
Liver (g)	14.43 ± 1.94			14.59 ± 1.89	7.72 ± 0.84			7.54 ± 0.64
Spleen (g)	0.79 ± 0.14			0.83 ± 0.18	0.59 ± 0.07			0.51 ± 0.01
Kidney (R)(g)	1.59 ± 0.09			1.65 ± 0.13	1.02 ± 0.09			1.02 ± 0.13
Kidney (L)(g)	1.61 ± 0.13			1.69 ± 0.17	1.03 ± 0.09			1.03 ± 0.12
Adrenal (R)(mg)	30 ± 3			29 ± 4	35 ± 3			35 ± 3
Adrenal (L)(mg)	32 ± 4			29 ± 1	38 ± 3			38 ± 3
Testis (R)(g)	1.75 ± 0.07			1.71 ± 0.20	NA			NA
Testis (L)(g)	1.73 ± 0.03			1.72 ± 0.14	NA			NA
Seminal vesicles (g)	1.57 ± 0.23			1.49 ± 0.18	NA			NA
Prostate (g)	1.29 ± 0.24			1.45 ± 0.31	NA			NA
Ovary (R)(mg)	NA			NA	37.3 ± 1.8			42.8 ± 7.0
Ovary (L)(mg)	NA			NA	40.9 ± 5.6			34.0 ± 6.2*
Uterus (mg)	NA			NA	704 ± 132			743 ± 86

Values are mean ± S.D.

NA: Not applicable

* : $p < 0.05$: Significant difference from control

Table 11. Relative organ weight in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
Brain (g/100g body weight)	0.39 ± 0.03	0.39 ± 0.02	0.40 ± 0.04	0.40 ± 0.03	0.66 ± 0.04	0.68 ± 0.07	0.66 ± 0.07	0.71 ± 0.06
Pituitary (mg/100g body weight)	2.4 ± 0.2	2.5 ± 0.3	2.6 ± 0.3	2.7 ± 0.4	5.2 ± 1.3	5.0 ± 0.8	5.4 ± 1.2	5.4 ± 0.8
Thyroid gland (R)(mg/100g body weight)	2.1 ± 0.5	2.2 ± 0.2	2.3 ± 0.4	2.3 ± 0.4	2.7 ± 0.5	2.8 ± 0.6	2.8 ± 0.7	3.0 ± 0.7
Thyroid gland (L)(mg/100g body weight)	2.1 ± 0.4	2.3 ± 0.3	2.0 ± 0.4	2.1 ± 0.4	2.8 ± 0.4	2.6 ± 0.7	2.6 ± 0.6	2.8 ± 0.5
Salivary gland (R)(mg/100g body weight)	73 ± 6	72 ± 5	76 ± 7	71 ± 8	87 ± 8	89 ± 12	83 ± 12	88 ± 10
Salivary gland (L)(mg/100g body weight)	73 ± 8	72 ± 4	75 ± 7	70 ± 8	86 ± 8	90 ± 14	81 ± 11	87 ± 11
Thymus (mg/100g body weight)	52 ± 8	47 ± 9	51 ± 11	56 ± 13	88 ± 20	74 ± 13	81 ± 17	77 ± 13
Heart (g/100g body weight)	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.28 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.32 ± 0.02	0.33 ± 0.02
Lung (g/100g body weight)	0.28 ± 0.03	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.02	0.39 ± 0.03	0.38 ± 0.04	0.37 ± 0.03	0.38 ± 0.02
Liver (g/100g body weight)	2.64 ± 0.15	2.71 ± 0.15	2.69 ± 0.19	2.75 ± 0.12	2.45 ± 0.10	2.32 ± 0.18	2.42 ± 0.22	2.44 ± 0.16
Spleen (g/100g body weight)	0.15 ± 0.01	0.15 ± 0.02	0.16 ± 0.03	0.15 ± 0.02	0.18 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02
Kidney (R)(g/100g body weight)	0.30 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.31 ± 0.03	0.32 ± 0.02	0.31 ± 0.03	0.33 ± 0.03	0.35 ± 0.02*
Kidney (L)(g/100g body weight)	0.30 ± 0.03	0.30 ± 0.02	0.31 ± 0.02	0.32 ± 0.04	0.32 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.35 ± 0.02**
Adrenal (R)(mg/100g body weight)	6 ± 1	6 ± 1	5 ± 1	6 ± 1	12 ± 1	12 ± 2	12 ± 3	12 ± 2
Adrenal (L)(mg/100g body weight)	6 ± 1	6 ± 1	6 ± 1	6 ± 1	13 ± 2	14 ± 4	13 ± 3	13 ± 2
Testis (R)(g/100g body weight)	0.33 ± 0.04	0.32 ± 0.03	0.31 ± 0.03	0.32 ± 0.03	NA	NA	NA	NA
Testis (L)(g/100g body weight)	0.33 ± 0.04	0.32 ± 0.03	0.31 ± 0.04	0.32 ± 0.02	NA	NA	NA	NA
Seminal vesicles (g/100g body weight)	0.28 ± 0.04	0.27 ± 0.04	0.27 ± 0.04	0.28 ± 0.04	NA	NA	NA	NA
Prostate (g/100g body weight)	0.24 ± 0.04	0.25 ± 0.05	0.25 ± 0.04	0.25 ± 0.04	NA	NA	NA	NA
Ovary (R)(mg/100g body weight)	NA	NA	NA	NA	14.8 ± 2.6	13.1 ± 3.1	14.0 ± 3.3	14.0 ± 3.0
Ovary (L)(mg/100g body weight)	NA	NA	NA	NA	14.6 ± 2.6	13.2 ± 2.8	12.1 ± 2.7	14.6 ± 3.0
Uterus (mg/100g body weight)	NA	NA	NA	NA	205 ± 40	186 ± 32	196 ± 50	200 ± 40
At Week 5 of Recovery								
Number of animals	6			6	6			6
Brain (g/100g body weight)	0.40 ± 0.04			0.41 ± 0.04	0.61 ± 0.08			0.66 ± 0.05
Pituitary (mg/100g body weight)	2.2 ± 0.1			2.3 ± 0.4	5.5 ± 1.3			5.6 ± 0.9
Thyroid gland (R)(mg/100g body weight)	2.1 ± 0.4			2.2 ± 0.5	3.0 ± 0.5			2.6 ± 0.3
Thyroid gland (L)(mg/100g body weight)	2.1 ± 0.3			2.2 ± 0.2	2.8 ± 0.4			2.3 ± 0.5
Salivary gland (R)(mg/100g body weight)	74 ± 8			78 ± 10	79 ± 9			86 ± 9
Salivary gland (L)(mg/100g body weight)	73 ± 10			76 ± 9	76 ± 8			85 ± 11
Thymus (mg/100g body weight)	30 ± 4			41 ± 13	59 ± 14			59 ± 11
Heart (g/100g body weight)	0.27 ± 0.03			0.29 ± 0.02	0.31 ± 0.04			0.33 ± 0.03
Lung (g/100g body weight)	0.29 ± 0.03			0.29 ± 0.03	0.36 ± 0.04			0.38 ± 0.02
Liver (g/100g body weight)	2.57 ± 0.24			2.73 ± 0.13	2.35 ± 0.26			2.49 ± 0.21
Spleen (g/100g body weight)	0.14 ± 0.02			0.16 ± 0.03	0.18 ± 0.02			0.17 ± 0.01
Kidney (R)(g/100g body weight)	0.29 ± 0.02			0.31 ± 0.02	0.31 ± 0.03			0.37 ± 0.06
Kidney (L)(g/100g body weight)	0.29 ± 0.02			0.32 ± 0.04	0.31 ± 0.03			0.34 ± 0.05
Adrenal (R)(mg/100g body weight)	6 ± 1			6 ± 1	11 ± 2			12 ± 2
Adrenal (L)(mg/100g body weight)	6 ± 1			6 ± 1	12 ± 1			13 ± 2
Testis (R)(g/100g body weight)	0.32 ± 0.02			0.32 ± 0.02	NA			NA
Testis (L)(g/100g body weight)	0.31 ± 0.02			0.32 ± 0.02	NA			NA
Seminal vesicles (g/100g body weight)	0.28 ± 0.03			0.28 ± 0.04	NA			NA
Prostate (g/100g body weight)	0.23 ± 0.03			0.27 ± 0.04	NA			NA
Ovary (R)(mg/100g body weight)	NA			NA	11.4 ± 1.4			14.2 ± 2.3*
Ovary (L)(mg/100g body weight)	NA			NA	12.6 ± 2.6			11.2 ± 1.6
Uterus (mg/100g body weight)	NA			NA	216 ± 47			247 ± 39

Values are mean ± S.D.

NA: Not applicable

* : $p < 0.05$; ** : $p < 0.01$: Significant difference from control

Table 12. Histopathological findings in rats following dietary administration of L-threonine.

Sex	Male				Female			
Concentration(%)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	12	12	12	12	12	12	12	12
At Week 13 of Treatment								
Heart								
Focal myocarditis (Total)	1	0	1	0	0	0	0	0
slight	1	0	1	0	0	0	0	0
Stomach								
Erosion in glandular stomach (Total)	0	1	0	0	1	0	0	1
mild	0	1	0	0	1	0	0	1
Epidermal cyst in submucosa (Total)	0	0	0	1	0	0	0	0
present	0	0	0	1	0	0	0	0
Cecum								
Granulomatous inflammation (Total)	0	0	0	0	0	0	0	1
mild	0	0	0	0	0	0	0	1
Liver								
Arteritis (Total)	1	0	0	0	0	0	0	0
slight	1	0	0	0	0	0	0	0
Focal necrosis with hemorrhage (Total)	0	0	0	0	1	0	0	0
slight	0	0	0	0	1	0	0	0
Pancreas								
Fibrosis in islet (Total)	3	0	0	1	NA	NA	NA	NA
slight	3	0	0	1	NA	NA	NA	NA
Interstitial cellular infiltration (Total)	1	0	0	0	NA	NA	NA	NA
slight	1	0	0	0	NA	NA	NA	NA
Spleen								
Focal inflammatory change (Total)	1	0	0	0	0	0	0	0
mild	1	0	0	0	0	0	0	0
Kidney								
Dilatation of renal pelvis (Total)	0	0	0	0	1	0	0	0
slight	0	0	0	0	1	0	0	0
Testis								
Atrophy of seminiferous tubule (Total)	1	0	0	0	NA	NA	NA	NA
slight	1	0	0	0	NA	NA	NA	NA
Eye								
Focal degeneration of retina (Total)	1	0	3	1	1	0	0	0
slight	1	0	3	1	1	0	0	0
Retinal atrophy (Total)	0	0	0	1	1	0	0	0
slight	0	0	0	1	1	0	0	0
Focal calcification in cornea (Total)	1	0	0	0	0	0	0	0
slight	1	0	0	0	0	0	0	0
At Week 5 of Recovery								
Number of animals	6			6	6			6
Stomach								
Erosion in glandular stomach (Total)	1			0	0			0
mild	1			0	0			0
Epidermal cyst in submucosa (Total)	0			0	0			0
present	0			0	0			0
Cecum								
Cellular infiltration in lamina propria mucosae (Total)	0			0	0			1
mild	0			0	0			1
Liver								
Diaphragmatic hernia (Total)	1			0	0			0
present	1			0	0			0
Pancreas								
Fibrosis in islet (Total)	2			0	NA			NA
slight	2			0	NA			NA
Interstitial cellular infiltration (Total)	1			0	NA			NA
slight	1			0	NA			NA
Spleen								
Granuloma (Total)	1			0	0			0
slight	1			0	0			0
Kidney								
Cyst (Total)	0			0	1			0
mild	0			0	1			0
Eye								
Focal degeneration of retina (Total)	1			0	0			0
slight	1			0	0			0

NA : Not applicable

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