SAFETY OF L-TRYPTOPHAN FOR PIGS


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ABSTRACT

Epidemic eosinophilia-myalgia syndrome (EMS) associated with excess L-tryptophan (Trp) consumption in humans has been declared a major public health problem. The EMS problem has not been observed in pigs, nor has comprehensive pathology associated with EMS in humans been described. Experiments were therefore conducted to evaluate the pathology and effects of excess dietary L-Trp for finishing (79 to 119 kg) pigs and to determine an LD$_{50}$ of Trp for pigs. In Exp. 1, addition of .1 or 1% Trp to com-soybean meal diets had no effect on growth performance or leukocyte and relative eosinophil counts or on plasma aspartate transferase, creatine phosphokinase, and lactate dehydrogenase activities. Likewise, untoward pathological effects of Trp feeding were not observed in the animals under study. In Exp. 2, supplementing the basal diet with 0, 2, and 4% Trp caused linear ($P < .05$) decreases in weight gain, feed intake, and gain:feed ratio. Mortality could not be produced by acute oral dosing in the LD$_{50}$ study (Exp. 3), wherein Trp doses between 2.00 and 5.71 g/kg of BW were administered by stomach tube. Vomiting occurred at oral doses greater than 5.71 g/kg of BW. These results suggest that oral ingestion of Trp in pigs is safe and that pigs can tolerate considerable excesses of Trp.

Key Words: Pigs, Tryptophan, Eosinophilia-Myalgia Syndrome, Leukocyte Disorders

Toxicity


Introduction

Eosinophilia-myalgia syndrome (EMS) associated with oral ingestion of L-Tryptophan (Trp) in humans has been declared an epidemic in the United States (Centers for Disease Control, 1989). Easily available in health food and drug stores, Trp has been used by humans as a treatment for insomnia (Lahmeyer, 1989) and depression (Coppen, 1972). Although the full complications of EMS are still unknown, a contaminant inherited from the Trp manufacturing process is thought to be responsible for the EMS symptoms (Slutsker et al., 1990). Indeed, all the EMS cases documented to date have been traced to only one of the three principal companies that manufacture Trp (Slutsker et al., 1990).

Tryptophan has been used for many years in animal nutrition for purposes of fortifying diets judged to be inadequate in this essential amino acid. Feeding 4% excess dietary Trp to young growing chicks (Edmonds et al., 1987) or pigs (Edmonds and Baker, 1987a) was without any ill effects other than anorexia and growth depression. Two percent Trp added to a com-soybean meal diet was innocuous for young pigs during a 28-d feeding period (Edmonds and Baker, 1987a).

Eosinophilia-myalgia syndrome has not been reported in meat-producing animals, nor has pathology associated with EMS in humans been comprehensively described. Because Trp is regularly fed to swine, it is important to clarify in pigs whether Trp per se, from a manufacturer whose product has not been associated with EMS, will or will not produce...
any of the EMS-like symptoms observed in humans. Therefore, the objectives of our study were 1) to describe the pathology of .1 and 1% excess dietary Trp for finishing pigs, 2) to study the effects of 2 and 4% excess dietary Trp on growth performance of finishing pigs, and 3) to determine an LD50 of Trp for pigs.

Materials and Methods

Diets. The basal diet (Table 1) was formulated to meet or exceed all nutrient requirements of finishing (50 to 110 kg) pigs (NRC, 1988) and contained 14% CP (determined by macro-Kjeldahl), 3,300 kcal of ME/kg, and .185% Trp (NRC, 1988). Tryptophan from Biokyoowa Corporation5 was supplied as feedgrade Trp (guarantee of 98.5% L-Trp). The purity of the batch of feedgrade L-Trp used in our study was analyzed by the manufacturer and determined to be 99.3% L-Trp. Tryptophan was added to basal diets at the expense of cornstarch.

General Protocol for Pig Trials. Crossbred (Duroc × Hampshire × Landrace) gilts were used in all experiments. From blocks based on ancestry and weight, individual pigs were assigned randomly to experimental treatments based on estimated breeding value. Gilts were housed individually in 1.4-m slatted-floor pens containing a nipple waterer and a self-feeder in an environmentally controlled finishing building. A 24-h constant light schedule was maintained. Twelve crossbred gilts averaging 79 kg were used in Exp. 1 to evaluate growth performance and pathology of L-Trp for finishing pigs fed the basal diet or the basal diet supplemented with Trp at .1% or 1% of the diet. Based on an estimated maximal supplemental Trp level of .033% in practical pig production, these oral doses were estimated to provide 3 times and 30 times the maximal dose, respectively. Each of the three experimental diets was fed individually to four pigs for 40 d. Pigs were bled from the anterior vena cava at trial initiation, weekly thereafter for 5 wk, and at trial termination. The weekly bleedings were carried out to observe changes in the pattern of blood counts and plasma enzyme activities over time. Lithium heparin blood samples were analyzed for total leukocyte counts6, including white cell differentials. A minimum of 300 leukocytes was identified to accurately determine relative distribution of cells. Plasma was used to quantify activities of aspartate transaminase (AST), formerly identified as glutamic oxaloacetic transaminase, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) according to procedures outlined by Boehringer Mannheim Corp. (1985, 1988, 1989). Enzymatic activities were measured using an automated analyzer7.

At the termination of the experiment, all 16 gilts were killed (electrical stunning followed by bleeding) and then necropsied. Complete pathological examinations were performed. All tissues sampled were fixed in 10% neutral-buffered formalin, with the exception of bone sections, which were fixed in formal-decal solution, and ocular tissues, which were fixed in Bouin’s solution. The following tissues were sampled: skin from the dorsal midline, muscle from the diaphragm, iliopsoas and glutus maximus, trachea and esophagus at the mid-cervical region, left apical and right diaphragmatic lung lobes, aorta at its base, right atrium, right and left ventricles, interventricular septum, tongue at the apex, mandibular

<table>
<thead>
<tr>
<th>TABLE 1. COMPOSITION OF THE BASAL DIETa</th>
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<tr>
<td>Ingredient</td>
</tr>
<tr>
<td>Cornstarch</td>
</tr>
<tr>
<td>Ground corn</td>
</tr>
<tr>
<td>Dehulled soybean meal</td>
</tr>
<tr>
<td>Dicalcium phosphate (feedgrade)</td>
</tr>
<tr>
<td>Ground limestone (feedgrade)</td>
</tr>
<tr>
<td>Trace-mineral mixbd</td>
</tr>
<tr>
<td>Vitamin mixc</td>
</tr>
</tbody>
</table>

aContains 14% CP, .185% tryptophan, and 3,300 kcal of ME/kg (NRC, 1988).

bTrace-mineral mix provided per kilogram of diet: Se, .10 mg; I, .35 mg; Cu, 8.0 mg; Mn, 20.0 mg; Fe, 90.0 mg; Zn, 100.0 mg; Co, .75 mg; and NaCl, 2.73 g.

cVitamin mix provided per kilogram of diet: vitamin A, 6,600 IU; vitamin D₃, 660 IU; vitamin E, 44 IU; vitamin K, 4.0 mg; riboflavin, 2.2 mg; d-pantothenic acid, 13.1 mg; niacin, 33.0 mg; choline chloride, 330 mg; and vitamin B₁₂, 35.1 mg.

5Biokyoowa Corp., St. Louis, MO.
6Sysmex F-800 Microcellcounter, TOA Medical Electronics Co., Ltd., Kobe, Japan.
7Hitachi 705, Automated Analyzer, Boehringer Mannheim, Indianapolis, IN.
salivary gland, palatine tonsil, stomach at the junction of stratified and glandular portions, duodenum, jejunum, ileum, cecum, spiral colon, liver, pancreas, kidney, urinary bladder, pituitary gland, adrenal gland, thyroid gland, spleen, mandibular and mesenteric lymph nodes, thymus, rib and bone marrow at the costo-chondral junction, cerebrum, thalamus, optic nerve and ocular musculature. In addition, bone marrow specimens were obtained from the rib near the costo-chondral junction and evaluated for abnormal numbers and/or morphology of eosinophils.

After fixation, tissues were trimmed, embedded in paraffin wax, sectioned at 5 μm, placed on microscope slides, and stained with hematoxylin-eosin. They were then examined for the presence of histologic changes with a light microscope. Tissues were examined without knowledge of the treatment group to which the animal had been exposed.

Excess Tryptophan Feeding Study. The objective of Exp. 2 was to examine the effects of excess dietary Trp on performance of finishing pigs. Nine crossbred gilts with an average initial weight of 70 kg were given ad libitum access to feed individually in a 16-d feeding trial. Levels of Trp (i.e., 0, 2, and 4%) supplied as feedgrade L-Trp were added to the basal diet at the expense of cornstarch. Before initiating and terminating the experiment, pigs were fasted overnight and weighed the following morning.

Tryptophan LD50 Study. Experiment 3 was conducted to determine the LD50 of Trp for pigs. Five crossbred gilts with an initial weight averaging 42 kg were used in an acute toxicity (LD50) assay, as described by Bruce (1985). Pigs were fasted overnight, weighed the following morning, and force-fed L-Trp via stomach-tube in a single oral dose. Animals were dosed one at a time with the best initial estimate of the LD50. They were observed for 2 d postdosing before dosing the next animal. The dose for each subsequent animal was increased based on the outcome of the preceding animal. Surviving animals were monitored for signs of disease and for delayed death (defined as deaths occurring more than 1 or 2 d postdosing) for a total of 7 d. Both feed (Table 1) and water were available ad libitum after oral dosing.

Assuming that all pigs could consume feed at 5% of BW, the estimated Trp dose for the first animal was 4% of the diet based on the results of Exp. 2; this level of excess dietary Trp decreased growth. Likewise, Edmonds and Baker (1987a) observed growth depression in young pigs fed 4% excess dietary Trp. The calculated Trp dose, for example, of the first animal (40 kg BW) was 80 g (40,000 g × .05 × .04), or 2 g/kg of BW. This dose was estimated to be four times higher than the maximal dose taken by humans who subsequently developed EMS (Centers for Disease Control, 1989). Four subsequent doses were determined by multiplying the previous pig’s dose by a constant factor of 1.3, up to a total dose of 5.71 g/kg (Bruce, 1985).

Statistical Analysis. Data from Exp. 1 and 2 were subjected to ANOVA procedures as appropriate for randomized complete-block designs. Single df comparisons were made to test the significance of treatment differences (Steel and Torrie, 1980).

Results

Experiment 1. The results of Exp. 1 are shown in Table 2. Supplementing the basal diet with 0, .1, or 1% L-Trp caused neither a growth increase nor a growth decrease. Likewise, voluntary feed intake and feed efficiency were similar across Trp doses. Total blood leukocytes and relative eosinophil counts were not different (P > .05) among treatment groups. Likewise, plasma AST, CPK, and LDH activities did not differ (P > .05) among diets. The weekly blood counts and plasma enzyme activities agreed with the final pattern shown in Table 2. Therefore, the weekly results are not presented.

Gross pathological evaluation revealed that all animals had mild ventral pulmonary consolidation. Histologically, there was moderate to large peribronchial accumulation of lymphocytes. In addition, several animals in the various test groups had airways filled with neutrophils. These changes are suggestive of enzootic pneumonia, most likely caused by Mycoplasma spp. Several animals scattered among the various test groups had a mild fibrinous exudate in their pericardial sacs.

Histologic changes were seen in a variety of organs, but these lesions were mild and of unspecific etiology. They occurred as frequently in the control group as in test groups. They included nonsuppurative inflammation of the renal interstitium and pericholangial region.
TABLE 2. PERFORMANCE, BLOOD CHEMISTRY, AND EOSINOPHIL COUNTS OF PIGS FED CORN-SOYBEAN MEAL DIETS SUPPLEMENTED WITH .1% OR 1% L-TRYPTOPHAN (EXP. 1)*

<table>
<thead>
<tr>
<th>Performance criteria</th>
<th>Blood counts</th>
<th>Plasma enzymesb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary addition</td>
<td>Gains/feeder g</td>
<td>Leukocytes, x 10^9/liter</td>
</tr>
<tr>
<td>Basalc</td>
<td>921</td>
<td>3,303</td>
</tr>
<tr>
<td>Basal + .10% L-Trp</td>
<td>1,031</td>
<td>3,472</td>
</tr>
<tr>
<td>Basal + 1.00% L-Trp</td>
<td>1,089</td>
<td>3,618</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>91</td>
<td>191</td>
</tr>
</tbody>
</table>

aData represent mean values of four individually fed gilts for a period of 40 d; average initial weight was 79 kg and average final weight was 119 kg. Blood data represent values at the end of the 40-d feeding period.
bAST = aspartate transferase; CPK = creatine phosphokinase; and LDH = lactate dehydrogenase.
cContained 14% CP and .185% Trp (NRC, 1988).

dosage. No other clinical signs were seen in any of the animals.

Discussion

A variety of gross and histologic changes were noted in individual animals in Exp. 1. These changes, however, were not consistently seen within dosage blocks, nor were increasingly severe lesions seen with increasing dosage of Trp. Clinical, clinicopathologic, or pathologic evidence suggesting myalgia, myositis, scleroderma, circulating eosinophilia, or abnormal eosinophilic infiltration of tissues, such as seen in humans (Flannery et al., 1990; Hertzman et al., 1990; Silver et al., 1990; Travis et al., 1990), was not present. Eosinophils, however, were abundant in the intestinal lamina propria of pigs on all four treatments in Exp. 1. Such infiltrates are frequently seen in normal porcine intestines.

TABLE 3. PERFORMANCE OF PIGS FED EXCESS LEVELS OF L-TRYPTOPHAN (EXP. 2)*

<table>
<thead>
<tr>
<th>Dietary addition</th>
<th>Daily gaind, g</th>
<th>Daily feedd, g</th>
<th>Gain/ fedd, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basald</td>
<td>987</td>
<td>3,008</td>
<td>330</td>
</tr>
<tr>
<td>Basal + 2.00% L-Trp</td>
<td>807</td>
<td>3,153</td>
<td>261</td>
</tr>
<tr>
<td>Basal + 4.00% L-Trp</td>
<td>429</td>
<td>1,779</td>
<td>243</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>68</td>
<td>366</td>
<td>23</td>
</tr>
</tbody>
</table>

aData represent mean values of three individually fed gilts for a period of 16 d; average initial weight was 70 kg and average final weight was 81 kg.

bLinear (P < .05) decrease.

c4.00% Trp < basal or 2.00% Trp (P < .05).
dContained 14% CP and .185% Trp (NRC, 1988).
TABLE 4. DETERMINATION OF LD_{50} FOR PIGS USING UP-AND-DOWN DOSE PROTOCOL\(^b\)

<table>
<thead>
<tr>
<th>Oral L-Trp dose(^a) (g/kg of BW)</th>
<th>No. of animals</th>
<th>Mortality</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1</td>
<td>No</td>
<td>Slight</td>
</tr>
<tr>
<td>2.60</td>
<td>1</td>
<td>No</td>
<td>Slight</td>
</tr>
<tr>
<td>3.38</td>
<td>1</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>4.39</td>
<td>1</td>
<td>No</td>
<td>Severe</td>
</tr>
<tr>
<td>5.71</td>
<td>1</td>
<td>No</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

\(^a\)Bruce (1985).
\(^b\)Administered in a single dose via stomach tube.

Tryptophan at the dosages administered failed to create gross, clinicopathologic or histologic changes in examined tissues. Recent evidence suggest that EMS associated with Trp ingestion may result from a contaminant inherited from the Trp production process, wherein a genetically altered Bacillus species was used (Slutsker et al., 1990). The contaminant has been isolated by HPLC and is presently referred to as peak E (Raphael, 1990). It seems that L-Trp per se is nontoxic in swine at levels greatly exceeding normal dietary supplementation, as well as in amounts that promote feed refusal.

Eosinophilia-myalgia syndrome associated with oral ingestion of Trp has been reported in epidemic numbers in the United States by the Centers for Disease Control (1989). The syndrome is characterized by peripheral-blood eosinophilia, severe muscle pain, skin rash, mouth ulcers, abdominal pain, and dyspnea (Hertzman et al., 1990). Increases of serum AST and LDH, but not of CPK, were reported by the same authors. Humans have developed EMS after taking 1,500 mg of Trp/d for half a month (Martin et al., 1990). Medsger (1990) suggested that myalgia may result from damage to peripheral sensory nerves because serum CPK is not increased, and histological evidence of myofibrillar degeneration is yet to be documented. Death caused by EMS complications was due to the ascending polyneuropathy with respiratory failure. Freese et al. (1988) reported that neurotoxicity is an adverse effect of Trp. Although increased serum and urine levels of kynurenine (Silver et al., 1990) and eosinophil-derived neurotoxin (Hertzman et al., 1990) may be important causative factors of neurotoxicity, the mechanism is still unclear. Stemberg et al. (1980) previously described a scleroderma-like illness in a patient receiving L-5-hydroxytryptophan and carbidopa for the treatment of intention myoclonus. Plasma kynurenine was also increased. Eosinophilia-myalgia syndrome shares many similarities with eosinophilic fascitis and localized forms of scleroderma in terms of nonacral distribution, subcutaneous and fascial involvement, and accumulation of eosinophils in peripheral blood (Medsger, 1989). Eosinophilic fascitis was reported in 1974 when Trp was first available in the United States (Shulman, 1975). This suggests that an EMS-like disease might have occurred before the recently reported epidemic (Centers for Disease Control, 1989).

Edmonds and Baker (1987a) observed that adding .5, 1, or 2% excess dietary Trp to corn-soybean meal diets had no effect on performance (i.e., weight gain, feed intake, gain:feed) of young (10-kg) pigs, although 4% supplemental Trp decreased both weight gain and feed intake, but not gain:feed ratio. Further studies by Edmonds et al. (1987) indicated that pigs preferred diets with excess threonine, lysine, arginine, or methionine over those containing an equal excess (i.e., 4%) of dietary Trp. When given a choice between a protein-free diet and a corn-soybean meal diet containing 4% excess Trp, pigs initially (d 0 to 4) preferred the protein-free diet, but later adapted to the extent that during the last 4 d of the 12-d feeding trial they consumed more of the Trp-imbalanced diet than of the protein-free diet (Edmonds et al., 1987). Chicks preferred diets with 4% excess methionine, threonine, or arginine over those containing an equal excess of Trp (Edmonds and Baker, 1987b). Also, laying hens fed 1% excess Trp for 4 wk showed no effects on weight gain, feed intake, or egg production (K. W. Koelkebeck and D. H. Baker, unpublished data). The effects of excess dietary Trp observed in young pigs are in contrast to those observed herein with finishing pigs (Exp. 2, Table 3) in that both weight gain and gain:feed were decreased linearly in the older pigs we used, whereas excess Trp levels up to 2% of the diet were innocuous in the younger pigs studied by Edmonds and Baker (1987a).

The results of our study suggest that there is a considerable margin of safety when Trp is used to fortify pig diets. In fact, at doses higher than 1% of the diet (about 30 times the maximum level of supplementation), the pig adjusts its feed intake downward as an
apparent compensatory mechanism (Table 3). With a high, single, acute oral dose (above 5.71 g/kg of BW), pigs in Exp. 3 vomited. Moreover, even with very high oral doses in Exp. 1 (chronic feeding) and Exp. 3 (LD₅₀ study), neither death nor EMS could be produced.

Implications

Oral ingestion of tryptophan from a single manufacturer (1989 batch) has been shown to cause eosinophilia-myalgia syndrome in humans. Clinical pathology failed to show any untoward pathologic effects in pigs fed considerable excesses of the tryptophan product used in our study. The results reported herein demonstrate that uncontaminated tryptophan is safe for pigs, particularly at the dosages normally used. Tryptophan excesses estimated at 30 times the maximal dose that would be used in practice produced no symptoms similar to those of eosinophilia-myalgia syndrome in 100-kg female swine.

Literature Cited


