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AVAILABILITY OF TRYPOTOPHAN IN SOME FEEDSTUFFS FOR SWINE[1,2]

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SUMMARY

Two experiments were conducted to determine the availability of tryptophan (Try) from corn (NC), *opaque-2* corn (OC), milo (M) and freeze dried alfalfa (FDA) when fed to swine. The first experiment consisted of a growth trial involving 96 baby pigs and eight treatments. The basal diet containing .06% Try and 24.5% protein was supplemented with .015%, .030% and .045% L-Try. These four diets were used to establish a standard curve for growth. The other treatments were formed by adding the feedstuffs as the source of Try to the basal diet. Availabilities were determined by comparing growth responses. Try availabilities from NC, OC, M and FDA were 70, 58.3, 70 and 83.3%, respectively, when gain was used as the response criterion, and 77, 65, 85 and 80% when gain/feed was the response criterion.

Plasma Try, muscle RNA and DNA and muscle polysome profiles were used as criteria for assessing the availability of Try. Dietary levels of Try showed a quadratic effect ($P<.01$) on plasma Try and no apparent effect on the muscle parameters.

In the second experiment, post-prandial changes in blood Try levels were used as a measure of Try availability from FDA. Analysis of blood from pigs fed the dietary treatments (growth curve) used in experiment 1 plus the treatment with FDA as the source of Try showed an increase in Try level that peaked irregularly between 15 and 45 min post-feeding followed by a marked decrease that plateaued at about 2 to 2.5 hours. The net decrease in plasma Try as well as the rate of the decrease (slope between the peak maximum and the plateau) appeared to be a function of the dietary level of Try. The Try availability from FDA was 97.9% and 110.5% using the decrease and the slope criteria respectively.

(**Key Words**: Tryptophan Availability, Swine, Corn, Milo, Alfalfa.)

INTRODUCTION

Considerable research attention has been given to protein and amino acid requirements of the different classes of swine but little to the biological availability of amino acids in feeds (Meade, 1972). This is especially true for Try which along with lysine is one of the two most limiting amino acids in commonly used swine diets. Also, methods of estimating amino acid availabilities either do not consider all the biological factors involved in the utilization of amino acids by the animal (*in vitro* methods), or if they do (biological methods), they are time consuming, require a large number of animals and are usually expensive.

The objectives of the present study were (1) to determine the biological availability of Try from normal corn (NC), *opaque-2* corn (OC), milo (M) and freeze dried alfalfa (FDA), and (2) to investigate the feasibility of using plasma tryptophan and muscle RNA/DNA and polysome profiles as response criteria for assessing the biological availability of tryptophan.

EXPERIMENTAL PROCEDURE

Experiment 1. This experiment was composed of two parts. The first part consisted of a 21-day growth assay to determine the availability of tryptophan from the different feedstuffs. It involved 96 baby pigs. In the second part, blood and muscle samples were collected from 24 of the baby pigs used in the growth assay.

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[2] Department of Animal Science. Acknowledgement is made to John Welch and Barbara Petersen for their laboratory assistance and to Dawes Laboratories Inc., Chicago, Illinois and American Cyanamid Co., Princeton, New Jersey for product support of the research.

TABLE 1. COMPOSITION OF THE BASAL DIET[a]

Ingredient	Ref. No.	G/kg of diet
Corn, yellow, grain grnd.	4-02-992	480
Corn starch, dehy grnd.	4-02-889	150
Gelatin		100
Zein		100
Sugar cane, sugar	4-04-701	75
Calcium phosphate dibasic, commerical	6-01-080	32
Swine, lard	4-04-790	30
Vitamin-antibiotic premix[b]		20
L-lysine HCl		7
Salt, NaCl, commercial (iodized)	6-04-152	5
Mineral premix[c]		1

[a]Composition: 24.5% protein, .06% tryptophan.

[b]Vitamin addition/kg of diet: vitamin A, 4,400 IU; vitamin D, 800 IU; choline chloride, 836 mg; niacin, 27.5 mg; pantothenic acid, 14.3 mg; riboflavin, 7.2 mg; vitamin B_{12}, .044 mg; ASP-250, 2.5 grams.

[c]Mineral addition/kg of diet: zinc, 200 mg; iron, 100 mg; manganese, 50 mg; copper, 11 mg; cobalt, 1 mg; iodine, 1.5 milligrams.

TABLE 2. EXPERIMENTAL TREATMENTS[a]

Diet	Treatment	% tryptophan added to basal diet from specified source
A	Basal diet	. . .
B	Basal + .015% L-try	.015
C	Basal + .030% L-try	.030
D	Basal + .045% L-try	.045
E	Basal + 15% normal corn[b]	.015
F	Basal + 15% *opaque-2* corn	.018
G	Basal + 15% milo	.015
H	Basal + 8% freeze-dried alfalfa	.041
. . .	Control diet[c]	

[a]Additions to the basal diet were made at the expense of corn starch.

[b]No. 2 yellow corn.

[c]16% corn-soy diet.

For the growth trial, pigs averaged 4.5 kg and were used in a completely block design involving eight treatments. Four pigs were randomly allotted by weight to each of eight pens per block. Three blocks were used. Each pen was formed by three gilts and one barrow. The pigs were housed in an environmentally controlled room with concrete floor pens (1/3 slotted) equipped with automatic waterers, self feeders and supplemental radiant heat lamps.

The experimental diets were as follows: The basal diet (A) contained 24.5% protein and .06% tryptophan. Diets B to D were formed by supplementing diet A with .015, .030 and .045% L-Try. Diets E to H were formed by adding .015, .018, .015 and .045% Try from NC, OC, M and FDA to diet A. The additions were made at the expense of corn starch. The small difference in protein content among the treatments was considered to have little effect on growth at this level of protein (Gupta and Elvehjem, 1957). The composition of the basal diet and experimental treatments are shown in tables 1 and 2. The protein and Try content of the feedstuffs appear in table 3. A Kjeldahl procedure for protein and a modified fluorometric method[3] (Duggan and Udenfriend, 1956) for Try analysis were used. Weight (of

[3]The modifications consisted in using 50 mg samples of each feedstuff very finely ground and in using polypropylene tubes (Cole-Parmer No. 6325) for the alkaline hydrolysis.

TABLE 3. TRYPTOPHAN AND PROTEIN CONTENT OF THE FEEDSTUFFS

Feedstuff	% tryptophan (as fed basis)	% protein (as fed basis)
Normal corn[a]	.10	8.3
Opaque-2 corn	.12	9.0
Milo	.10	7.8
Freeze-dried alfalfa[b]	.51	25.2

[a]No. 2 yellow corn.

[b]Freeze-dried alfalfa meal courtesy of G. Kohler and A. Livingston, U.S.D.A., WRRL, Berkeley, California.

the individual pig) and feed consumption (pen average) were recorded weekly.

For the second part of the experiment, one pen of pigs previously fed treatments A, B, C, D, F and H (from one of the blocks used in the growth assay) was continued on each of the same treatments for 1 week during which muscle and blood samples were collected and analyzed. All 24 pigs were then shifted to a 16% corn-soy diet (control diet) for one week at the end of which they were sampled again. The purpose of the change in diets was to study the effect of amino acid imbalanced (the diets fed in the growth trial were considered ultimately to be imbalanced) *vs* balanced diets on protein synthesis and on concentration of plasma tryptophan.

In the first sampling, two pigs of each pen, selected at random, were subjected to a biopsy of the *gracilis* muscle by procedures developed by Moser *et al.* (1972). Two biopsies were performed on each pig. The sample from the left thigh was immediately processed for determining the polysome profile by use of a procedure outlined by Drysdale and Munro (1967) and as modified by Aprahamian (1973). The muscle sample from the right thigh was immediately frozen for later analysis for RNA and DNA as outlined by Prasad *et al.* (1972).

Blood samples (6 to 8 ml) were obtained from the brachial region of the other two pigs of each pen by means of a Becton-Dickson vacutainer tube (10 ml with 143 USP units of sodium heparin). The blood samples were centrifuged at 2,000 rpm for 10 min and the plasma analyzed for Try (Duggan and Udenfriend, 1956) and for total amino acid nitrogen (Danielson, 1965).

For the second sampling (1 week after the pigs had been on the control diet) thy procedure was repeated but this time the pigs previously bled were biopsied and vice versa. The pigs were fed *ad libitum* during the time of the sampling.

Statistical Analysis. One-way analysis of variance was performed on the gain, feed intake and feed cconversion data. Regression analyses of gain and feed efficiency on dietary tryptophan were also calculated in order to determine the equation of the straight lines to use as standard curves for calculating tryptophan availabilities.

The data on muscle RNA/DNA, muscle polysome ratios and plasma Try and total amino acid nitrogen were analyzed in two forms. One-way analysis of variance of the data for each sample time separately and a two-way cross classification analysis of variance was used on the complete data.

Experiment 2. The purpose of this experiment was to further investigate the response of plasma Try concentration as a function of dietary levels of Try, as well as to evaluate the usefulness of this response as a criterion in determining the availability of Try in feeds.

Since the objectives of the experiment required frequent blood sampling, a venous catheterization technique developed by Zimmerman *et al.* (1973) was used to affix 12 pigs with an in-dwelling cannula in the jugular vein hopefully to avoid stress in the animals when taking repeat sampling.

At the end of a 10-day period of recovery from the surgery, six barrows averaging 21 kg were assigned to a 6 × 6 latin square arrangement of treatments in a completely randomized block design. The animals were individually housed in an environmentally regulated research unit. Each pen (1.5 × 1.2 m) with a totally slatted floor had an automatic drinking fountain and a galvanized metal feeder.

The experimental diets were the same diets A, B, C, D and H used in experiment 1 plus a corn-soy control. They were fed on the day of the experiment at a rate of 3.5% of (body weight)$^{.75}$, that is, at a rate commensurate with metabolic weight (Kleiber, 1947). Each diet was fed only once to a particular pig and no more than two different diets were fed to the same pig in the same week. The remaining days of experiments the pigs were fed a 16% corn-soy diet as a maintenance ration, 5% of (body weight)$^{.9}$. Prior to each day of the experiment the pigs were fasted for 24 hr and then fed the experimental treatments. Blood samples were collected before presentation of the meal (time zero) and then every 15 min for 2 hr, every 30 min for the subsequent 2 hr, and every hour for the last 2 hr of the 6-hr post-feeding period. Each blood sample was of 3.6 ml and was withdrawn with a syringe containing .4 ml of a heparin solution. Plasma Try was analyzed as described for experiment 1. Total cell volumes were determined periodically to see if adverse changes were occurring due to frequent blood sampling.

RESULTS AND DISCUSSION

Experiment 1. A treatment difference ($P<.01$) in gain and gain/feed was observed (table 4). The pigs fed the basal diet (A) that contained .06% Try lost an average of 150 g during the 3 weeks. As Try was added to A (treatments B to D) the growth rate and feed efficiency increased in linear fashion.

It was felt that an amino acid imbalance must have been present in all dietary treatments because gain and feed intake were depressed to an extent which cannot be explained by only the low level of Try in the diet, i.e.: diet D (.105% Try) supplied about 60% of the Try requirements of the 5 to 10 kg pig. The National Research Council (1973) recommends .18% Try for this class of swine and suggests an ADG of 300 grams. The pigs on diet D showed an ADG of 34 g when one would expect a value of 120 to 160 g or about 60% of the optimum gain. The negative effect of an amino acid imbalance on feed intake has been reported by Stahly (1975). Thus in the forthcoming results and discussion, the dietary treatments of this experiment are also designated as imbalanced diets.

A linear effect ($P<.01$) of supplemental Try on gain and gain/feed was observed. The equation of the straight line and the correlation coefficient (r), were: $Y = -.16 + 19.37x$, $r = .91$ and $Y = -.051 + 5.51x$, $r = .92$ in each case. Y represents gain in g or feed efficiency (G/F) as a function of x. And x, in turn, represents the percentage of L-Try added to diet A. The Try availability from the feedstuffs was calculated using both the gain and feed efficiency standard curves. The results are shown in table 5.

It can be observed that when the gain/feed standard curve was used to calculate the Try availabilities greater values were obtained consistently for all the feedstuffs. The same observation has been reported by Gupta *et al.* (1958) in the rat and by Smith (1968) and Campbell (1966) in the chick. They concluded also that amino acid availabilities based on feed efficiency responses are more accurate and reliable than those based merely on body weight gain. The reason is that gain alone does not consider possible differences in feed intake while gain/feed eliminated feed intake as a source of error.

Accepting that the trypotophan availability values calculated from the feed efficiency standard curve were more representative of the actual values, it can be noticed that they fall in a range between 65 to 85% for the four feedstuffs tested, the greater value being for M and in a decreasing order FDA, NC and OC.

TABLE 4. EFFECT OF DIETARY TRYPTOPHAN LEVELS ON PERFORMANCE OF THE GROWING PIG

Diet	A	B	C	D	E	F	G	H
Source of added try	...	L-try	L-try	L-try	NC[a]	OC[a]	M[a]	FDA[a]
% added try	...	.015	.030	.045	.015	.018	.015	.041
Gain[b,c,d] (g)	-150	123	418	717	36	36	36	418
Feed intake[d] (kg)	2.86	3.53	3.81	3.62	2.67	2.96	2.86	3.24
Gain/feed[b,c,d]	-.052	.035	.109	.197	.014	.013	.013	.128

[a]NC = normal corn (no. 2 yellow corn), OC = *opaque-2* corn, M = milo, FDA = freeze-dried alfalfa.

[b]Treatment difference ($P<.01$).

[c]Tryptophan (A, B, C, D) linear effect ($P<.01$).

[d]Data based on average of three pens; four pigs/pen int. wt, 4.5 kg, duration of trial, 21 days.

These values are in agreement with values reported for wheat ranging from 71% (Hepburn *et al.*, 1966) to 93.2% (Kuiken and Lyman, 1948) of available tryptophan. Other feedstuffs have been studied including soybean meal, fish meal and dried skim milk (Meade, 1972). Most of the values reported fall between 80 to 95% of available tryptophan and some extremely low values (27 to 35%) in heat damaged feeds (Pongpaew and Guggenheim, 1968).

The activity of protein synthesis is directly related to RND/DNA ratio of the particular tissue under study (Allison, 1963; Moser, 1972). In a similar way the polysome profile is an estimation of the protein synthetic activity of the tissue (Symmons *et al.*, 1972).

A polysome profile is the tracing of the UV absorption at 260 nm of the different ribosomal structures that have been separated by means of a density gradient centrifugation. Fig. 1a-b are two of the polysomes obtained from the *gracilis* muscle preparations of the baby pigs. In order to quantitatively compare the activity of protein synthesis of different muscle samples, the area A_1 formed by monosomes and disomes is considered inversely related with protein synthesis since these light ribosomal structures do not actively participate in the process. Heavier ribosomal aggregates (active during protein synthesis) form area A_2 which is correlated with the activity of the process. The polysome ratio that was used as a quantitative estimation of protein synthesis activity is simply the A_2 / A_1 ratio and has the same meaning of the RNA/DNA ratio. That is, the greater the value the more active the process of protein synthesis. The effect of dietary Try levels on the activity of protein synthesis in muscle tissue and on plasma Try concentration is shown in table 6.

There was no difference in the RNA/DNA or polysome ratios due to treatment. Thus these response criteria are perhaps not as sensitive as growth for recognizing small dietary differences in Try content. The RNA/DNA ratios in this experiment ranged from .48 to 2.01 and are very low compared with values from 3.43 to 8.25 reported by Moser (1972) for baby pigs fed balanced protein diets.

A treatment different ($P<.05$) in plasma Try was observed (table 6). Try concentration showed extremely low values ranging from 1.7 to 3.0 mcg/ml while values for well-fed pigs may vary from 7-15 mcg/ml (Lewis, *personal communication*). However, low values were expected considering that all diets were first limiting in Try. A quadratic effect ($P<.01$) of dietary Try on plasma Try was also observed. Plasma Try decreased from 2.9 to 1.7 mcg/ml when the Try content of the diet increased from .06 to .075% and increased linearly thereafter (diets B to D) up to 3.0 mcg/ml. This response is not in complete agreement with typical plasma amino acid response curves. Lewis and Speer (1974) and Young *et al.* (1971) have reported a sigmoid type of curve of plasma Try in swine and man respectively.

Total amino acid nitrogen (TAAN) in blood

TABLE 5. CALCULATED AVAILABILITIES OF TRYPTOPHAN

Feedstuff	% try in the feedstuff	% feedstuff in the diet	% try supplemented	% try from std. curve	% try available Mean		Standard error[b]
Normal corn[a]	.10	15	.015	.0105	70	±	12.9 (A)
				.0115	77	±	17.1 (B)
Opaque-2 corn	.12	15	.018	.0105	58.3	±	15.4 (A)
				.0117	65	±	15.5 (B)
Milo	.10	15	.015	.0105	70	±	22.8 (A)
				.0127	85	±	25.5 (B)
Freeze-dried alfalfa	.51	8	.041	.0299	73.3	±	8.5 (A)
				.0328	80	±	8.5 (B)

(A) Using gain standard curve.

(B) Using feed efficiency standard curve.

[a]No. 2 yellow corn.

[b]Results are the mean of three pens.

TABLE 6. EFFECT OF DIETARY TRYPTOPHAN LEVELS AND SOURCE ON PROTEIN SYNTHESIS, PLASMA TRYPTOPHAN AND TOTAL AMINO ACIDS IN THE BABY PIG[a]

Treatment	A	B	C	D	F	H
% try added	...	.015	.030	.045	.018	.041
Source of try added	...	L-try	L-try	L-try	OC[b]	FDA[b]
RNA/DNA	1.19	1.30	2.01	.58	.48	1.24
Polysome ratio	.38	.35	.47	.43	.32	.26
Plasma try[c,d] (mcg/ml)	2.9	1.7	2.1	3.0	2.4	2.0
Total amino acid nitrogen (mg %)	7.66	10.30	7.56	7.17	8.94	8.61

[a]Values are the mean of two observations.

[b]OC = *opaque-2* corn; FDA = freeze-dried alfalfa.

[c]Treatment difference (P<.05).

[d]Tryptophan (A, B, C, D) quadratic effect (P<.01).

was also studied to determine if it had any relation with the plasma Try changes. TAAN was not affected by either dietary or plasma Try.

In order to further study the effect of imbalanced protein on protein synthesis and plasma Try, blood and muscle samples were obtained from the same pigs following a week of feeding a 16% corn-soy (balanced) diet and the same response criteria were evaluated. The results are shown in table 7. Sample time I represents the responses when the imbalanced diets were fed. These are the same results of table 6 shown again to facilitate the comparison. Sample time II represents the responses 1 week after the change in diets. An increase in all the response criteria was observed in sample time II *vs* sample time I. There was a slight increase in the RNA/DNA ratio and almost a threefold increase (P<.01) for the polysome ratio and plasma Try. The balanced diet promoted an increase in protein synthesis that can be readily noticed by comparing the muscle polysome profiles of pigs fed either an imbalanced diet (figure 1-A) or a balanced one (figure 1-B). Notice that the area of polysomes (A_2) represents a greater percent of the total area in the polysome at the right, meaning a higher protein synthesis in that pig. On the average, the polysome ratio increased from .37 to .92 by shifting the diets from imbalanced to balanced.

The level of plasma Try increased on the average from 2.35 to 6.50 mcg/ml as a result of the change in diets. No difference due to previous dietary treatment was found for any of the response criteria considered.

In summary, and in view of the results obtained, the possibility of using polysome and RNA/DNA ratios as substitutes for growth responses to determine amino acid availabilities does not appear promising. They are not extremely sensitive to small differences in dietary concentrations of amino acids. Plasma Try in contrast looks like a promising response criterion to use in amino acid studies. It is sensitive and relatively easy to measure.

Experiment 2. Figure 2 shows the typical plasma Try response curves to two different dietary treatments in the same pig on two different days of the experiment. The responses to both the control (balanced) diet and the imbalanced diets (A to D and H) were remarkably consistent in all the pigs and featured the

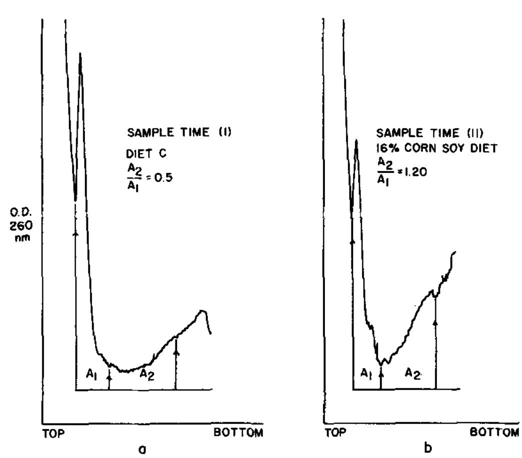

Figure 1. Example of muscle polysome profiles of baby pigs after being fed (a) dietary treatment C followed by (b) a 16% corn-soy diet.

TABLE 7. EFFECT OF IMBALANCED (I) *VS* BALANCED (II) PROTEIN ON PROTEIN SYNTHESIS AND PLASMA TRY IN THE BABY PIG

Criteria	Sample Time[b]	Treatment[a] A	B	C	D	F	H	Sample time avg
	I / II			← 16% corn-soy diet	→			
RNA/DNA[c]	I	1.19	1.30	2.01	.58	.48	1.24	1.15
	II	1.76	2.54	1.00	2.49	1.06	1.30	1.67
Polysome[c,d]	I	.38	.35	.47	.43	.32	.26	.37
	II	1.03	.67	1.14	.94	.79	.90	.92
Plasma try[c,d] mcg/ml	I	2.9	1.7	2.1	3.0	2.4	2.0	2.35
	II	5.7	6.8	7.2	6.3	6.6	6.4	6.50

[a]See table 5 for description.

[b]I = Sample taken after pigs were fed imbalanced diets for 3 weeks; II = Sample taken after pigs were fed a 16% corn-soy diet for 1 week following imbalanced diets.

[c]Values are the mean of two observations.

[d]Sample time difference (P<.01).

following characteristics: when the control diet was fed, plasma Try increased continuously and reached a maximum between 2 to 2½ hr post-feeding. The level dropped slightly 4 and 6 hr post-feeding. In contrast, when the imbalanced diets were fed, plasma Try increased and reached a peak soon after ingestion (between 15 to 45 min post-feeding) followed by a marked decrease that leveled off at 2 to 2½ hr post-feeding. Notice (figure 2) that the level of plasma Try at this time was even lower than the fasting level. This low level of Try persisted during the whole sampling period 6 hr post-prandial. This type of plasma amino acid (PAA) response, which is due to a dietary deficiency of the amino acid in question, was first described by Longenecker and Hause (1959) and confirmed by many workers thereafter (Munro, 1970).

It was observed that as dietary tryptophan increased (diets A to D) the decrease in plasma Try following the maximum was less pronounced. In other words, as Try increased in the diet the shape of the curve looked more like the control curve. And at the same time the net decrease in plasma Try was inversely related to the concentration of Try in the diet.

In order to quantitatively compare the plasma Try response curves to the different dietary treatments, the net decrease of plasma Try and the rate of that decrease (slope) were calculated between the peak maximum and two hr post-feeding (at about this time plasma Try pla-

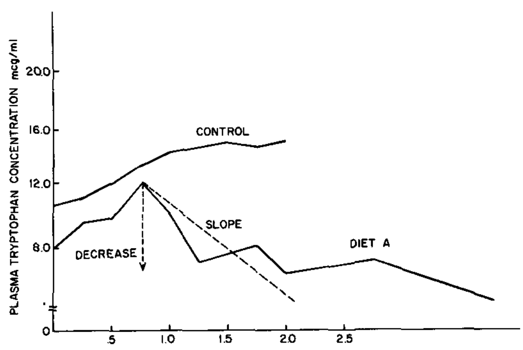

Figure 2. Plasma tryptophan response curve. Postprandial changes of plasma tryptophan concentration of a cannulated pig fed either the dietary treatment A or the control diet (16% corn-soy). The vertical broken line represents the net decrease in plasma tryptophan concentration between the peak and two hr post-feeding. The slope is the regression line between the same two points and represents the rate of plasma tryptophan decrease.

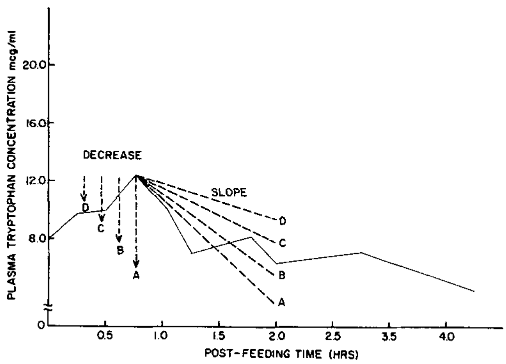

Figure 3. Plasma tryptophan response curve. Hypothetical representation based on the actual results of the plasma tryptophan decrease and the rate of that decrease (slope) as a function of the dietary treatments A to D.

teaued). The decrease and the slope are represented with dotted lines in figure 2. The slope was calculated as the regression line between the points involved.

The selection of "decrease" and "slope" as parameters to evaluate the post-prandial changes of plasma Try, as well as the method of evaluating them, may seem somewhat arbitrary, but they are two single factors that very well define the shape of the curve during the time when major changes of plasma Try occurred. That is, the first two hr post-feeding. The main effect of dietary Try on post-prandial changes of plasma Try was observed to be in the overall shape of the response curve.

Figure 3 is a graphic representation of the magnitude of the "decrease" and "slope" changes as related to the dietary concentration of Try in treatments A to D. In order to clearly notice the changes it was assumed that the peak maxima coincided at the same point in all the response curves and only one curve was drawn. The numerical data are shown in table 8.

The net decrease of plasma Try and the negative value of the slope were inversely related to the amount of Try supplemented to diet A. Regression analysis of each set of data on the percent of Try added was performed (see footnotes c and d of table 8). When tested for linearity, both regression lines failed to show a significant linear effect due to level of supplemental Try, corroborating the low correlations obtained (43% and 49%) for the "decrease" and "slope" curves respectively. The lack of agreement between the results of the statistical analysis and the apparently linear relationship observed when only the treatment means were considered was due to a large variation within treatments of both the "decrease" and "slope" criteria. The coefficient of variation was on the average 47% for the four treatments in both cases and as high as 97% in treatment C for the "decrease." In turn, the observed variation was attributed to a notably different and definite pattern in eating habits that was observed in each pig. The concentration of blood Try during the absorptive phase is a function not only of the level of Try in the diet but is also affected by the rate of absorption of amino acids. This latter factor is difficult to control in experimental conditions since it depends on the rate of food consumption and becomes a real task for the researcher interested in measuring plasma amino acid responses as influenced *only* by dietary amino acid levels.

When the decrease and slope standard curves were used to calculate the Try availability from FDA, values of 97.9 and 110.5% of available

TABLE 8. EFFECT OF DIETARY TRYPTOPHAN ON POST-PRANDIAL CHANGES OF PLASMA TRYPTOPHAN

Diet	A	B	C	D	H
Source of try added	...	L-try	L-try	L-try	FDA
% try added	...	.015	.030	.045	.041
Decrease in plasma try[a,b,c] (mcg/ml)	5.11 (5)	4.53 (4)	3.68 (2)	3.14 (4)	3.45 (2)
Slope[a,b,d] X (−1000)	51 (5)	49 (4)	37 (2)	26 (4)	28 (2)

[a]Number in parentheses indicates the number of observations.

[b]The regression on dietary tryptophan levels (A, B, C, D) was not significant.

[c]Equation and correlation coefficient of the regression line: $Y = 5.13 - 44.5x; r = .43$.

[d]Equation and correlation coefficient of the regression line: $Y = -.054 + .58x; r = .49$.

TABLE 9. CALCULATED AVAILABILITY OF TRYPTOPHAN FROM FREEZE-DRIED ALFALFA

Criterion	% try supplied by alfalfa	% try as read from std curve	% try available
Decrease	.0408	.0395	97.9
Slope	.0408	.0451	110.5

Try were obtained (table 9). These results are high compared with 83.3 and 80% that were obtained using the gain and feed efficiency responses in experiment 1.

In order to observe if any abnormal changes in blood composition were occurring due to frequent blood sampling, packed cell volumes (PCV) were determined at the beginning and at the end of the experiment on five pigs. Initial and final PCV were 33.4 and 35.6%, respectively. From these determinations it appears that frequent blood sampling did not produce an observable effect on erythrocyte content of the blood.

It was concluded that the determination of the availability of tryptophan based on growth response and particularly feed efficiency produced more reliable results than when other criteria were used. The suggested amino acid imbalance should not compromise the validity of the data since it is present in all dietary treatments. Thus no difference should exist in the relative utilization of tryptophan regardless of whether it was supplemented as L-Try or from the test feedstuffs.

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