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Changes in Free Amino Acid Levels in the Blood of Growing Swine as an Index to Protein Quality and Availability

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SUMMARY

Paper chromatography was used to study the free amino acid pattern in blood serum of four Hampshire gilts.

1. The free amino acids present in swine blood serum showed a characteristic pattern of increasing and decreasing at intervals after the pigs were fed a practical type ration containing supplemental isoleucine.
2. The isoleucine-leucine content in the blood serum of Hampshire gilts fed an isoleucine supplemented ration increased at 3 hours after feeding, decreased at 6 hours, increased slightly again at 12 hours, and decreased markedly at 18 hours.
3. The approach described could aid in determining protein quality and availability but is too laborious and inexact to be recommended for routine use.
4. Methionine appears to be the limiting amino acid in the ration used.

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INTRODUCTION

The plasma amino concentration of several species has been used as an aid in the study of their protein utilization. Much of the work has been done with dogs (Howell, 1906; Van Slyke *et al.,* 1912; Denton and Elvehjem, 1952; Dent and Schilling, 1948; Denton *et al.,* 1953; Denton and Elvehjem, 1953). Information from the rat is available from the study of Gibson *et al.,* 1951; on the chick from Charkey *et al.,* 1953, and Richardson, 1953. One of the early studies with humans was that of Munro *et al.,* 1952, which showed that glucose lowered the blood amino acids within one hour after its administration. Butter had no effect on blood amino acid composition. When this study was initiated, no work had been reported with pigs; therefore, the purpose of the experiments described herein was to see if similar methods could be used for examining the amino acid content of the blood of growing pigs and to determine if these techniques had promise for future studies.

MATERIALS AND METHODS

The two rations used in this study are shown in Table 1. They were identical except for isoleucine content.

Four Hampshire gilts weighing approximately 36 pounds were self-fed ration 760 (Table 1) for two weeks. Each gilt was removed from its pen and fasted for 18 hours. A blood sample was taken and the pig was fed all of ration 760-A it would consume in 30 minutes. The pig was then placed in a collection crate and blood samples were taken at intervals of 3, 6, 12, and 18 hours after feeding. The samples taken while gilts were on ration 760 and after they had been fasted were obtained through a 16 gauge needle in the ear veins. When the pigs were in collection crates, blood from the pigs' tails was collected. Five milliliters of blood were collected into 15 milliliter centrifuge tubes. The blood was centrifuged at 3000 rpm for thirty minutes.

A protein-free filtrate was prepared by the method of Folin and Wu (1919), frozen, and stored in a freezer at -10°F until ready for analysis by paper chromatography according to the following method.

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[1] The data reported herein are taken from a Master's thesis submitted to the Graduate Faculty, University of Missouri, 1958.

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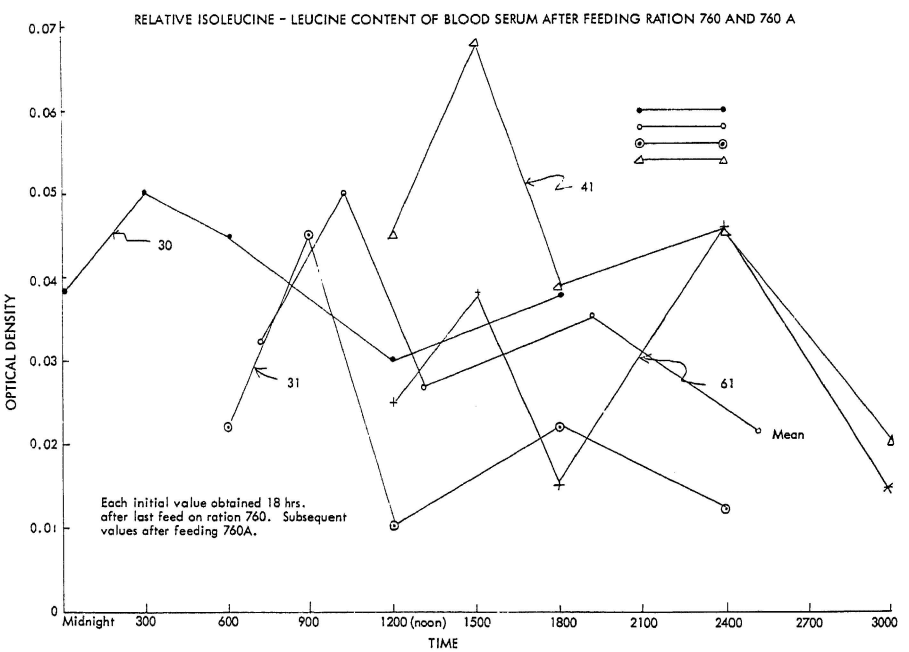
line of developed spots designated as zero hours shows the free amino acid pattern in blood serum after an 18-hour fast. Other verticle lines of developed spots show the free amino acid pattern in blood serum of the same gilt at intervals after she was fed ration 760-A. The combined isoleucine-leucine content is shown by the horizontal line of developed spots designated 1; numbers 2, 3, and 4 correspond to spots developed for methionine, tryptophan, and lysine plus histidine, respectively.

The relative isoleucine-leucine content in blood serum from each gilt is shown in Figure 2. Although considerable variation existed among the serum isoleucine-leucine contents of the four gilts, the general pattern, as shown by the mean values, was an increase at 3 hours and a decrease at 6 hours after feeding.

The content of isoleucine-leucine in swine blood serum after an 18-hour fast was determined as 9.4 micrograms per milliliter. An analysis of blood samples taken three hours after the pigs were fed ration 760-A showed the isoleucine-leucine content as 19.0 micrograms per milliliter of blood serum. At 6, 12, and 18 hours after feeding pigs ration 760-A, the blood serum levels of isoleucine-leucine were 7.9, 10.8, and 6.5 micrograms per milliliter, respectively. The concentrations of the other amino acids were not determined quantitatively, but it appears that the concentration of tryptophan and methionine are very low and that lysine was present in large amounts.

FIGURE 2



The free amino acid pattern of swine blood serum at intervals after feeding pigs rations containing crystalline isoleucine is in agreement with the findings of Denton and Elvehjem (1953) on dogs.

The results of this experiment suggest that the supplemental isoleucine was rapidly absorbed from the small intestines, resulting in an increase of free isoleucine in the blood. As glucose and amino acids liberated from the ingested ration became available, the free isoleucine in the blood was lowered by the anabolic processes of the body. The slight increase in the isoleucine-leucine content, 12 hours after feeding ration 760-A, may have corresponded to a reduced glucose supply followed by hydrolysis of labile body proteins. The second decrease in the isoleucine-leucine content of swine blood serum may have been a result of a deficiency of glucose or reduced TPN to furnish energy for synthetic reactions and amino acids may have been deaminized.

The isoleucine-leucine content of swine blood serum was greater 18 hours after feeding ration 760 than it was 18 hours after feeding ration 760-A. The gilts were fed ration 760 *ad libitum* for two weeks before the first 18-hour fast. One blood sample was taken at the end of the first fast and the gilts were fed all of ration 760-A they would consume in one-half hour. A second 18-hour fast was started immediately and during this fast four blood samples were taken. It is logical to believe that the supply of free amino acids in the blood was at a lower level at the start of the second fasting period than at the start of the first fast. Also, the added stress placed on the gilts during the second period may have helped reduce the free amino acids in the blood. It would have been preferable to have obtained values at intervals on each ration.

The differences in the serum isoleucine-leucine content of the four Hampshire gilts may have been due in part to a diurnal variation. At each interval, blood samples were taken at the same time of day from Hampshire gilts 31 and 61. Blood samples were taken from the other gilts at a different time of day for the various intervals. There was less variation in the blood serum isoleucine-leucine content of Hampshire gilts 31 and 61 than for the other gilts, except at the 12-hour interval.

Qualitative examination of the chromatographs suggested that methionine was the limiting amino acid in this ration and that lysine was being made available in amounts exceeding the pig's ability to use it.

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