FEATHER MEAL:
EVALUATION OF THE EFFECT OF PROCESSING CONDITIONS
BY CHEMICAL AND CHICK ASSAYS

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FEATHER MEAL: EVALUATION OF THE EFFECT OF PROCESSING CONDITIONS BY CHEMICAL AND CHICK ASSAYS

Proefschrift

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 9 mei 1984
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MANTHOS C. PAPADOPOULOS

FEATHER MEAL: EVALUATION OF THE EFFECT OF PROCESSING CONDITIONS BY CHEMICAL AND CHICK ASSAYS

Thesis

submitted to fulfil the requirements for the degree of
Doctor of Agricultural Sciences,
on the authority of the Rector Magnificus,
Dr. C.C. Oosterlee,
to be defended in public
on Wednesday 9th May 1984 at 16.00 hrs,
in the Auditorium of the
Agricultural University in Wageningen

WN 8201, 980

THEOREMS

I

Processing can have beneficial effects on some characteristics of feather meal protein and deleterious effects on others. Consequently, the processing conditions can only be optimized after defining the criteria by which the product will be judged.

This thesis.

II

The losses of certain amino acids in processed feather meal may not be of much nutritional significance unless other structural modifications to the protein affect *in vivo* digestibility/availability of the amino acids.

This thesis.

III

In the formulation of balanced poultry diets, it is essential that the dietary feather meal protein has to be evaluated on the basis of digested amino acids, because the variations between the individual amino acids in their digestibility are sufficiently extensive to justify this.

This thesis.

IV

Because the lanthionine content of differently processed feather meals is inversely proportional to their amino acid digestibility, it is a reasonable indicator of the effect of processing on the test feather meals.

This thesis.

V

From the practical point of view fecal analysis is not only simpler but also as reliable as ileal analysis for routine determination of amino acid digestibility in poultry.

Achinewhu, S.C. and D. Hewitt, 1979. Br. J. Nutr., 41: 559.
Picard, M., S. Bertrand, M. Duron and R. Maillard, 1983. Proc. 4th Eur. Symp. on Poultry Nutrition, Tours, France.
This thesis.

VI

The value of feather meal in ruminant nutrition is still underestimated in practice.

Church, D.C., D.A. Daugherty and W.H. Kennick, 1982. J. Anim. Sci., 54: 337.
Aderibigbe, A.O. and D.C. Church, 1983. J. Anim. Sci., 56: 1198.

VII

Improved processing of by-products and waste materials should be given a high priority within any poultry improvement program in developing countries.

VIII

Important contributions to science are not based only on significant effects.

IX

Unfortunately, belief is still an important factor in animal nutrition.

X

Further development of Greek agriculture depends not only on studying the methods developed by other advanced countries, but more importantly adjusting them to local conditions.

XI

The development of a culture is better off with appropriate elements of several cultures than with the choice of one culture alone.

XII

Eventually all things come together in a constantly progressing universe:
Ta panta rhei (*Everything flows*).

Heracleitus.

Thesis; Manthos C. Papadopoulos
Feather meal: evaluation of the effect of processing conditions by chemical and chick assays.

Wageningen, 9th May, 1984.

"The facts have not yet been sufficiently established. If ever they are, then credit must be given to observation rather than to theories, and to theories only insofar as they are confirmed by the observed facts".

Aristotles

To my wife Ria,
our children Thomas and Kostis,
and our parents

Papadopoulos, M.C., 1984. Feather meal: evaluation of the effect of processing conditions by chemical and chick assays.
Doctoral thesis, Department of Animal Science, Agricultural University, Wageningen, The Netherlands; English and Dutch summaries.

FOREWORD

The research described in this thesis was carried out during the tenure of a post-graduate fellowship in the Department of Animal Science. I am very happy to take this opportunity of expressing my obligation to the Agricultural University for enabling me to undertake this work.

I am deeply grateful to my promoter Prof.Dr.Ir. E.H. Ketelaars, for his confidence in me throughout and for many valuable discussions and suggestions during the preparation of this manuscript.

My special thanks are due to Dr. A.R. El Boushy for his contribution to experimental hypotheses, organizational support and good comradeship. His interest in my work and encouraging discussions are greatly appreciated.

Various parts of the work were carried out at the experimental station of the Department of Animal Science and at the laboratories of the Departments of Animal Physiology, Food Technology and Microbiology, and I wish to thank these Departments for providing me with the facilities for the studies. To the staff of the laboratories and specially to Mr. H. Koopman, I wish to express my gratitude for most helpful discussions and assistance. The gift of the raw feathers by K. de Vries & Zn, b.v., Barneveld, is acknowledged with many thanks.

I am greatly indebted to Mr. A.E. Roodbeen for his contribution in the initial phases of this study and help on a variety of problems, and to Ir. W. de Boo for his contribution on computer programming and fruitful discussions.

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CONTENTS

chapter 1

GENERAL INTRODUCTION

At present the principal sources of animal protein for livestock feeding are fish and meat meals. These are expensive and it would be advantageous if other cheaper animal by-products could find a use in animal feeds. Poultry industries now exist in many countries and generate large amounts of residues that require disposal. Of these, a considerable quantity consists of poultry feathers. Although this keratinous material has a high protein content (85-100%) it is virtually indigestible in its natural state. Consequently a great deal of interest has been aroused over the possibility of processing it to make it more digestible. If this could be achieved economically it would provide an additional and cheap source of animal protein for livestock feeding to help meet the growing demand for animal products by an ever increasing world population.

Feather keratin is very rich in the sulfur-containing amino acid cystine (Block and Bolling, 1951). Because the cystine disulfide bonds within the keratin contribute to the insolubility of this protein, they must be destroyed before feather protein can be digested by chickens (Moran et al., 1966).

Feather meal is processed under different conditions to increase its digestibility. Whereas some treatments might increase protein digestibility they could, at the same time, reduce the nutritional value of the dietary proteins. This reduction can be correlated with changes in the amino acid pattern. These changes may not be of much nutritional significance unless other modifications to the protein affect the availability of most of the amino acids. In treated proteins part of the amino acids may become linked to other substances or form new cross-linkages within the protein molecule (Hurrell et al., 1976; Whitaker, 1980) by bonds resistant to hydrolysis by the digestive enzymes, though liberated by acid hydrolysis such as applied in analytical procedures. This chemical analysis gives only the total amino acid content. The information required, to assess nutritive value is the amount of each amino acid that is biologically available.

Hydrolyzed feather meal in chicken diets has been used for several years. However, because it is deficient in several amino acids (methionine, lysine, histidine and tryptophan) and also varies in quality, discrepancies have been

found in the amount of meal which can be effectively used under practical conditions. Our knowledge up to now of the evaluation of the protein quality of hydrolyzed feather meal is limited. Only differences between the cystine contents of raw and commercially prepared products are known while changes in the other amino acids are not fully understood. Furthermore, precise information on the influence of treatment upon the digestibility of the amino acids, particularly the limiting ones, is lacking.

This study deals primarily with the questions as to how various processing conditions may affect amino acid contents and what is the relationship between the method and degree of feather meal processing and amino acid digestibility. In addition, the amino acid profile along the small intestine and in the blood plasma of chicks was investigated, in order to obtain more information concerning feather meal digestion and absorption.

chapter 2

THE EFFECT OF DIFFERENT PROCESSING CONDITIONS ON AMINO ACID COMPOSITION AND PROTEIN SOLUBILITY OF FEATHER MEAL

2.1 Introduction

Feathers have been of interest in nutritional studies, since up to 97% of their weight consists of protein, mainly as keratin. Harrap and Woods (1964) reported that approximately 85 to 90% of the protein from feather keratin is composed of uniform units of mol. wt. 10 400. Because of their resistance to hydrolysis by proteolytic enzymes of the digestive system, keratin proteins have generally been considered to be of little or no nutritive value in the feeding of most animals.

Mangold and Dubiski (1930) failed to show any digestion of white goose feathers by cats, owls, dogs and rats. Routh (1942) reported that powdered chicken feathers as the sole source of protein for rats were capable of supporting a moderate growth rate but only when supplemented by tryptophan, histidine and lysine, while Moran et al. (1966) found that ground raw feathers failed to support growth in chicks even after supplementation with amino acids. Furthermore, McCasland and Richardson (1966) showed that rats fed on ground raw feathers as the sole source of protein lost weight and had a mortality rate of 100%. This was reduced to 25% by amino acid supplementation.

Since feather protein in its natural state is very poorly digested various methods have been developed for processing feathers to convert their keratinous proteins to a more digestible form. The results of the various methods are summarized in the following reports from the literature.

2.2 Literature

2.2.1-Characteristics of feather meal protein

Feather keratin

The high content of keratinous sources such as feathers, hair, wool, hoofs,

nails, scales and horns, has been recognized. The keratin is fibrous and differs from other proteins in its greater resistance to denaturation or other chemical and physical alterations, and the existence of a high concentration of cystine. Analysis of feather protein showed that 8.8% of the protein is cystine (Block and Bolling, 1951). Cystine forms cross-linkages between different peptide chains or different parts of the same chain within a protein. It is believed that this characteristic is responsible for the mechanical stability and chemical inertness of keratin.

Treatments for rendering keratins more digestible include grinding to a fine powder, reaction with chemical agents, use of enzymes and heat treatment. Routh and Lewis (1938) found that wool after grinding was 'digested' by trypsin and pepsin, while Olcott (1943) reported that ground hoofs were attacked by pancreatin. Heating under steam and pressure (Draper, 1944; Binkley and Vasak, 1950), chemicals such as sodium sulfide, thioglycolate (Draper, 1944; Moran et al., 1966) and enzymes, such as bacterial keratinase (Noval and Nickerson, 1959; Kuchaeva et al., 1963), resulted in disulfide bond cleavage.

Amino acid composition

The major difference in amino acid composition between raw and processed feather meal, is the drastic reduction in cystine concentration after treatment (Block and Bolling, 1951; Gregory et al., 1956; Davis et al., 1961; McCasland and Richardson, 1966; Moran et al., 1966). This is an indication that disulfide linkages have been broken, thus making the feather protein more soluble and susceptible to proteolytic enzymes.

Block and Bolling (1951) analyzed the protein of raw feathers and reported that it contained large amounts of glycine, cystine, arginine and phenylalanine. The cystine content was as high as 8.8% of the protein.

As far as the amino acid pattern of processed feathers was concerned, Gregory et al. (1956) reported that the amino acids in commercially hydrolyzed feathers (determined by the method of Binkley and Vasak, 1950) were relatively stable during processing with steam and pressure, with the exception of arginine, phenylalanine, isoleucine and cystine. Of these the only considerable loss was in cystine content.

Davis et al. (1961) studied the time-pressure combinations necessary for hydrolyzing feathers and evaluated several methods to determine their effect on amino acid composition of the processed feathers. The methods evaluated were 185 kilopascal pressure (kPa) for 16 hours, 310 kPa for 30 minutes to 4 hours

and 515 and 720 kPa for 20 and 6 minutes respectively. They reported that cystine appeared to be lowered in extreme processing conditions. They also observed the appearance of the unnatural amino acid lanthionine in feather meal, but not in feathers. The fact that the amount found approximated to the loss of cystine during processing, indicates that most of the cystine lost is converted to lanthionine [HOOC-CH(NH_2)-CH_2-S-CH_2-CH(NH_2)-COOH]. In their tests the other amino acids did not appear to be affected substantially by the processing treatments employed. Very little is known about the nutritional value of lanthionine (Robbins et al., 1980; Baker et al., 1981).

Morris and Balloun (1973b) presented results indicating that the level of the limiting amino acids (lysine, methionine and histidine) in the processed feather meal was correlated with the conditions of time, temperature and pressure in which the treatment was carried out. The maximum level was attained by hydrolysis for 1 hour at 445 kPa pressure and with intermittent stirring. Similar results for the sulfur amino acids have been reported by Wheeler and Latshaw (1980).

It is generally concluded that the cystine content in the feather meal decreases as processing time and pressure increase.

Amino acid composition of chemically treated feathers has been reported by Eggum (1970). In his trials, the addition of 1% HCl-solution to hydrolyzed feathers reduced the fall in cystine compared with feather meals processed under the same conditions of heat and pressure without HCl. It was also shown that the addition of HCl reduced the contents of other amino acids and that the decreases were most pronounced in the case of lysine, tyrosine, arginine and tryptophan when compared with feather meals treated without HCl.

Wolski et al. (1980) observed that in feathers treated with dimethylsulphoxide (DMSO) the contents of all the amino acids increased in comparison with the non-modified feathers, except for cystine, methionine, lysine and histidine. They suggested that the lowering of the contents of these amino acids might be caused by the effect of DMSO.

Concerning the effect of microorganisms on the amino acid contents of feather meal, Elmayergi and Smith (1971) published results showing that levels of methionine, tyrosine, lysine and histidine, usually present in small quantities in feather meal, were increased considerably during fermentation with *Streptomyces fradiae*. They concluded that cystine concentration decreased because it was used for methionine synthesis.

It is obvious from the available information that there are no conclusive data on changes in the content of feather meal amino acids resulting from different treatments.

2.2.2 Methods of processing feather meals

Hydrolyzed feather meal

The commercial use of feathers up to the present time has been confined mainly to hydrolyzed (autoclaved) feather meal and several reports concerning different methods of hydrolyzing feathers have been published.

In early studies, Draper (1944) observed that autoclaving the feathers for various periods from 2 to 8 hours, at different pressures between 200-240 kPa, appeared to have little positive effect on their nutritive value, measured by chick growth assays.

A method developed by Binkley and Vasak (1950) for processing feathers into a friable, high density meal, stimulated new investigations into the nutritive value of feather keratin. This method is essentially a wet cooking process in which the feathers are treated with saturated steam at pressures of 275-415 kPa for 30 to 60 minutes with constant agitation. The feathers were dried and ground to produce a free flowing meal of relatively high density. They also noted that with a steam pressure above 415 kPa and constant agitation the feathers tended to 'gum', leading to a non-free-flowing meal.

Sullivan and Stephenson (1957) found that variations in processing methods - with 200 to 340 kPa for 20 to 60 minutes - influenced the nutritive value of hydrolyzed feather meal as measured by chick growth. Moran et al. (1966) showed that commercial feather meal, hydrolyzed at 142°C for 30 min, with appropriate amino acid supplementation supported chick growth equivalent to that of soybean protein. Raw feathers autoclaved at 121°C for 18 hours however did not show the same ability to support growth. Morris and Balloun (1973a) demonstrated in chick growth trials that feather meal cooked for 60 min at 445 kPa with intermittent agitation contained more 'available' amino acids, than 'standard' feather meal processed at 340 kPa for 30 min with constant agitation.

Feathers treated with chemicals

Chemically treated feather meal has not been studied extensively. Few reports have been published on the use of this product as a protein source for animal and poultry feeding. Draper (1944) in his studies used sodium sulfide treated feathers by adding 700 g feathers to 454 g Na_2S and 16 g NaOH dissolved in 6 l water. The mixture was allowed to stand for 24 hours, with occasional stirring. The author reported that 50% supplementary protein from sodium sulfide treated feathers added to a basal cereal diet resulted in a significantly higher growth

rate than that produced by the basal diet alone when fed to chicks and rats.

Moran et al. (1966) treated feather meal samples with reducing agents such as sodium thioglycolate and sodium sulfide. They concluded that the meal prepared with the lowest concentrations of the sodium thioglycolate (¼ of the molar quantity of cystine) when supplemented with methionine, histidine, tryptophan, lysine and glycine and fed to chicks at a 15% level in a diet as the sole source of protein, gave a similar growth response to that of commercial feather meal similarly supplemented. When higher levels of sodium thioglycolate and a single level of sodium sulfide (1 mole/mole cystine) were used in the preparation of the feather meal, the chick growth response was depressed suggesting that toxic factors may have been present.

Treating feathers with sodium hydroxide during autoclaving has been reported by Gruhn and Zander (1977). They used low pressures of 200 and 300 kPa for 2 hours with different concentrations of sodium hydroxide from 0.25 to 1.0%. Their results from feeding trials with laying hens led them to suggest that treated feather meals could be used more widely.

Feathers treated with enzymes

The literature contains some reports on enzymes and microorganisms with keratinase activity, but the rate and extent of keratin hydrolysis by such enzymes is quite limited. Noval and Nickerson (1959) reported that the enzyme from *Streptomyces fradiae*, isolated from soil, seems to be an effective protease with keratinolytic activity. They concluded that the unusual ability of the organism to decompose keratin (wool and chicken feathers) rapidly and completely, may be due to its ability to reduce disulfide bonds in keratin. Day et al. (1968) and Yu et al. (1968) reported the isolation of an enzyme from *Trichophyton granulosum*, a fungus of human and mammalian dermatophytes, with keratinase activity as well as proteolytic activity. Hersiczky (1978) demonstrated a multistage degradation process and obtained a concentrated hydrolysate from the feathers of low volume as a result of enzymatic treatment with alkaline protease at pH 9 and 60°C.

While these reports indicated that some proteolytic enzymes hydrolyzed keratins, reports on the nutritive value and utilization of these hydrolysates as a protein source for poultry are quite limited. Elmayergi and Smith (1971) compared commercial feather meal, fermented by *Streptomyces fradiae* with unfermented meal, in feeding trials with chicks. They found that no significant difference in nutritional value existed between the two products, although the fermented meal was 90% digestible by pepsin-HCl solution as compared with 65-70% for unfermented meal.

Apparently, the nutritional value of enzymatically-treated feather meal in poultry nutrition is still unknown.

2.2.3 Chemical evaluation of feather meal protein quality

Hydrolyzed feather meal is characterized by variable nutritive quality, dependent upon processing methods. Consequently the animal feed industry, needs a rapid quality-control method in order to produce meals of good quality. For this purpose, crude protein analysis and digestibility determinations *in vitro* are often used in practice. However, a crude protein analysis does not distinguish between raw and hydrolyzed feathers and gives relatively little or no information about the quality of the product for animal feeding, which depends largely upon the efficiency of the hydrolytic process in the digestive tract.

Several attempts have been made to simulate *in vitro* conditions of digestion *in vivo* so as to predict the relative digestibility of proteins, since digestibility of protein is related to its usefulness as a source of individual amino acids. Gehrt et al. (1955) developed a simple enzymatic method for measuring relative digestibility of animal proteins, by the use of pepsin-HCl, originally described by Almquist et al. (1935). This method, commonly known as the 'protein digestibility' method, has been used by different investigators, usually in conjunction with other biological or chemical tests for estimating relative digestibility of feedstuffs. Standardization of the pepsin test proved to be difficult due to the impurity and low activity of the commercial pepsin preparations, and due to the use of varying levels of pepsin-HCl solution for pepsin digestion.

Feather meal showed a wide range of digestibility values when the pepsin-HCl test was applied (Naber et al., 1961; Morris and Balloun, 1973b; Johnston and Coon, 1979b; Aderibigbe and Church, 1983). The data suggested that there was a definite trend for feather meals to have higher pepsin digestibility values and increased degradation of cystine, as processing time and pressure increased. However, no standard definition of a desirable pepsin digestibility of the feather meal protein has been adopted.

Scope of the study

The published information suggests that: a) feather meal has to be treated in order to increase its digestibility for use as a feedstuff in animal nutrition and b) autoclaving hydrolysis seems to be the most used method.

Systematic studies on the influence of processing time/pressure on feather meal protein-amino acid quality are lacking. Moreover, other process variables have not been investigated. The moisture content of the raw feathers could be related to the time needed for drying the final product and therefore it might be of economic importance. Chemical and enzymatic modifications have been applied to food- and feedstuffs to give them the desired nutritional and functional properties. However, in feather meal processing the use of chemicals has been tested to only a very limited extent. Enzymes can be used as digestive aids in the processing of various foods but their use in feather meal processing has not been investigated. Therefore, the object of the research reported here was to study in detail the effect of different processing factors, namely time, moisture, sodium hydroxide (practically applicable), and enzyme (a new attempt) on the amino acid composition and protein solubility of feather meal.

2.3 Materials and methods

2.3.1 Preparation of the raw feathers

White feathers of broiler chicks of about 45 days old and weighing approximately 1400 g were obtained from a local poultry processing plant as soon as possible after plucking; they were then cleaned and freed of foreign matter. In order to protect these samples from fungal growth and insect infestation, they were dried directly after receiving in a circulating air oven at 60°C for 48 hours, to a final moisture content of about 5%, and stored in a deep-freezer at -25°C until they were processed in an autoclave.

2.3.2 Experimental design

Four experiments were conducted to determine the effect of different processing conditions on feather meal protein quality. The raw feathers were treated as follows:

Experiment 1: 9 combinations of processing time and moisture content.

Experiment 2: 15 combinations of processing time, moisture content and sodium hydroxide concentrations and

Experiment 3: 15 combinations of processing time, moisture content and enzyme concentrations. The processing conditions are further described in detail.

The treatment combinations, tested in a two-dimensional (Experiment 1) and three-dimensional (Experiments 2 and 3) central composite design (Box, 1954),

involved five lengths of processing times (30, 40, 50, 60 and 70 min), five moisture contents (50, 55, 60, 65 and 70%) and five levels of NaOH (0.2, 0.3, 0.4, 0.5 and 0.6%) and enzyme (0.2, 0.3, 0.4, 0.5 and 0.6%). Coded values running consecutively from -2 to 2 were assigned to each of the five levels of each variable (Table 2.1). The treatment combinations and their coded values (Tables 2.2 and 2.3) are also presented graphically (Fig. 2.1).

Experiment 4: This experiment was conducted to evaluate processing effects on lanthionine contents of feather meals, as well as to evaluate the extent of digestion *in vitro* with different pepsin concentrations. Some chemical indices were also calculated. The processing conditions were carefully selected to obtain maximum information with a minimum number of variables in order to keep the number of test samples for analyses within practical limits. The 9 selected treatments were as follows:

Treatments nr 5, 6 and 7, from Experiment 1 (Table 2.2) coded as FM_{50}, FM_{30} and FM_{70}, respectively

Treatments nr 9, 10 and 11, from Experiment 2 (Table 2.3) coded as $FM_{50}CH$, $FM_{30}CH$ and $FM_{70}CH$, respectively

Treatments nr 9, 10 and 11, from Experiment 3 (Table 2.3) coded as $FM_{50}EN$, $FM_{30}EN$ and $FM_{70}EN$, respectively.

Each of the prepared samples was replicated 4 times. Finally equal amounts of sample, measured on a dry matter basis, were taken from each replicate and mixed.

Table 2.1. Levels of variables studied and coded values.

Coded value		-2	-1	0	1	2
Processing time	(X_1)	30[a]	40	50	60	70
Moisture content	(X_2)	50[b]	55	60	65	70
NaOH-added	(X_3)	0.2[c]	0.3	0.4	0.5	0.6
Enzyme-added	(X_3')	0.2[d]	0.3	0.4	0.5	0.6

[a] Number of minutes
[b] Percent moisture content
[c] Percent added NaOH
[d] Percent added enzyme

2.3.3 Processing conditions

General autoclaving procedure

The stored dry feathers were taken out of the deep freezer and allowed to

Table 2.2. Treatment combinations with actual and coded values (Experiment 1).

Treatment no.	Time (X_1)		Moisture (X_2)		Number of Replications
	Min	Code	%	Code	
1	40	-1	55	-1	3
2	40	-1	65	1	3
3	60	1	55	-1	3
4	60	1	65	1	3
5	50	0	60	0	4
6	30	-2	60	0	2
7	70	2	60	0	2
8	50	0	50	-2	2
9	50	0	70	2	2

Table 2.3. Treatment combinations with actual and coded values (Experiment 2).

Treatment no.	Time (X_1)		Moisture (X_2)		NaOH (X_3)*		Number of Replications
	Min	Code	%	Code	%	Code	
1	40	-1	55	-1	0.3	-1	3
2	40	-1	55	-1	0.5	1	3
3	40	-1	65	1	0.3	-1	3
4	40	-1	65	1	0.5	1	3
5	60	1	55	-1	0.3	-1	3
6	60	1	55	-1	0.5	1	3
7	60	1	65	1	0.3	-1	3
8	60	1	65	1	0.5	1	3
9	50	0	60	0	0.4	0	4
10	30	-2	60	0	0.4	0	2
11	70	2	60	0	0.4	0	2
12	50	0	50	-2	0.4	0	2
13	50	0	70	2	0.4	0	2
14	50	0	60	0	0.2	-2	2
15	50	0	60	0	0.6	2	2

*The actual and coded values are the same for Experiment 3, but with one single treatment per combination.

equilibrate with the atmospheric moisture at room temperature. Two hundred grams of dry raw feathers were moistened with water just before processing in order to adjust the moisture content according to the experimental design.

The feathers were processed in a laboratory rotary pressure cooker (autoclave) consisting of a pressure vessel with a double wall and a safety valve, a variable speed shaft carrying the drum holder and the main stainless steel drum, readily removable, with a horizontal section, 28 cm in diameter and 12.5 cm deep. All the

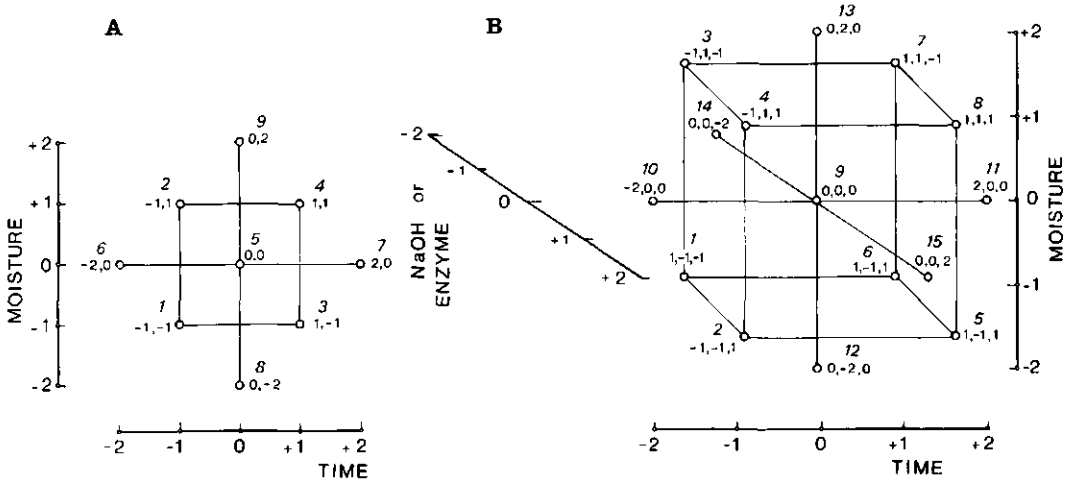

Fig. 2.1. A: Two-dimensional central composite design showing the coded values in the order of processing time (min) and moisture content (%) for each treatment combination (Experiment 1).
B: Three-dimensional central composite design showing the coded values in the order of processing time (min), moisture content (%) and NaOH (%) (Experiment 2) or enzyme (%) (Experiment 3), for each treatment combination.

processing conditions were governed by a control panel housing all the recording instruments needed to give the complete processing data. Triple steam pressure gauges gave accurate pressure readings within the inner and outer chambers and the direct temperature reading of the test material was made by a thermocouple and recorded by an electronic potentiometer.

Because no agitator was used during autoclaving hydrolysis, a modification was made so that the steam could penetrate quickly and uniformly into the feathers by using stainless steel balls (15 mm in diameter) and by adjusting a stainless steel wing on the inner wall to let the balls fall down and hit the material during processing.

After the autoclave was closed, the air was vented and the temperature/pressure raised as quickly as possible (in 2 to 3 minutes) to the required levels (146°C/436 kPa absolute pressure) by steam injected directly into the chambers. The process was timed from the time temperature/pressure was reached until the pressure was released (Fig. 2.2). The steam was exhausted as rapidly as possible (less than one min) to facilitate the removal of water when the batch was emptied. The treated feathers were transferred to a circulating air oven at 60°C, where they were dried to a moisture content of 3 to 5 percent. The dry material was ground in a hammer mill, to pass a 1-mm mesh sieve, giving a powder suitable for chemical analysis. This powder was then stored.

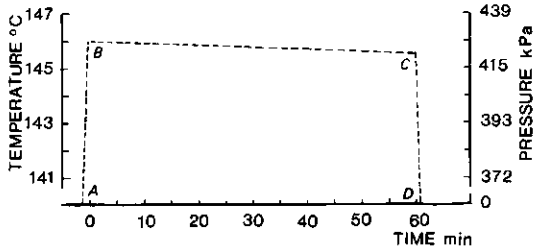

Fig. 2.2. Processing data, showing the variations in time required to reach temperature/pressure and to reduce pressure, as well as the variations during processing for 60 min autoclaving hydrolysis of feather meal. A: Loading autoclave; B-C: 60 min; D: emptying autoclave.

Chemical treatment

Sodium hydroxide (10%) was dissolved in water, in the concentration described in the experimental design (Table 2.3). It was sprayed as a mist on 200 g air-dry raw feathers just before processing and the material was thoroughly mixed. The autoclaving procedures took place as previously described.

Enzymatic treatment

The following parameters were used in the enzymatic hydrolysis.

Substrate: A powdered feather meal substrate was prepared by heat and pressure under different processing conditions of time and moisture content. The procedures have already been described in detail (see: General autoclaving procedure).

Enzyme: Maxatase[1], P 330 000, a commercial proteolytic enzyme preparation produced by a special strain of a spore-forming *Bacillus* free from pathogenic microorganisms and active at an alkaline range of pH, was obtained as a sample from a manufacturing company.

Temperature and *pH* were adjusted to their optimum ranges, as listed by the enzyme manufacturer.

Reaction time: Incubation time (2 hrs) was based on results obtained in a preliminary trial designed to determine the optimum conditions. The trial is described later.

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[1] Gist-Brocades N.V., Delft, the Netherlands

Enzymatic hydrolysis (procedure): Two hundred grams of feather meal suspended in 100 ml of hot water ca 58°C, was immersed in a waterbath. The pH was maintained with concentrated ammonia (25%) at 8.5 and the required amount of enzyme related to the original weight (section 2.3.2, Table 2.3) was added to the aqueous suspension of the feather meal. The mixture was gently agitated at 52°C for 2 hours, then heated at 87°C for 5 minutes to inactivate the enzyme and cooled rapidly. During incubation, care was taken to minimize evaporative loss of water, by covering the beaker. One half of the suspension was then freeze-dried. The other half was centrifuged at 18 000 x g for 60 minutes. The solids were washed once with distilled water and centrifuged again, under the same conditions. The total supernatant liquid was freeze-dried, as shown in Figure 2.3.

Determination of reaction time (preliminary trial): This experiment was designed to determine the optimum duration of enzymatic hydrolysis of the autoclaved feather meal under the recommended conditions of pH and temperature. The reaction was allowed to proceed for 5 hours and samples were withdrawn periodically. A control series without added enzyme was run concurrently with the test series. The criterion for assessing the effectiveness of the enzyme's action was the ability to increase the solubility of the feather meal protein. This is defined as:

$$\text{percent enzymatic conversion} = \frac{\text{wt of the supernatant (freeze-dried)}}{\text{wt of the sludge + supernatant (freeze-dried)}}$$

The extent of that conversion increases with time (Fig. 2.4). The increase is rapid in the first half-hour, followed by a gradual increase up to 2 hours. When time is longer, up to 5 hours, there is only a slight increase in solubility of the feather meal.. Therefore, 2 hours of enzymatic hydrolysis was chosen as the optimum reaction time for the enzymatic treatment of feather meals.

2.3.4 Analytical methods

Amino acid analysis

Amino acids were determined by ion-exchange chromatography procedure as described by Spackman et al. (1958). The test sample was hydrolyzed with 6 N HCl at 110°C for 22 hours. A Biotronic, model LC-6000, automatic amino acid analyzer was used for the analysis. The sulphur-containing amino acids, methionine and cystine, were determined as methionine sulfone and cysteic acid, respectively, after performic acid oxidation (Moore, 1963) followed by acid hydrolysis. Duplicate determinations were made for each sample. Calculations were based on the

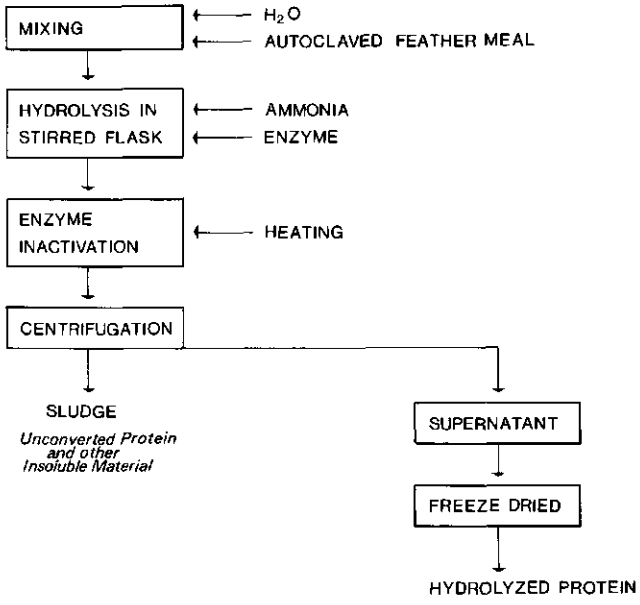


Fig. 2.3. Diagrammatic representation of the preparation of enzymatic hydrolysis.

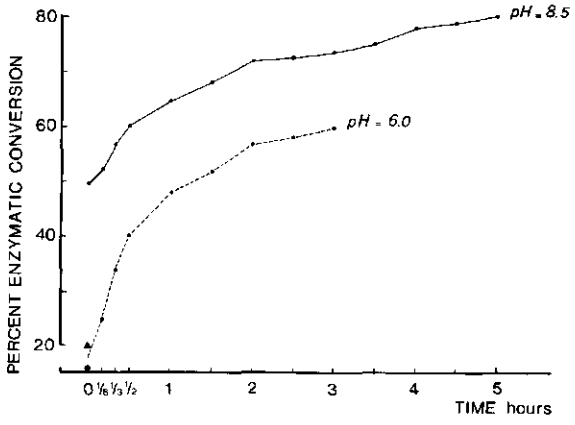


Fig. 2.4. The percentage of enzymatic conversion in relation to time, when enzyme (0.4%) was incubated with feather meal at 52°C with pH 8,5 and pH 6.
▲ and ● : Control samples without enzyme at pH 8.5 and pH 6, respectively.
Each point represents an average of two determinations.

comparison with a standard mixture containing all the amino acids in the concentration of 0.150 µmoles/ml, except for cystine (0.075 µmoles/ml), as specified in the instruction manual supplied with the instrument. Nor-leucine was used as an internal standard.

For correction of the destruction of threonine and serine and of the incomplete hydrolysis of isoleucine and valine, factors were used as given by Slump et al. (1977). Lanthionine was measured as described by Friedman et al. (1977) by using special chromatographic conditions to separate the two lanthionine peaks ((R)-L-lanthionine and meso lanthionine) from glutamic acid/proline. The virtually complete stability of lanthionine during acid hydrolysis of feather meal was proved by adding a measured amount of crystalline L-DL-lanthionine to the test feather meal sample and hydrolyzing the mixture. Blank hydrolyzates (with lanthionine only) were also prepared. Duplicate quantitative recoveries of lanthionine are shown below.

No	Sample/Hydrolyzate	Lanthionine recovery (%)
1	feather meal A + Lanthionine	98.41 and 100.30
2	feather meal B + Lanthionine	98.90 and 97.60
3	Lanthionine (Blank)	99.70 and 98.90

The raw and processed feather meals were also analysed for crude protein, ash and moisture by procedures described in the AOAC (1975).

In vitro protein digestibility

Protein digestibility of the processed feather meals *in vitro* was determined by pepsin-hydrochloric acid treatment.

Samples of 1 g feather meal in 390 ml of distilled water were incubated with 9 ml 4 N HCl and 50 ml of a freshly prepared solution of 0.2% pepsin (activity 1 : 10 000) at 40°C. After 48 hours, 15 ml 25% HCl were added and the digest was cooled, brought to 500 ml with distilled water, and filtered. Duplicate aliquots of 50 ml of the filtrate were analyzed for nitrogen by the Kjeldahl method. Additionally, 'pepsin blank values' were determined. The corrected pepsin digestibility of the feather meal protein (PDP) was calculated as the ratio of the weight of protein (N x 6.25) recovered from the filtrate to the weight of the protein in the test meal, multiplied by 100.

In addition, a higher (2)% and a lower (0.02%) pepsin concentration were used in Experiment 4.

Nitrogen solubility

Solubility in alkali: The nitrogen solubility in 0.02 N NaOH (NSS) was determined by the method outlined by Lyman et al. (1953) with slight modifications. Samples of approximately 1 g (weighed to the nearest mg) ground to pass a 1-mm mesh sieve were shaken for 1 hour at 160 strokes/min with the solvent in a water-bath maintained at 37°C. The suspension was centrifuged for 15 min, at 3000 r.p.m. and the soluble nitrogen determined by Kjeldahl estimations on 25-ml portions of the supernatant liquid. The nitrogen solubility was expressed as the percentage of total nitrogen.

Solubility in acid: The nitrogen solubility in 6 N HCl (NSH) was determined by the same procedure as with sodium hydroxide.

Chemical indices (CI) were calculated taking into consideration the nitrogen solubility in alkali and acid respectively, in relation to cystine destruction of the test materials: CI = [NSS or NSH] ÷ [cystine (%)] x 100.

The chemical score (CS) of each meal (Experiment 4) was also calculated. The amino acid (AA) with the highest percentage deficit from the corresponding one in a reference protein was the first limiting amino acid and was used to determine the chemical score: CS = (AA test protein) ÷ (AA reference protein) x 100. The amino acid requirements of 3-week old broilers (NRC, 1977) were those taken for reference.

2.3.5 Statistical methods

In this study a multiple regression model was constructed, using the coded values (Tables 2.2 and 2.3), to estimate linear, quadratic and interaction effects, as follows. The models were:

$$\hat{Y} = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2,$$

for Experiment 1

$$\hat{Y} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

for Experiments 2 and 3, and

$$\hat{Y} = b_0 + C_1 + C_2 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 +$$
(all interactions of C_1 and C_2 with X_1, X_2, X_1^2, X_2^2 and X_1X_2)

for the combination of the three Experiments,

where $\hat{Y}$ is the measured response, the b's are the partial regression coefficients, X_1, X_2 and X_3 are the actual values for the independent variables, and C_1 and C_2 are the contrasts between treatments without additions (Experiment 1) with the chemical treatment (Experiment 2) and enzymatic treatment (Experiment 3), respectively.

The higher degree effects of the variables have been omitted because their analysis provided very weak information.

Regression coefficients were tested for significance by the F-test. The respective response surfaces were calculated and generated by computer, using the significant partial coefficients of the prediction equation, where independent variables varied over the entire experimental range.

2.4 Results

The amino acid analysis of the basic material (raw feathers) is given in Table 2.4. Observed values for individual amino acids and other response criteria of the processed feather meals are in Appendices 1-3. The statistical evaluation of the data is presented in detail in Tables 2.5, 2.6 and 2.7.

Table 2.4. Amino acid composition (%) of raw feathers[1].

Essential amino acids		Non-essential amino acids	
Threonine	4.66 ± 0.08[2]	Aspartic acid	6.21 ± 0.13
Cystine[3]	6.87 ± 0.29	Serine	11.13 ± 0.25
Valine	7.38 ± 0.10	Glutamic acid	9.32 ± 0.19
Methionine	0.57 ± 0.04	Proline	8.81 ± 0.19
Isoleucine	4.90 ± 0.09	Glycine	6.25 ± 0.12
Leucine	7.41 ± 0.08	Alanine	4.27 ± 0.04
Tyrosine[3]	2.79 ± 0.06		
Phenylalanine	4.35 ± 0.25		
Lysine	1.97 ± 0.08	NH_3	1.30 ± 0.03
Histidine	0.60 ± 0.03	Nitrogen	15.50 ± 0.03
Arginine	6.45 ± 0.13		

1) All values are on dry matter basis.
2) Mean ± standard error with five observations per mean.
3) Semi-essential amino acid (Scott et al., 1983), for this and all subsequent tables and figures.

2.4.1 Effect of time and moisture (Experiment 1)

Amino acids

Processing time (T) and moisture content (M) showed varied positive and negative effects on the different response criteria (see partial regression coefficients in Table 2.5).

According to the significance of the estimates, the most pronounced effect of processing time was on the contents of cystine and the three limiting amino acids: methionine, lysine and histidine. However, their responses to T were not the same. Cystine was severely depressed by increasing T and the coefficients of determination (R^2) indicate that the linear component accounted for 83.3% of the variation observed. Methionine and lysine were curvilinearly influenced by T in a positive and negative coefficient, respectively, while histidine had a positive linear coefficient. Figure 2.5 clearly illustrates the variations of the significant components on the concentrations of the limiting amino acids.

Moisture content had a positive linear effect on methionine and lysine, and a negative quadratic effect on histidine. Moreover, the moisture content seems to be the major factor influencing the non-essential amino acids in a negative way.

Cystine and histidine were the only amino acids which were significantly affected by T x M interaction. Variation of those amino acids was explained to only a small extent by the regression equation used, as may be seen from their R^2 values (Table 2.5).

Nitrogen characteristics

Processing time was the main factor influencing nitrogen solubilities in alkali (NSS) and acid (NSH) environments. A large part of the variation was explained by the positive linear components (Table 2.5).

Moisture content showed a negative linear effect on crude protein (CP) and a positive curvilinear effect on pepsin digestible protein (PDP) (Fig. 2.5).

2.4.2 Effect of time, moisture and chemical (Experiment 2)

Amino acids

It is obvious from Table 2.6 that the amino acids showed considerable variation in their response to different processing variables, T, M and sodium hydroxide.

Table 2.5. Multiple regression and correlation coefficients for amino acids and nitrogen characteristics, for linear, quadratic and linear x linear effects (Experiment 1).

Regression equation[1]: $\hat{Y} = b_0 + b_1T + b_2M + b_{11}T^2 + b_{22}M^2 + b_{12}TM$

Predicted responses ($\hat{Y}$)	Multiple regression coefficients									
		Linear				Quadratic				Interaction	
	b_0	b_1	R^2	b_2	R^2	b_{11}	R^2	b_{22}	R^2	b_{12}	R^2
Essential											
Thr	4.782	-0.001_{***}	0.0	-0.059^{**}	32.4	-0.022	4.4	-0.011	1.3	-0.003_{*}	4.5
Cys	3.972	-0.240^{***}	83.3	0.008	0.1	0.010	0.0	0.014	0.3	-0.076^{*}	3.6
Val	7.771	0.027	2.9	-0.059_{*}	14.1	-0.032_{*}	9.3	0.014	0.9	-0.049	4.2
Met	0.586	0.001_{**}	0.1	0.006_{*}	18.2	0.005	33.1	-0.003	5.2	-0.004	3.7
Ile	5.015	0.051^{**}	29.6	-0.034^{*}	13.1	-0.017	5.6	0.001	0.0	-0.039	7.5
Leu	7.543	-0.005	0.5	-0.018	5.7	-0.011	10.4	0.018	6.9	-0.028	6.2
Tyr	2.315	-0.014	1.4	0.051	18.1	0.024	0.5	0.035_{***}	10.2	0.016	0.8
Phe	4.199	0.035	2.8	0.028_{*}	1.8	0.012_{**}	7.4	0.120^{***}	40.5	-0.004	0.0
Lys	1.956	-0.003_{*}	0.9	0.009^{*}	7.8	-0.013	18.0	-0.005_{*}	2.6	0.002_{*}	0.1
His	0.687	0.006^{*}	12.8	-0.001	0.4	-0.002	0.2	-0.005^{*}	9.9	0.008^{*}	8.4
Arg	6.569	-0.009	0.4	-0.028	4.2	-0.024	1.6	-0.021	2.8	-0.031	2.2
Non-Essential											
Asp	6.521	-0.001	0.0	-0.053^{*}_{*}	17.4	-0.031	0.2	-0.055^{*}_{*}	22.6	-0.014	0.5
Ser	11.596	0.020	0.7	-0.105^{**}	19.5	-0.030	0.7	-0.096^{*}	19.5	-0.023	0.4
Glu	10.937	0.060	6.3	-0.151^{**}	39.7	-0.017	0.0	-0.032	2.1	-0.016	0.2
Pro	8.997	0.015	0.5	-0.053	6.4	-0.016	0.4	-0.011_{*}	0.3	-0.036	1.3
Gly	7.062	0.027	4.3	-0.017	1.7	-0.023	0.3	-0.061^{*}	26.7	-0.009	0.2
Ala	4.293	0.008	1.0	-0.020	7.1	-0.005	1.5	0.005	0.6	-0.008	0.4
CP[2]	96.915	-0.073	0.9	-0.362^{**}_{**}	21.1	-0.231	18.4	0.085	1.4	-0.333_{*}	7.7
Ash[3]	1.228	0.022	6.8	-0.048^{**}	33.1	0.005_{*}	0.1	0.016_{*}	4.3	-0.048^{*}	13.8
PDP[4]	92.330	0.184_{***}	2.2	-0.345_{***}	7.9	0.478_{***}	7.4	0.437_{***}	15.1	0.453	5.8
NSS[5]	73.415	6.640_{***}	88.5	-1.364^{**}	3.7	-1.146	1.2	-1.130^{***}	3.1	-0.153_{*}	0.0
NSH	37.071	5.378^{***}	95.3	0.488^{**}	0.8	0.210	0.5	-0.234	0.2	-0.543^{*}	0.4

[1] In the equation $\hat{Y}$ is the predicted response, b's are the partial regression coefficients and T and M are the coded time and moisture, respectively.

[2] Crude protein; [3] Pepsin digestibility protein; [4] Nitrogen solubility in 0.02 N NaOH; [5] Nitrogen solubility in 6 N HCl

*P < 0.05, **P < 0.01, ***P < 0.001.

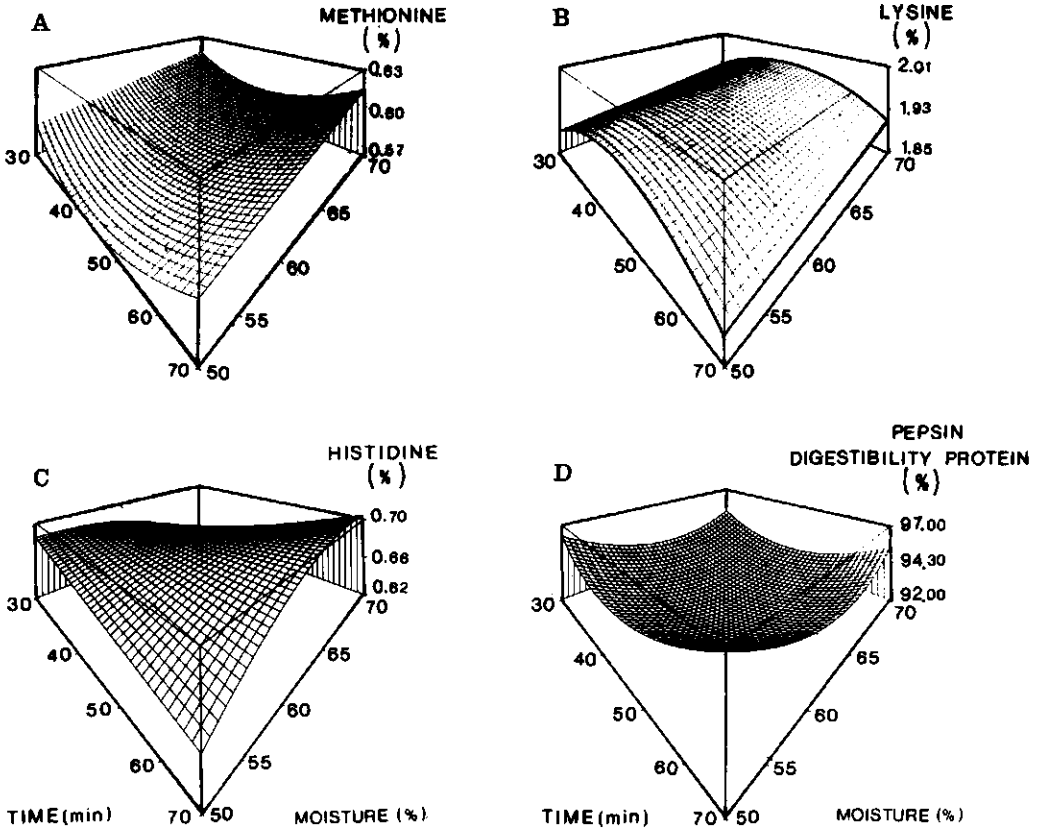


Fig. 2.5. Three dimensional response surfaces of methionine (A), Lysine (B), Histidine (C) and Pepsin digestibility protein (D) for feather meals treated under varying processing time and moisture content.

Significant effects of T were those on threonine, cystine and non-essential amino acids which had negative linear coefficients. A positive effect of time was shown on histidine and lysine, expressed in a linear and curvilinear way, respectively.

The effect of M was most pronounced on methionine and histidine. Both amino acids were significantly affected by negative quadratic coefficients.

Sodium hydroxide (C) had a highly significant effect on threonine, cystine and lysine which had negative linear coefficients. Isoleucine, leucine, tyrosine, phenylalanine and lysine behaved in a similar curvilinear way from the C effect, and had negative coefficients.

The most significant interactions were the T x M for methionine and the M x C for arginine.

Table 2.6. Multiple regression and correlation coefficients for amino acids and nitrogen characteristics, for the linear, quadratic and linear x linear effects (Experiment 2).

Predicted responses ($\hat{Y}$)	Regression equation[1]:								
		Linear							
	b_0	b_1	R^2	b_2	R^2	b_3	R^2	b_{11}	R^2
Essenstial									
Thr	4.492	-0.076***	24.1	-0.029*	3.6	-0.060***	15.0	-0.004	0.1
Cys	2.876	-0.167*	32.5	-0.080*	7.5	-0.193***	43.6	0.034	1.8
Val	7.623	-0.035*	5.1	0.040**	6.4	0.013*	0.7	-0.005	0.4
Met	0.529	0.000	0.0	-0.009**	10.5	-0.007*	7.1	0.003	0.1
Ile	5.124	0.025*	3.1	0.006	0.2	-0.022	2.4	-0.020	0.7
Leu	7.796	0.035***	5.0	0.012	0.6	-0.007	0.2	-0.028	3.0
Tyr	2.767	0.056***	14.0	-0.006	0.2	-0.024	2.6	-0.003	6.4
Phe	4.797	0.024	1.3	-0.016	0.6	-0.023***	1.2	0.028*	14.0
Lys	1.947	-0.003***	0.2	-0.003***	0.2	-0.029***	18.9	0.014*	9.7
His	0.604	0.011***	12.6	-0.015***	23.5	-0.002	0.2	-0.004	4.6
Arg	6.322	0.005	0.1	-0.001	0.0	-0.017	1.4	0.021	0.3
Non-Essential									
Asp	6.067	-0.122***	40.2	-0.023	1.5	0.012	0.4	0.019	0.4
Ser	10.927	-0.154***	15.5	-0.018	0.2	-0.060	2.3	0.023	1.5
Glu	11.128	-0.026	1.6	0.047**	5.0	0.028**	1.8	-0.006	1.4
Pro	9.057	-0.003**	0.0	-0.141**	12.1	0.117**	8.4	0.090	6.6
Gly	6.898	-0.053***	8.7	0.014	0.6	0.058**	10.6	0.009	3.1
Ala	4.153	-0.044***	13.4	-0.022	3.3	0.001	0.0	0.011	1.2
CP[2]	95.673	-0.187*	5.4	0.134***	2.8	-0.478***	35.3	-0.019	1.4
Ash[3]	2.450	-0.025*	0.6	0.099***	8.7	0.304***	82.7	-0.010	0.0
PDP[4]	94.579	-0.383***	6.8	0.360***	6.0	0.174*	1.4	0.033***	1.6
NSS[5]	73.905	9.021***	81.2	-2.310***	5.3	0.669***	0.5	-1.777**	6.5
NSH[5]	57.290	9.009***	60.2	2.859***	6.0	6.433***	30.7	0.361**	0.0

[1] In the equation $\hat{Y}$ is the predicted response, b's are the partial regression coefficients and T, M and C are the coded time, moisture and chemical, respectively.

[2], [3], [4], [5] See Table 2.5, footnotes 2), 3), 4) and 5), respectively.

*P < 0.05, **P < 0.01, ***P < 0.001.

$\hat{Y} = b_0 + b_1T + b_2M + b_3C + b_{11}T^2 + b_{22}M^2 + b_{33}C^2 + b_{12}TM + b_{13}TC + b_{23}MC$

Multiple regression coefficients

Quadratic				Interaction					
b_{22}	R^2	b_{33}	R^2	b_{12}	R^2	b_{13}	R^2	b_{23}	R^2
-0.021	1.5	-0.005	0.1	-0.011*	0.3	-0.028	2.0	-0.004	0.0
0.001	0.1	-0.009	0.1	0.046*	1.5	-0.001**	0.0	-0.020	0.3
-0.091	0.1	-0.031	3.1	-0.025***	1.5	-0.075**	13.6	-0.010*	0.3
0.003	0.2	0.004**	1.6	0.016*	21.8	0.006	3.0	-0.008*	6.1
-0.032***	0.0	-0.061***	15.0	-0.053	8.2	-0.010	0.3	-0.013	0.5
-0.072*	3.8	-0.085***	23.7	-0.037	3.4	-0.040	3.9	-0.005*	0.1
-0.033*	0.2	-0.079***	22.3	-0.007	0.1	0.002	0.0	0.040*	4.2
-0.013	5.4	-0.119***	25.9	0.016	0.4	0.013	0.2	0.020	0.6
0.010	11.4	-0.023**	9.8	0.004*	0.3	0.005	0.4	-0.005	0.4
0.001	0.3	0.004	1.6	-0.007***	2.8	-0.006	2.1	0.004**	1.1
0.018	0.2	0.024	2.2	-0.084***	21.1	-0.033	3.1	0.063**	11.9
0.003	0.2	0.023	1.2	-0.035	2.0	-0.050*	4.0	-0.004	0.0
-0.053*	1.6	-0.010	0.1	-0.055	1.2	-0.042	0.7	-0.062	1.5
-0.071*	10.2	-0.014	0.4	-0.013	0.3	-0.061	5.1	0.014	0.3
0.043	3.3	-0.058	1.6	0.077	2.2	0.093**	3.1	0.078	2.2
-0.029	0.7	-0.029	2.1	-0.048	4.3	-0.069**	9.0	0.014	0.4
-0.003	0.1	0.001	0.0	-0.028	3.4	-0.049**	10.3	-0.012	0.6
0.116	0.9	0.083	0.9	0.290*	7.8	-0.178	2.9	-0.039	0.1
0.022	0.1	0.019	0.3	-0.018	0.2	-0.013*	0.1	0.029	0.5
-0.291	2.7	-0.116***	0.5	0.366***	3.8	0.539*	8.1	0.157***	0.7
0.395	0.1	1.281***	1.3	1.428***	1.2	-0.348***	0.1	1.421**	1.2
0.153	0.1	0.863***	0.4	-0.240	0.0	1.580***	1.1	0.355**	0.1

Response surfaces for the dependent variable were also drawn, but Figure 2.6 illustrates only the most representative ones.

Nitrogen characteristics

The increase of T and C caused a significant decrease in CP, which was dominated by the C-linear component (R^2 = 35.3%).

PDP was significantly influenced by a negative linear effect for T and had a positive interaction coefficient for T x C (Fig. 2.6).

For NSS and NSH, processing time was the main influencing factor.

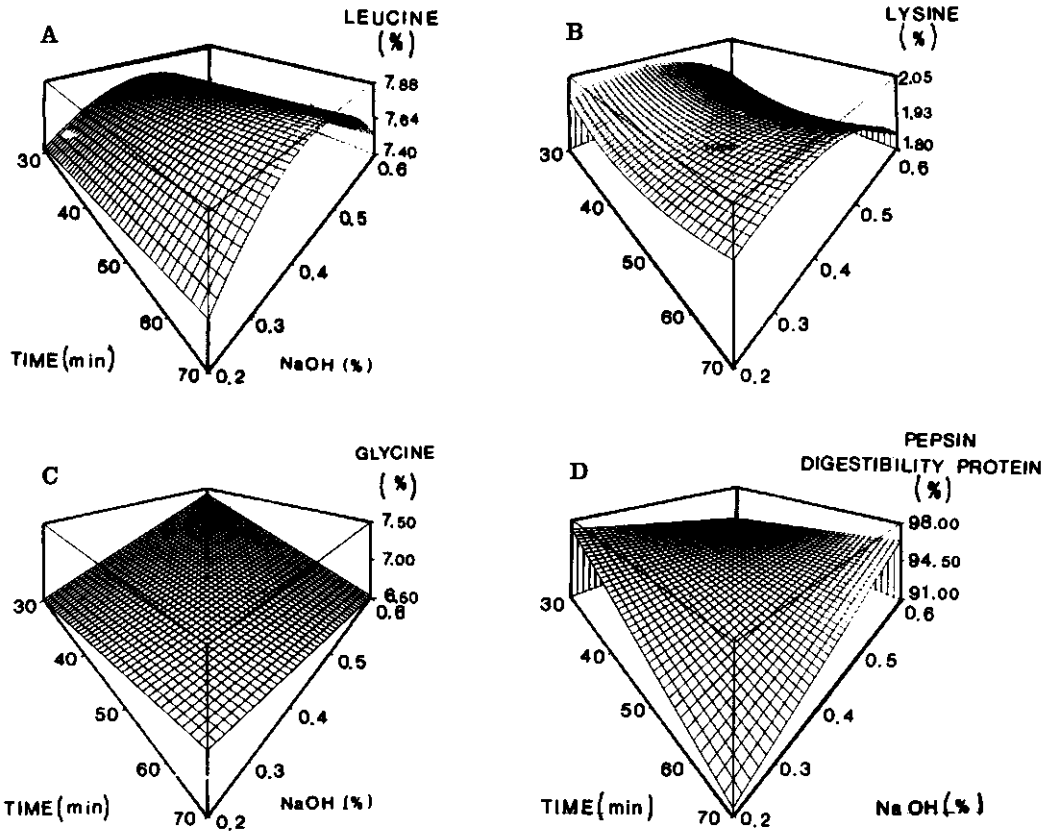


Fig. 2.6. Three dimensional response surfaces of leucine (A), lysine (B), glycine (C) and pepsin digestibiliy protein (D) for feather meals treated under varying processing time and NaOH content.
The graphical description of the two independent variables (time, NaOH) has been made by holding the third variable (moisture at a constant intermediate value.

2.4.3 Effect of time, moisture and enzyme (Experiment 3)

Amino acids

Table 2.7 shows the statistical evaluation of the data of the enzymatically treated feather meals. From these results it is obvious that enzymatic hydrolysis (E) provides very little information about its significance in the regression model used. However, there were some exceptions. The positive quadratic coefficient of E was significant for threonine. From the interactions, only M x E was significant and had a negative effect, mainly on threonine, isoleucine, aspartic and glutamic acids.

Nitrogen characteristics

The most pronounced effect of E was on the enzymatic protein conversion (EPC), which increased with increasing enzyme levels (Table 2.7).

2.4.4 Differences between treatments with and without additions

The statistical analyses of the three experiments did not distinguish the differences between feather meals treated without additions and samples treated with NaOH or enzyme. Therefore a statistical analysis of the three experiments combined was made in order to test these differences. The results are demonstrated in Table 2.8. The analysis of variance shows that there are significant differences between the experiments (no additions vs chemical or enzyme). These differences are more pronounced than those of the time and moisture effects within experiments. Moreover, the effect of chemical is more pronounced than that of enzyme. Most of the amino acid contents, with the exceptions of leucine, tyrosine, phenylalanine and proline, were significantly lower and the protein (N x 6.25) solubilities higher in the chemically or enzymatically treated feather meals by comparison with the samples with no additions.

2.4.5 Lanthionine content and other tests (Experiment 4)

The results of the lanthionine contents and the other laboratory tests in Experiment 4, are given in Table 2.9.

Lanthionine: It is obvious from these data that the lanthionine content increased with increasing processing time of feather meals and is inversely proportional to the cystine content. The highest values of lanthionine content were

Table 2.7. Multiple regression and correlation coefficients for amino acids and nitrogen characteristics, for the linear, quadratic and linear x linear effects (Experiment 3).

Predicted responses ($\hat{Y}$)	Regression equation[1]:							
		Linear						
	b_0	b_1	R^2	b_2	R^2	b_3	R^2	b_{11}	R^2
Essential									
Thr	4.451	-0.022***	4.2	0.028	6.9	-0.027	6.3	-0.026	30.6
Cys	3.844	-0.279***	71.5	-0.031	0.9	-0.016	0.2	-0.001	2.0
Val	7.172	-0.028	13.1	0.001	0.0	0.012	2.3	-0.029	39.2
Met	0.557	0.006	6.9	-0.009	19.1	0.003	2.1	-0.008	3.2
Ile	4.866	-0.023	6.2	0.038*	17.1	0.006	0.5	-0.039	15.7
Leu	7.638	-0.025	0.1	0.341*	26.8	-0.009	0.0	-0.066	2.9
Tyr	2.453	-0.026	9.8	-0.005	0.4	0.000	0.0	0.038	1.2
Phe	4.381	-0.004	0.2	0.020	4.9	-0.028	9.3	0.019	9.1
Lys	1.823	-0.007***	2.7	-0.002	0.2	-0.006	1.8	0.003	20.2
His	0.629	0.011***	48.2	0.005	9.5	0.000	0.0	-0.006	15.0
Arg	6.261	0.004	0.2	0.019	3.8	-0.028	8.1	0.035	5.4
Non-Essential									
Asp	5.886	-0.006	0.7	-0.005	0.5	0.018	5.8	-0.014***	16.6
Ser	10.497	0.066	3.6	0.069	3.9	-0.006	0.0	-0.227***	75.0
Glu	10.240	0.101	14.3	0.012*	0.2	-0.089	11.3	-0.043	18.4
Pro	8.800	-0.045**	7.6	0.089*	29.6	-0.016	1.0	0.023	4.4
Gly	6.259	0.061**	34.4	-0.039	13.8	0.014	1.7	-0.012	14.9
Ala	4.072	0.066	14.3	0.079	20.9	0.006	0.1	-0.038	0.0
CP[2]	97.557	-0.027	0.4	-0.099	5.6	-0.028	0.5	-0.290**	48.8
Ash[3]	1.393	-0.010	3.9	0.000	0.0	0.013	6.0	-0.022	0.3
PDP[4]	95.377	0.311***	16.2	0.214**	7.7	-0.116*	2.2	0.085*	13.1
NSS[5]	88.443	3.225***	77.4	-0.933**	6.5	0.831*	5.1	-0.579*	1.1
NSH[6]	80.382	6.964**	70.5	-0.564	0.5	1.975*	5.7	-2.543	11.2
PEC[6]	85.286	8.268**	79.2	-0.688	0.0	3.068*	10.9	-1.027	4.1

[1] In the equation $\hat{Y}$ is the predicted response, b's are the partial regression coefficients and T, M and E are the coded time, moisture and enzyme, repsectively.

2), 3), 4), [5] See Table 2.5, footnotes 2), 3), 4) and 5), respectively.

[6] Percent enzymatic conversion.

*P < 0.05, **P < 0.01, ***P < 0.001.

$$\hat{Y} = b_0 + b_1T + b_2M + b_3E + b_{11}T^2 + b_{22}M^2 + b_{33}E^2 + b_{12}TM + b_{13}TE + b_{23}ME$$

Multiple regression coefficients

Quadratic				Interaction					
b_{22}	R^2	b_{33}	R^2	b_{12}	R^2	B_{12}	R^2	b_{23}	R^2
0.010	5.1	0.049*	14.2	-0.009	0.3	-0.001	0.0	-0.064*	17.7
-0.038	0.0	-0.062	2.4	0.069	2.2	-0.059	1.6	0.004	0.0
0.011	0.0	0.019	3.9	0.011***	1.1	-0.006	0.3	-0.029	6.8
-0.006	0.4	-0.007	7.6	0.019***	38.2	-0.009	8.3	0.001*	0.2
-0.023	7.6	-0.007	0.0	-0.028	4.6	-0.020	2.4	-0.063*	23.8
-0.396	42.8	-0.006	0.0	-0.015	0.0	-0.005	0.0	-0.063	0.5
0.055	3.4	0.062	37.8	0.015	1.6	0.020	2.8	-0.015	1.6
0.042	0.0	0.062	33.1	-0.015	1.4	0.000	0.0	-0.010	0.6
0.023	3.7	0.025	24.2	-0.004	0.4	0.022	13.1	-0.004	0.4
-0.002	2.8	0.000	0.0	0.000	0.0	0.000	0.0	-0.005	4.8
0.015	0.1	0.026	5.1	-0.048	12.1	-0.033	5.7	-0.038	7.5
0.018	3.7	0.008	0.9	-0.013	1.5	-0.023	4.8	-0.060**	34.0
0.027	0.0	0.035	0.7	0.023	0.2	0.000	0.0	-0.148**	9.0
0.031	2.2	0.103	10.3	0.041	1.2	0.051	1.9	-0.194**	26.4
-0.049	22.8	0.034	3.0	0.028	1.4	-0.018	0.6	-0.013	0.3
0.025	0.4	0.029	5.3	0.000	0.0	-0.013	0.7	-0.033	4.8
-0.090	32.0	-0.002	0.0	-0.009	0.1	-0.014	0.3	-0.041	2.8
-0.081*	2.5	-0.031	0.4	-0.269**	20.4	0.104	3.0	0.091	2.4
-0.040*	34.3	-0.020	11.2	-0.015	4.4	0.010	1.9	0.013	3.0
-0.200*	1.5	-0.175	3.5	0.199***	3.3	-0.361*	10.9	-0.054	0.2
-0.508*	1.6	-0.140	0.1	1.225***	5.6	-0.613*	1.4	0.020	0.0
-0.662	0.9	0.101	0.0	1.393	1.4	-0.950	0.7	-0.398	0.1
0.459	0.0	0.932	0.7	-0.520	0.2	-0.515	0.2	0.648	0.0

Table 2.8. Multiple regression and correlation coefficients for amino acids and nitrogen characteristics, for the linear, quadratic and linear x linear effects (Experiments 1, 2 and 3).

Regression equation[1]: $\hat{Y} = b_0 + C_1 + C_2 + b_1T + b_2M + b_{11}T^2 + b_{22}M^2 + b_{12}TM +$ (all interactions of C_1 and C_2 with T, M, T^2, M^2 and TM)

Predicted responses ($\hat{Y}$)	Multiple regression coefficients							
	b_0	C_1	C_2	b_1	b_2	b_{11}	b_{22}	b_{12}
Essential								
Thr	4.611	-0.211**	-0.300***	-0.033^{*+}	-0.020	-0.027*	-0.017	-0.018
Cys	3.507	-0.279***	-1.113**	-0.229***	-0.034	0.029	0.007	0.013
Val	7.517	-0.553***	-0.209**	-0.012	-0.006^{+}	-0.021	0.005	0.021**
Met	0.554	-0.047***	-0.050***	0.002	-0.004^{++}	0.001	-0.001	0.010^{++}
Ile	4.960	-0.151*	-0.013	0.018	0.003***	-0.015	-0.008***	-0.040
Leu	7.594	0.089***	0.092***	0.002^{+}	0.112^{+++}	-0.019	-0.134^{+++}	-0.027
Tyr	2.510	0.291**	0.293***	0.005^{+}	0.017	0.020^{+}	0.019**	0.008
Phe	4.431	0.336*	0.361*	0.019	0.010	0.026^{+}	0.056**	-0.001
Lys	1.913	-0.072***	-0.056***	-0.004***	0.001$_{++}$	0.000$_{*}$	0.008	0.001
His	0.643	-0.057**	-0.074***	0.009***	-0.004^{++}	-0.005*	-0.003	0.000$_{*}$
Arg	6.421	-0.243**	-0.199**	-0.000	-0.003	0.001	-0.006	-0.054*
Non-essential								
Asp	6.180	-0.616***	-0.408***	-0.043^{**++}	-0.027	-0.014$_{*}$	-0.017	-0.020
Ser	11.029	-1.012***	-0.689***	-0.023^{+}	-0.018^{++}	-0.084^{+++}	-0.047	-0.003
Glu	10.843	-0.444**	0.164	0.045	-0.031^{++}	-0.042	-0.044	0.004
Pro	8.941	-0.113***	-0.054**	-0.011^{+}	0.059	0.034	-0.003	0.023
Gly	6.744	-0.732***	-0.211**	0.012^{+}	-0.023	-0.010	-0.023$_{*}$	-0.019
Ala	4.172	-0.225**	-0.139*	0.010^{++}	0.013^{++}	-0.010	-0.029^{++}	-0.015
CP[2]	96.745	0.566	-1.075**	-0.096	-0.109^{+}	-0.187*	0.033	-0.104^{+}
Ash[3]	1.687	0.115**	1.261**	-0.004	0.017	-0.001	0.000	-0.027
PDP[4]	93.875	2.617***	2.017**	0.037***	0.076	0.256***	0.040	0.339
NSS[5]	79.326	14.683***	3.051**	6.295^{+++}	-1.535***	-1.350^{+++}	-0.597*	0.834*
NSH	58.906	43.560***	21.944***	7.117^{+}	0.928	-0.823	-0.413	0.203

[1] In the equation $\hat{Y}$ is the predicted response, b's are the partial regression coefficients and T and M are the coded time and moisture, respectively.

C_1 is the contrast NA vs CH and C_2 is the contrast NA vs EN. NA, CH and EN: feather meals treated without additions, with chemical and enzyme, respectively. All b's (except b_0) are tested for b = 0, indicated by *, and a test is made for the equality of the b's per experiment. The significance for the last test is indicated by +.

2), 3), 4), 5) See Table 2.5, footnotes 2), 3), 4) and 5) respectively.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2.9. Chemical analysis and results of laboratory tests of feather meals (Experiment 4)[1].

Feather meal[2]	N[3]	Cys[3]	Lan[3]	N-pepsin digestibility[4]				N-solubility[4]		Chemical score[5]			Chemical index[6]	
				Pepsin concentration (%)				0.02 N	6 N	Limiting amino acid[7]				
				2.00	0.20	0.02	0.00	NaOH	HCl	Met	Lys	His	Alkali	Acid
FM_{30}	15.5	4.63	1.96	93.6	88.1	65.6	13.1	47.4	26.9	32	39	48	10	6
FM_{50}	15.6	4.04	2.33	90.3	91.6	72.2	19.2	64.5	36.8	30	38	50	20	9
FM_{70}	15.5	3.69	2.28	93.5	93.4	79.6	27.0	81.1	50.8	32	38	50	22	14
$FM_{30}CH$	15.2	3.44	3.28	94.3	94.3	82.6	20.4	43.8	43.6	32	39	46	13	13
$FM_{50}CH$	15.2	3.04	3.31	93.4	93.0	88.9	28.4	73.3	65.9	32	38	49	24	22
$FM_{70}CH$	15.3	2.67	3.05	94.2	94.5	93.4	34.5	85.3	81.6	31	38	49	32	31
$FM_{30}EN$	15.5	4.65	2.08	94.9	91.9	80.9	50.9	73.1	60.3	31	38	48	16	13
$FM_{50}EN$	15.6	3.51	2.25	93.8	93.2	91.3	60.5	87.8	77.5	33	38	51	25	22
$FM_{70}EN$	15.5	2.94	2.27	96.2	95.1	93.4	68.9	91.4	87.1	31	38	48	31	30

1) All values are on dry matter basis.
2) See coded values of Experiment 4, section 2.3.2.
3) Means of duplicate observations.
4) Lan: lanthionine
Means of triplicate observations.
5), 6) See section 2.3.4.

$$\text{Chemical score} = \frac{\text{amino acid in test protein}}{\text{amino acid requirements of 3-week old broilers (NRC, 1977)}}$$

$$\text{Chemical index} = \frac{\text{Nitrogen solubility (NS) \%}}{\text{total cystine expressed as \%}}$$; Alkali: NS in 0.02 N NaOH, Acid: NS in 6 N HCl.

7) Met, Lys and His: 1st, 2nd and 3rd limiting amino acid, respectively.

found in the chemically treated feathers.

Pepsin digestion: The decreased pepsin levels increased the sensitivity of the test. Feather meals digested with 2% and 0.2% pepsin showed a range of 5.9% and 7.0% in PDP respectively, while the samples digested with 0.02% pepsin had a 27.8% range in PDP. The use of 0.02% pepsin instead of the currently recommended level of 0.2% clearly increases the range between the samples as affected by an increased processing time within the test groups.

Chemical scores: The chemical scores were based on the amount of the first limiting amino acids. Taking into account the amino acid composition (g AA/100 g protein) all the test feather meals showed the first limiting amino acid to be methionine, while the second and the third limiting amino acids were lysine and histidine respectively.

Chemical indices: Chemical indices were increased by increasing the processing time in the case of both alkali and acid tests. Enzymatically and chemically treated feather meals gave higher values for indices than feather meals without additives.

2.5 Discussion

2.5.1 Effect of treatments on feather meal amino acid composition

Time effect

The present study shows a clear tendency towards reduced cystine content in the hydrolyzed feather meals with an increase in processing time. In none of the test samples was the cystine level as high as in raw feathers. Our results are supported by those of Davis et al. (1961), Morris and Balloun (1973b) and Wheeler and Latshaw (1980).

Concerning the other amino acids of the treated feather meals the literature showed some variability in the amino acid composition of heat prepared samples, but this information is very limited. Gregory et al. (1956) reported some loss of phenylalanine, isoleucine and arginine in autoclaved feathers, compared with the raw material. Morris and Balloun (1973b) showed in hydrolyzed feather meals that, by increasing the time from 30 to 60 min, methionine, lysine and histidine contents decreased under a pressure of 375 kPa, but these amino acids increased at 445 kPa. However, no statistical evaluation of their data was presented.

Changes in the amino acid composition of processed proteins were also obtained by other workers. Loss of cystine, lysine, arginine, threonine and serine as

a result of heat treatment has been shown in several animal protein sources by Udes et al. (1971), Varnish and Carpenter (1975a), Ford (1976) and Gumbmann et al. (1983). Increased levels of glycine and alanine in heated casein have been found by Pienazek et al. (1975b). In these studies the heated protein sources were compared with untreated samples but the variations during treatments have not been investigated systematically. These results, therefore, cannot be adequately evaluated.

In heat processing, time is a controllable variable related to the nutrient destruction rate in feeds (Bluestein and Labuza, 1975). The deviations occurring in the amino acid pattern of the processed feather meals in our study could probably be explained by different orders of kinetic parameters for individual nutrients which might occur in processing (Karel, 1979).

Moisture effect

There is no available information with which to compare our results on the amino acid changes caused by different moisture contents of the processed feather meals.

The effect of moisture content in processing feeds, without additives, has been a subject of discussion by a number of research workers. Our results, based on Experiment 1 suggested that there is a positive linear effect of moisture on the sulfur amino acids, cystine and methionine. Similar results were reported by Pieniazek et al. (1975b) for heated casein with 4% and 80% moisture content.

The findings of Renner et al. (1953) and McNaughton and Reece (1980) that lysine contents in heated soybean meal decreased with increasing added moisture levels, from 0-16% and 0-50% respectively, were not in line with ours. The type of protein subjected to the technological processes and the different moisture levels may explain the differences. We also found that most of the essential and all the non-essential amino acids were lower in more highly moistened meals than in those of lower moisture content. Myklestad et al. (1972) showed the same trend for autoclaved fish meal with moisture percentages of 7.5% and 27%.

Our results imply that the behaviour of amino acids during heat treatments might be affected by moisture. A number of relations have been suggested to describe the dependence of reaction rates on moisture content or on water activity (the availability of water for the reactions), since its interaction with food components is directly influenced by the chemical reactions that can take place (Karel, 1979). Water can play different roles including the following as reviewed by Karel (1979): a) as a solvent for reactants and products, b) as

a reactant (in hydrolytic reactions), c) as a product of reactions (in condensation reactions), and d) as a modifier of the activities of catalysts and inhibitors. Because the effect of water on chemical reactions is complicated, the influence of water on the rate of amino acid degradation in a feed processing system is not clear. The published data have not been complete enough to allow an estimation of the parameters needed to predict the susceptibility of nutrients, to thermal processing. We conclude therefore that more work is needed to explain the differences reported in nutrient changes.

Chemical effect

Our results suggest that there was a significant variation in the amino acid responses to the effect of sodium hydroxide. Samples treated with sodium hydroxide showed that cystine was decreased drastically below its level in the raw material. Similar results were reported for feather meals treated with hydrochloric acid (Eggum, 1970) and dimethylsulphoxide (Wolski et al., 1980). In addition, Eggum (1970) reported that the amino acid composition with the exception of cystine, did not show any change either with increasing acidity or time of hydrolysis. Our results are not comparable with these findings, probably due to different chemical technological processes.

The pH conditions in the alkali treatment of the protein have been demonstrated to affect the rate of loss of amino acids, the amino acids being less stable at high pH values (De Groot and Slump, 1969; Whitaker, 1980). This was confirmed in our work by the negative linear effect of increasing sodium hydroxide concentrations.

Enzyme effect

There is a lack of information in any study on the effect of enzyme treatment on the various functional characteristics of feather meal protein as well as on its amino acid pattern. Since our enzymatic hydrolysis took place after autoclave hydrolysis of the samples, our work must be discussed carefully, because we actually measured the combined effect of the different enzymatic parameters used. Evaluation of enzyme-treated feather meals, such as is described here, was not sufficient to determine the full potential of the method employed. This preliminary study attempts to form the groundwork for more research on the development of the product treated with enzymes.

2.5.2 Lanthionine content

In our studies we found that the unusual amino acid lanthionine is present in hydrolyzed feather meals treated with or without additives. Our results are in agreement with those reported by other workers using autoclaved feather meals (Davis et al., 1961; Wheeler and Latshaw, 1980; Baker et al., 1981) and alkali treated wool keratin (Asquith and Otterburn, 1977; Friedman, 1977). The general trend to increased lanthionine content with increasing time as demonstrated in our results has been shown also by Davis et al. (1961). The addition of sodium hydroxide generally resulted in an increase in lanthionine, although in the samples treated for 70 min lanthionine formation was partially inhibited. This latter observation cannot be explained satisfactorily. Probably some partial decomposition of lanthionine took place during this prolonged process in the presence of alkali. Asquith and Otterburn (1977), using wool keratin as a model, found a lower lanthionine content at high temperatures (180°C) than at temperatures below 160°C.

The fact that the amount of lanthionine found approximated to the loss of cystine during processing, indicates that most of the lost cystine is converted to lanthionine.

2.5.3 Causes of amino acid changes due to processing

In the present study it was shown that processing conditions have a substantial effect on the amino acid composition of the test materials. This leads to the conclusion that autoclaving hydrolysis of feather meals, with or without additives, has altered the protein structure. An indication of the nature of these chemical changes is given in the fourth trial describing the formation of the unusual amino acid lanthionine in parallel with a drastic destruction of cystine. Possible mechanisms for the effects of thermal and alkali treatment on proteins can result from different types of reactions. Simple modifications of amino acid residues, namely, destruction of amino acids and formation of new cross-linked compounds have been discussed by several investigators (Hurrell et al., 1976; Bender, 1977; Whitaker, 1980). In the absence of reducing substances, such as carbohydrates and fats, there are many possible protein cross-linking reactions. From the formation of new isopeptide bonds and from the degradation of cystine, with the simultaneous formation of new amino acids such as lysinoalanine, lanthionine and ornithoalanine may result. The new amino acids

formed in proteins by alkali treatment can be explained by the initial formation of the common intermediate dehydroalanine which probably comes through β-elimination reaction (Bohak, 1964; Nashef et al., 1977). The formation of new amino acids is possible not only in pure protein systems, described by most investigators, but also in protein feed products (De Groot and Slump, 1969; Sternberg et al., 1975; Baker et al., 1981).

An interesting feature of this study is that the damage to individual amino acids varies with the different processing factors. This is emphasized by the marked degradation of cystine, which is by far the most heat sensitive of the amino acids under prolonged processing conditions, and by smaller losses of other amino acids.

Loss of cystine probably occurs through desulfurization reactions that may lead to unstable residues of dehydroalanine which may then condense with cysteine to form lanthionine, or with the ε-amino group of lysine to form lysinoalanine, as a result of thermal degradation (Bjarnason and Carpenter, 1970). Our findings on the drastic degradation of cystine and formation of lanthionine are in line with those in literature studies. Apparently there is no information in the literature on lysinoalanine in hydrolyzed feather meals, however, evidence for that as well as ornithoalanine has been obtained by other workers using wool keratin as a model, with steaming and alkali treatment (Asquith and Otterburn, 1977; Friedman, 1977).

Loss of lysine, as observed in our studies, could be explained by the addition of an ε-amino group of lysine residues with dehydroalanine (Ziegler et al., 1967; Bjarnason and Carpenter, 1970). Further, the decomposition of threonine and serine found in this work is probably a result of the β-elimination reaction (Asquith et al., 1969; Whiting, 1971).

Degradation of the arginine content in heat/alkali treated feathers could be explained by the formation of ornithine, as has been suggested by Ziegler et al. (1967), Asquith and Otterburn (1969), using wool keratin, probably due to a hydrolytic reaction (Whitaker, 1980).

The essential amino acids, valine, leucine, tyrosine and phenylalanine, were shown to be rather stable in thermal processing (Experiment 1). Our results are in agreement with those of Myklestand et al. (1972), Pienazek et al. (1975b). In alkali treatment (Experiment 2), the above-mentioned amino acids were shown to be sensitive and negatively affected by sodium hydroxide. These findings could not be explained in this study, due to the absence of literature covering the assessment of the chemical response of these amino acids to the heat/alkali treatment.

2.5.4 Effect of treatments on protein characteristics

The reduction in protein content of the test samples under processing conditions might have been the result of the drastically decreased cystine content or the loss of non-protein nitrogen. A decrease in crude protein with increased processing time has also been shown in feather meals by Morris and Balloun (1973b) and Aderibigbe and Church (1983) and in other feedstuffs by Udes et al. (1971). Increased ash content with increasing NaOH levels seems to be due mainly to Na retained in the samples, although their Na contents were not determined.

The laboratory assays have been carried out in an attempt to test additional criteria for feather meal protein evaluation. It is known that heating can improve the digestibility of protein by structural changes through denaturation, and that, in general, a protein has to be in solution for its potential to be realized. Our results suggest that the solubility characteristics of protein varied with varying processing conditions.

Pepsin digestion as carried out in the first three experiments did not give a clear picture of the feather meal protein quality, although the variations found in pepsin digestibility due to processing conditions, were similar to those in the reports of Morris and Balloun (1973b), Johnston and Coon (1979a). When decreased pepsin levels were used (Experiment 4), the sensitivity of the test was increased and digestibility differences between short-term and long-term processed feather meals, with or without additives, were increased. However, the pepsin-hydrochloric acid test measures only solubilization of protein and not the extent of its digestion and absorption by an animal. Such an *in vitro* test should be compared with *in vivo* digestibility measurements of protein and amino acids in order to prove its validity. The test is still used in the laboratory and animal feed industry as a quality-control method, mainly for animal protein feedstuffs.

There are no published data on soluble nitrogen in feather meals, processed under different conditions, with which to compare our results. Alkali and acid solubility of nitrogen measures the extent of the chemical attack by NaOH and HCl but does not account for the amino acid composition of the tested protein. The difference between the alkali and acid solubilities is to be anticipated, because by decreasing the proportion of basic groups to acid groups, a decrease in acid solubility is to be expected and conversely, the increase in acid groups accompanied by decrease in basic groups leads to an increase in alkali solubility.

In general, feather meal is treated under different conditions in order to

increase the digestibility of protein. An interesting feature of this study is that although the protein solubility (in pepsin-HCl solution and alkali or acid solvents) of the test feather meals was increased as a result of added NaOH or enzyme (Table 2.8), the amino acid contents, especially cystine, were decreased. This suggests that the amount of cystine (the most sensitive of the amino acids) may be a reasonable index of the degree of processing, since feather meals with lower cystine contents showed higher protein solubility values. This inverse relationship should be taken into account in the evaluation of feather meal protein quality.

2.5.4 Concluding remarks

The effects of different processing conditions on feather meal protein by a central composite experimental design (Box, 1954), have not been described before. In our study, this procedure appears to offer an efficient approach to the examination of the effect of different processing conditions (time, moisture, chemicals, enzyme) on feather meal protein quality as evaluated chemically.

These investigations have demonstrated that:

a) there are losses of amino acids during processing of feather meal, particularly of cystine (which is believed to be related - via cross-linking sulfur groups - to the insolubility and indigestibility of feather protein);
b) substantial differences in amino acid reduction have been found in the samples from chemical treatments and to a smaller extent in the enzymatic ones, by comparison with the treatments without additives;
c) there is heterogenous behaviour of the individual response criteria (amino acids, protein solubilities) to the different processing variables; it is therefore difficult to optimize treatment procedures.

The practical implication of these findings is that in most cases it is difficult to distinguish the effect of processing on feather meal protein from the corresponding effect on the amino acid patterns. Biological experiments therefore are necessary in order to study whether other modifications to protein during processing affect the nutritional quality of feather meal.

chapter 3

THE EFFECT OF DIFFERENT PROCESSING CONDITIONS ON AMINO ACID AND NITROGEN DIGESTIBILITY OF FEATHER MEAL, DETERMINED BY CHICK BIOASSAY METHOD

3.1 Introduction

Amino acid rather than total protein requirement should be the primary consideration of the nutritionist in formulating poultry rations, and therefore the amino acid content of the feed ingredients should be taken into account. Published figures for amino acid contents of individual feedstuffs have, in general, been obtained by physicochemical methods of analysis. Because these determine the total amount of amino acid present they are of limited value only, since not all of each amino acid in a protein is made available to the animal in the course of digestion, absorption and metabolism.

Several methods, essentially classified as *in vivo* or *in vitro* have been suggested for assessing the nutritional quality of protein in feedstuffs. However there is inadequate information on which methods are valid predictors of production responses. The features of the various methods in use for measuring amino acid digestibility and availability in feedstuffs are discussed in the following section from data reported in the literature. For the purposes of this study, apparent digestibility is defined here as the difference between the amount of the amino acid in the diet and in the excreta or ileal digesta, divided by the amount in the diet. True digestibility is defined in a similar way except that the amounts of amino acids of endogenous origin in feces or ileal digesta (measured on nitrogen-free diets) are subtracted from the total amount of amino acids in the feces or ileal digesta. Absorbability is defined as the degree to which amino acids are passed through the intestinal wall. Availability is defined by the degree to which amino acids are present in a form suitable for digestion, absorption and metabolic processes.

3.2.1 Estimations of digestible/available amino acids in feedstuffs

Digestibility *in vivo*

Fecal analysis: The digestibility of individual amino acids was first studied by Kuiken and Lyman (1948), using rats as test animals. In poultry Bragg et al. (1969) proposed a rapid procedure for determining the digestible amino acids in feeds, using Fe_2O_3 as a marker. The method, with slight modifications, has been used for a wide range of plant and animal protein sources in chicks (Ivy et al., 1971; Burgos et al., 1974; Nwokolo et al., 1976; El Boushy and Roodbeen, 1980). The technique has been questioned because of practical considerations in the quantitative collection of the test excreta and because of failure to distinguish adequately between ingredients of widely different quality.

The assay for true metabolizable energy (TME) by excreta analysis (Sibbald, 1976) has also been used for measuring digestible amino acids in several feedstuffs (Likuski and Dorrell, 1978; Muztar et al., 1980; Parsons et al., 1982). The accuracy of the method has been studied by several workers in order to overcome some criticisms associated with the amount of amino acid excretion as affected by the diet composition (Sibbald, 1980; Muztar and Slinger, 1980b) and the duration of quantitative collection of the test excreta (Kessler and Thomas, 1981; Parsons et al., 1981). However the results are promising and interest is growing in using this method because it is simple, rapid and inexpensive.

It has been questioned if the mixed fecal and urinary materials in poultry may influence evaluation of amino acid digestibility. Research studies, however, support the view that the common excretion of urine and feces has little effect under the conditions of the assay. Bragg et al. (1969) concluded that the results obtained with normal chicks were more reproducible and suitable for making such studies than those obtained with surgically modified chicks with an artificial anus. Similar conclusions were obtained by Gruhn (1974) and Terpstra (1977), from their studies using normal and colostomised hens and cocks respectively.

Ileal digesta analysis: The main criticisms of the fecal analysis method for determining amino acid digestibility are related to the effect of the intestinal bacteria. Several approaches have been made to study whether or not the fermentation effect of the microflora substantially influences fecal excretion of nitrogen or individual amino acids. Payne et al. (1968) measured undigested

amino acids in the ileum of chicks and suggested that the assay may represent a sensitive index of amino acid digestibility. Nesheim and Carpenter (1967), Soares et al. (1971) and Salter and Coates (1971) reported that the fecal analysis method may overestimate amino acid digestibility due to microbial action.

Further studies with germ-free and conventional chicks reported no significant differences in amino acid digestibility (Erbersdobler and Riedel, 1972; Salter and Fulford, 1974; Elwell and Soares, 1975). In addition, Varnish and Carpenter (1975b) and Achinewhu and Hewitt (1979) compared the fecal and ileal assays and reported insignificantly lower amino acid digestibility values in ileal content analysis. Recently, Picard et al. (1983) showed that there is no significant difference between the true digestibility values of amino acids obtained with cecectomised or intact birds.

It may be suggested that the microbial fermentation in poultry has less effect on protein degradation than in such other monogastric animals as pigs (Zebrowska, 1973; Sauer et al., 1977) and rats (Valle-Riestra and Barnes, 1970; Mason and Palmer, 1973). This may be explained by the short digestive tract, the small capacity of the large intestine, compared with the entire digestive tract, and the rapid rate of passage of digesta in chickens.

Digestibility *in vitro*

Enzymatic methods: Several attempts have been made to simulate conditions of biological digestion on an *in vitro* basis and for several years a number of different proteolytic enzymes have been tested for their ability to release amino acids from the test material (Sheffner et al., 1956; Akeson and Stahmann, 1964; Splitter and Shipe, 1976). Some workers developed enzymatic methods for specific amino acids such as lysine (Payner and Fox, 1976; McBee and Marshall, 1978), tryptophan (Holz, 1972) and methionine and cystine (Pienazek et al., 1975a).

Enzymatic methods give a relative rather than a quantitative measure of amino acid digestibility. Although these methods are rapid and reproducible, it seems to be impossible with the organism to imitate the complex and dynamic conditions in the intestinal tract.

Availability *in vivo*

Growth assays: The growth assays used by several workers to estimate the availability of amino acids of a dietary protein have been generally similar. The growth rate obtained by the chicks when fed a control diet deficient only

in the amino acid under study was compared with those fed the diet to which known levels of the limiting amino acid had been added in crystalline form. The calculations were based upon the relationship between growth response and dietary level of the tested amino acid, expressed a percentage of the diet (Ousterhout et al., 1959) or in terms of amino acid consumed per chick (Campbell, 1966).

Applications of the growth assay were reviewed by Netke and Scott (1970), Nijke et al. (1975), Robel (1980). The method has been used to evaluate new protein sources in poultry feeding (Reddy et al., 1979; Cave and Williams, 1980) and remains the only direct means of checking the validity of amino acid availability values obtained by *in vitro* procedures (Waibel et al., 1977; Major and Batterham, 1981).

In chick growth assays the response measured is not only dependent on the availability of dietary amino acids. In developing a method many factors have to be considered. The rate of feed consumption, the level of dietary protein, and the interactions among the amino acids can affect growth rate and, therefore, the availability values. In addition, the question of whether the growth rate, feed conversion, carcass protein gain or nitrogen retention should be used as criteria in availability determinations, remains open. Moreover, the method is limited in evaluating only one amino acid at a time and this tends to make the method very expensive and time-consuming.

Plasma amino acids: Because the blood serves as a carrier of the products of digestion of dietary protein, several workers have attempted to develop a method for measuring amino acid availability based on plasma free amino acid analysis. However, the recommended procedures by using monogastric test subjects, as rats (Guggenheim et al., 1960; Stockland and Meade, 1970), chicks (Smith and Scott, 1965b), pigs (Stockland et al., 1970; Windels et al., 1971) and humans (Longenecker and Lo, 1974) have not been fully validated to date.

The plasma techniques have not been successful in determining amino acid availability because they are susceptible to several influences. The results of the analysis depend on the time and site of blood sampling in relation to the time of ingestion of the test proteins, the amino acid composition of the dietary protein, the rate of release of free amino acids from the digested protein and the rate of absorption from the gastrointestinal tract. Other factors such as the extent of metabolism of the amino acids by the intestinal tissues and withdrawal of amino acids from the plasma for protein synthesis also complicate the response of the animals in the test.

Concentrations of free amino acids in blood plasma may provide useful in-

formation about the relative digestibility of dietary protein, but it seems to be difficult to interpret such changes as practical quantitative measures of amino acid availability.

Availability *in vitro*

Chemical assays: The most frequently used chemical method is that of Carpenter (1960) which is based on the measurement of the percentage of the free epsilon-amino group of lysine in the test proteins by fluorodinitrobenzene (FDNB). The method has been criticized because of being time consuming and its poor performance when applied to vegetable proteins. In spite of some theoretical criticism of the FDNB method (Finot, 1973) it remains after some modifications (Roach et al., 1967; Booth, 1971) the most appropriate laboratory method with good reproducibility (Carpenter and Woodham, 1974; Couch, 1975), and the possibility of a rapid estimation of the heat damage in processed animal protein feeds (Waibel et al., 1977; Payner and Fox, 1978).

Other attempts have been made to estimate available lysine chemically. Kakade and Liener (1969) used trinitrobenzene sulfonic acid (TNBS) based on the same principle as that of the FDNB method. This procedure needs a shorter period of hydrolysis and has been used, after some modifications, by several workers (Eklund, 1976; Holguin and Nakai, 1980) who reported reasonable accuracy.

Chemical determination of available methionine has been reported by Ellinger and Duncan (1976), Lipton and Bodwell (1977) who showed the distinction between methionine and its sulphoxide form, which is biologically unavailable (Smith, 1972). Methods for determining other amino acids have not been published.

Consequently although chemical methods may be used to determine available lysine, there is no possibility of evaluating other amino acids, with the possible exception of methionine.

Microbiological assays: The introduction by Ford (1960) of microbiological methods using certain microorganisms, such as the bacterium *Streptococcus zymogenes*, because of its considerable proteolytic powers, stimulated interest in determining the availability of certain amino acids, excluding lysine (Ford, 1962; Miller et al., 1965; Carpenter et al., 1972). Although the reproducibility of the method has been questioned (Boyne et al., 1975), it is used in some laboratories for available and total amino acids assays (Ford, 1976).

The phagotrophic protozoan *Tetrahymena pyriformis* (Fernell and Rosen, 1956) was also used to estimate available lysine, methionine, arginine and histidine (Stott and Smith, 1966). The method has been subjected to some modifications

and has been found to be highly correlated with the FDNB-method (Shorrock, 1976) and in good agreement with chick growth-assays (Shepherd et al., 1977). However, a simple and standardized microbiological technique for the determination of available amino acids, applicable to all types of feeds, still remains to be developed.

It can be concluded that the various methods described cannot fulfil completely the requirements for reliable evaluation of the nutritional protein-amino acid quality. However, the growing interest in the use of by-products and novel feedstuffs in animal nutrition, and the extent to which protein amino acid quality can be changed by processing underlines the need for a rapid and accurate method of assessment. The *in vitro* methods are, in general unsatisfactory in many ways (they are not specific or limited to only one or a few amino acids). On the other hand the *in vivo* methods are expensive, time consuming and, in the case of availability estimations, not fully proven as reliable predictors. Although availability is clearly different from digestibility, as noted earlier, the fecal and ileal assays appear to be promising since the digestibility of all of the amino acids in a feedstuff can be determined simultaneously by these methods. Moreover, the fecal method seems to be the more practical criterion in routine amino acid digestibility determinations, because the values obtained by it are not substantially different from those obtained by using ileal assays.

3.2.2 Amino acid digestibility/availability of feather meal

Feather meal is subjected to various processing treatments to make it digestible but the resulting products are not uniform in quality because of differences in the treatments. One factor involved is the effect of heating that, though beneficial in some respects, can reduce the nutritional value of the feather meal protein. If the digestibility and/or the availability of its amino acids were known, the value of feather meal as a component of animal feeds could then be properly assessed.

For several years, research workers have tried to determine the amino acid digestibility/availability of hydrolyzed feather meals for several monogastric species, but the reports have been relatively few. In digestion experiments with pigs (Dammers, 1964), feather meal had a rather high true digestibility of the individual amino acids of between 80 and 90%. Similar results were obtained by Eggum (1970) using rats in his studies.

In chick growth assays, Smith (1968), found that the availability of most of the amino acids in commercial feather meal was poor compared with fish and soybean meals. This applied especially to lysine and histidine. In contrast, MacAlpine and Payne (1977) reported that lysine had an availability of 72.5%, estimated by a chemical (FDNB) method. Similar results on 'available' lysine present in pepsin-digested (*in vitro*) feather meal, were found by Morris and Balloun (1973b). They also showed that the more severely processed feather meals (445 kPa for 60 min) contained more 'available' lysine, methionine and histidine, than did feather meals processed under milder conditions.

In digestion experiments with laying hens Vogt and Stute (1975) showed that the apparent digestibility of amino acids in feather meal was 79%. The true digestibility of amino acids from feather meal, determined by fecal analysis in chicks, has been found by Burgos et al. (1974) and Kirby et al. (1978) to be as high as 97% and 94%, respectively.

Kim et al. (1980) studied the available sulfur amino acid content of five feather meals, different in terms of plant source and processing method, using the growth of chicks as the criterion. They found that the range in availability of the sulfur amino acids was from 41% to 82%. Furthermore, Parsons et al. (1982), using fecal analysis method, reported the average true digestibility of amino acids in commercial feather meal of 82% compared to 76% for cystine.

Scope of the study

From the above it is obvious that the values of amino acid digestibility/availability of the feather meals vary and definite date are lacking. In addition, the effects of different processing conditions on feather meal digestibility have not been studied. The objectives of this study, therefore, were to investigate systematically, by fecal analysis method, the amino acid digestibility of feather meal, and to assess the influence of various processes on it. In addition, an attempt was made to find if crude protein digestibility and other *in vitro* tests could reflect the amino acid digestibility.

3.3 Materials and methods

3.3.1 Processing of the test feeds and experimental design

For the present study 9 differently processed feather meals were prepared. The processing conditions were selected to obtain maximum information with a

minimum number of variables in order to keep the number of samples for biological tests and analysis within practical limits. Thus, the processing variables applied in the experiments described in Chapter 2, we used three periods (short, medium and long) of time, while moisture, sodium hydroxide and enzyme were kept constant at their intermediate levels, i.e. 60% for moisture and 0.4% for NaOH and enzyme, respectively. Table 3.1 describes the test feather meals used in the balance experiments with chicks. The general procedure employed in the preparation of raw feathers and their treatments, has been described in detail earlier (Chapter 2, section 2.3.2).

Table 3.1. Feather meals used in digestion experiments with chicks.

Feather meal No.	Processing conditions (Autoclaving hydrolysis)	Code
1	Time; 30 min	FM_{30}
2	" 50 min	FM_{50}
3	" 70 min	FM_{70}
4	" 30 min with added NaOH (0.4%)	$FM_{30}CH$
5	" 50 min " " "	$FM_{50}CH$
6	" 70 min " " "	$FM_{70}CH$
7	" 30 min with enzymatic hydrolysis (0.4% enzyme)	$FM_{30}EN$
8	" 50 min " " "	$FM_{50}EN$
9	" 70 min " " "	$FM_{70}EN$

3.3.2 Animal experimental procedure

The bioassay was conducted by the TME (true metabolizable energy) method of Sibbald (1976) applied to digestible amino acid determinations (Likuski and Dorell, 1978). On the basis of preliminary studies, some modifications to the method were made.

Housing and management of the chicks

One day-old Hybro male broiler chicks were obtained from a commercial hatchery and housed in a battery brooder with wire floors; temperature was controlled according to age. The chicks were raised for 21 days on a commercial broiler starter ration, in a finely ground form. At this time the chicks were weighed (chicks were fasted overnight prior to weighing) and 72 of them, with a body weight distribution from 510 to 560 g, were assigned to the experimental treatments. The chicks were randomized and kept individually in balance cages

in an air conditioned windowless room. These cages (22 cm wide x 45 cm long x 47 cm high) were designed for mature cocks, but they were adapted to the body-size of young chicks. They were made from galvanised iron, coated with a nylon preparation (rilsan) to prevent corrosion, and equipped with mesh floors covered in the front part with a perforated plastic carpet. The cages were placed on a stand 1.2 m above floor level with a 10 cm partition between them. The temperature in the experimental room was maintained at 24-26°C with a relative humidity of approximately 50-60%, while a 24-hours light period was used. The chicks were fed the same diet (broiler starter) and kept in the cages for acclimatization and a training period until the experiment began. Water was supplied ad libitum, throughout the experiment by means of a nipple type fountain alongside each cage. All chicks were judged to be normal in condition and consistency of feces.

Feeding procedure

After the adjustment period, when the chicks were 28 days of age, they were starved for 24 hours to empty their alimentary tracts of feed residues. The chicks were then force-fed the test feather meals as the sole feed. The force-feeding technique was applied because otherwise the chick were unwilling to eat reasonable amounts of the feed under study, which has to be administered under strict control to avoid feed waste. The feather meal was given to each chick in 2 doses administered over a period of 3 hours with each dose containing 6 g of dry matter, which would be an acceptable feed intake on a voluntary basis. The amounts of feed given to the chicks were established in preliminary experiments, taking into consideration its form in order to avoid regurgitation. A stainless-steel funnel, with 40 cm long stem and an external and internal diameter of 12 mm and 9.7 mm respectively, and a ramrod were used to introduce the feed into the crop through the oesophagus. Examination of different test chicks in preliminary trials, did not show any injury to the oesophagus or crop after feeding with the tube. When the feeding was completed the oesophagus was squeezed to remove particles of feed which might have been adhering to the outside of the stem of the funnel. After feeding, water was given by a dropper to assist the chicks in swallowing remaining feed particles. The amount of feed input per chick was accurately measured to 5 mg. The time of feeding was recorded. The procedure was repeated until there were nine experimental groups for each of the test feeds with 7 chicks per treatment. An additional group of 9 chicks received a nitrogen-free diet (NFD) in the same manner as the feather meal test-groups, and served as negative controls to give an estimate of the metabolic

fecal plus endogenous urinary excretion of amino acids. This method assumes that metabolic excretion is related to the amount of dry matter consumed and is not influenced by the presence of protein in the diet. The NFD was formulated to contain 81% glucose, 3% cellulose, 10% corn oil and adequate quantities of minerals and vitamins (Bragg et al., 1969).

Excreta collection

As soon as the chick was fed it was placed in the cleaned wire cage, to prevent possible contamination of the feces. A porcelain dish, of 25 cm diameter, was placed in a large, smooth, removable plastic tray, measuring 35 by 40 cm and 5 cm deep, underneath the cage, which allowed collection of all the excreta. Care was taken to collect residues of droppings on the wire-floors.

In order to determine the time of excreta collection, the clearance time - i.e. the time between ingestion and the excretion of the last residue - was measured in a series of preliminary trials. In these trials the duration of starvation prior to test feed-input, the amount of feed-input and the physical form of the ingredient, were considered. In addition, information was obtained in pre-experimental studies on the possible retention of feed residues in different parts of the digestive tract by opening the alimentary canal of the chicks at different periods of time. On the basis of these trials 36 hours after first feeding was the time chosen to collect the feces. Every 6 hours the excreta were transferred quantitatively into a plastic container with the aid of a broad-bladed spatula and distilled water. The excreta were then freeze-dried and stored until amino acid analyses were carried out. To facilitate homogenising and grinding of the NFD-excreta, approximately 2 g of silica gel were added to the wet droppings before they were frozen. During the periods of excreta collection, to allow quantitative collection of the droppings, the chicks were tied to the front of the cage with a neck-chain attached to a rod that prevented them from turning round. However, they were able to drink and to lie down. When collecting the excreta, feathers and scales were removed. Clipping of the feathers around the vent aided in accurate quantitative excreta collection. Throughout the starvation and experimental periods the chicks had free access to water.

3.3.3 Analytical methods

Amino acid analysis

At the end of the trial, the freeze-dried excreta from the individual chicks were allowed to equilibrate with the atmospheric moisture at room temperature, weighed and ground to pass through a 1 mm screen. Samples of the processed feather meals were also ground to the same particle size as the excreta. A detailed description of the general procedure for amino acid analyses can be found in Chapter 2 (section 2.3.4). Tryptophan was omitted because of the extra steps required in its determination. Lanthionine has been analyzed in pooled excreta. Because during acid hydrolysis of poultry excreta, uric acid is partly converted into glycine (Soares et al., 1971), the values for this amino acid were considered as unreliable and omitted.

Estimation of nitrogen digestibility

Samples of excreta from each chick within the test group were pooled for nitrogen digestibility estimations. The excreta were mixed in a blender and stirred between the removal of each subsample. Because poultry excreta are a mixture of feces and urine, fecal nitrogen was determined by the lead acetate method of Terpstra and de Hart (1974).

In vitro tests

The methods used for nitrogen pepsin-HCl digestibility and nitrogen solubility in alkali and acid respectively, were principally those described in detail in Chapter 2. Additionally, a higher (2%) and lower (0.02%) pepsin concentration was used.

Calculation formulae

The apparent and true amino acid digestibility values, AAAD and TAAD respectively, were calculated for each amino acid using the following equations:

$$AAAD = \frac{AA_I - AA_{TE}}{AA_I} \times 100$$

$$TAAD = \frac{AA_I - (AA_{TE} - AA_{CE})}{AA_I} \times 100$$

in which:

AA_I = Amino acid (AA) intake of the chicks (intake of feed, g x % amino acid of the test feed).

AA_{TE} = Amount of AA (g) voided in excreta by the test-fed chicks during the 36-hrs period after first feeding.

AA_{CE} = Amount of AA (g) excreted by the nitrogen-free diet control chicks during the 36-hrs period after first feeding.

The chemical scores and indices were also calculated (see Chapter 2, section 2.3.4).

3.3.4 Statistical methods

The data of the digestibility studies were statistically analyzed by a multiway analysis of variance (Steel and Torrie, 1980). The independent variables were three processing time periods (30, 50 and 70 min, respectively) and three treatment regimes (without additives (NA) and with additives of chemicals (CH) and enzymes (EN), respectively). The dependent variables were the apparent and true digestibility values of the individual amino acids. There were seven individual replications per test group. Significant differences among the means were determined by Duncan's New Multiple Range Test (1955). Simple correlations were determined between *in vitro* tests and selected *in vivo* tests.

The following model was used for the multiway analysis of variance:

Source of variation		Degrees of freedom	Sum of Squares	Mean of Squares	F-value
Mean		1			
Time[1)]	(T)	2			
Linear	(TL)	1			
Quadratic	(TQ)	1			
Treatment-conditions[2)]	(P)	2			
Contrast-1[3)]	(C1)	1			
Contrast-2[4)]	(C2)	1			
Interaction		4			
TL x C1		1			
TL x C2		1			
TQ x C1		1			
TQ x C2		1			
Residuals		54			
Total		63			

[1] Processing time in three periods of 30, 50 and 70 min, respectively.
[2] Treatment conditions at three levels: a) by time only (NA), b) by time + chemical (CH), and c) by time + enzyme (EN).
[3] Contrast between: (NA) vs (CH + EN).
[4] Contrast between: (CH) vs (EN).

3.4 Results

The average moisture, ash and crude protein contents, and the average amino acid contents in the processed feather meals are given in Table 3.2. A more comprehensive discussion on the chemical characteristics of the differently processed samples in this study is given in Chapter 2.

Table 3.2. Chemical composition of experimental feather meals[1].

Item	Feather meals[2]								
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$
Proximate analysis %									
Dry matter	93.42	93.21	92.81	93.05	93.08	92.44	91.65	92.90	92.14
Crude protein[3]	96.56	97.63	96.88	94.94	95.81	95.38	96.81	97.69	97.00
Ash	1.21	1.19	1.26	2.47	2.42	2.46	1.28	1.19	1.31
Essential amino acids, %									
Threonine	4.88	4.81	4.66	4.72	4.67	4.52	4.80	4.79	4.54
Cystine[4]	4.63	4.04	3.69	3.44	3.04	2.67	4.65	3.51	2.94
Valine	7.51	7.41	7.36	7.41	7.36	7.20	7.44	7.36	7.14
Methionine	0.66	0.64	0.67	0.66	0.67	0.64	0.64	0.69	0.65
Isoleucine	5.14	5.09	5.08	5.08	5.04	4.95	5.07	5.08	4.93
Leucine	7.68	7.64	7.71	7.66	7.58	7.52	7.62	7.57	7.48
Tyrosine[4]	2.88	2.86	2.87	2.86	2.91	2.72	2.87	2.89	2.77
Phenylalanine	4.77	4.79	4.78	4.79	4.79	4.66	4.72	4.74	4.61
Lysine	1.96	1.93	1.94	1.94	1.90	1.87	1.93	1.94	1.90
Histidine	0.70	0.74	0.74	0.67	0.72	0.71	0.70	0.75	0.70
Arginine	6.76	6.68	6.62	6.59	6.55	6.37	6.61	6.59	6.66
Non-essential amino acids, %									
Aspartic acid	6.41	6.26	6.26	6.35	6.32	6.28	6.25	6.22	6.14
Serine	11.37	11.24	11.13	11.24	11.51	11.77	11.17	10.77	11.07
Glutamic acid	10.72	10.63	11.03	11.03	10.89	10.93	10.47	10.54	10.69
Glycine	6.81	7.05	6.89	6.85	6.77	6.98	6.68	6.61	6.63
Proline	8.62	8.40	8.80	8.67	8.60	8.38	8.87	8.55	8.19
Alanine	4.36	4.33	4.44	4.34	4.34	4.37	4.27	4.27	4.32
Lanthionine, %	1.96	2.33	2.28	3.28	3.31	3.05	2.08	2.25	2.27

[1] All values are on dry matter basis. Means represent duplicate or triplicate analyses.
[2] For feather meal codes in this and all subsequent tables, see Table 3.1.
[3] Percent Kjeldahl-N x 6.25.
[4] Semi-essential amino acid (Scott et al., 1983), for this and all subsequent tables and figures.

3.4.1 Amino acid excretions

Data of the mean amino acids (AA) excreted by the control birds fed N-free diet (NFD), expressed as a percentage of the sum of the AA and mg/bird, are represented in Table 3.3. This amino acid pattern reflects the composition of the metabolic fecal and endogenous urinary amino acids (M + E AA) which were excreted by the chicks. The AA excreted in the greatest amounts were glutamic acid, aspartic acid, leucine and lysine in order of decreasing abundance with serine and threonine being next most abundant in the present experiment. The AA excreted in the lowest quantities were methionine, histidine, cystine and tyrosine.

Table 3.3. Values of metabolic plus endogenous amino acids in chicks fed on nitrogen-free diet.

Amino acid	percent of the sum of the amino acids	mg/bird
Essential		
Threonine	6.69 ± 0.15[1)]	32.83 ± 2.90
Cystine	3.80 ± 0.16	18.70 ± 1.69
Valine	6.06 ± 0.09	30.00 ± 2.61
Methionine	2.00 ± 0.12	9.70 ± 1.12
Isoleucine	4.94 ± 0.19	24.83 ± 2.65
Leucine	8.69 ± 0.55	45.83 ± 3.93
Tyrosine	4.42 ± 0.12	21.93 ± 2.12
Phenylalanine	4.66 ± 0.08	23.13 ± 2.16
Lysine	7.19 ± 0.35	35.53 ± 3.58
Histidine	2.56 ± 0.08	12.57 ± 1.14
Arginine	5.51 ± 0.30	27.07 ± 2.30
Non-essential		
Aspartic acid	9.99 ± 0.19	49.37 ± 4.35
Serine	7.15 ± 0.10	35.07 ± 2.76
Glutamic acid	13.46 ± 0.09	65.97 ± 4.95
Proline	6.78 ± 0.30	32.83 ± 2.36
Alanine	6.13 ± 0.11	30.30 ± 2.63
Sum amino acids		496.03 ± 40.58

[1)] Mean and standard errors of nine observations.

The average amino acid composition of excreta in the experimental groups fed the test feather meals, expressed as percent of the sum of the AA can been seen in Table 3.4. The digestibility of exogenous and endogenous proteins, the level of microbial fermentation as well as urinary amino acids will determine the

Table 3.4. Amino acid composition of excreta in chicks fed on differently treated feather meals[1), 2)].

Amino acid	Feather meals															
	FM_{30}		FM_{50}		FM_{70}		$FM_{30}CH$		$FM_{50}CH$		$FM_{70}CH$		$FM_{30}EN$		$FM_{50}EN$		$FM_{70}EN$	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Essential																		
Thr	5.89	0.05	5.81	0.07	5.95	0.05	5.98	0.03	5.87	0.04	5.80	0.03	5.95	0.05	5.94	0.03	5.78	0.03
Cys	7.35	0.28	6.89	0.23	5.53	0.11	5.78	0.12	5.04	0.11	4.49	0.10	8.04	0.11	6.71	0.09	5.52	0.06
Val	6.27	0.25	6.05	0.19	6.07	0.17	6.00	0.15	6.35	0.18	6.39	0.07	6.13	0.15	6.44	0.20	6.61	0.13
Met	0.98	0.03	1.07	0.03	1.02	0.03	0.93	0.03	1.01	0.02	0.95	0.04	0.96	0.05	1.05	0.03	1.03	0.02
Ile	3.39	0.15	3.31	0.10	3.40	0.06	3.29	0.06	3.44	0.14	3.61	0.05	3.23	0.05	3.68	0.10	3.41	0.09
Leu	7.42	0.17	6.97	0.13	6.53	0.08	7.16	0.10	6.79	0.14	6.69	0.06	7.21	0.05	6.76	0.12	6.61	0.06
Tyr	3.07	0.08	3.16	0.04	3.18	0.06	3.40	0.06	3.16	0.04	3.16	0.05	3.35	0.08	3.24	0.05	3.12	0.06
Phe	3.72	0.11	3.52	0.03	3.73	0.06	3.93	0.04	3.89	0.04	4.08	0.03	3.56	0.04	3.74	0.05	3.83	0.07
Lys	3.72	0.13	4.10	0.09	4.22	0.08	3.83	0.15	4.11	0.13	3.95	0.09	3.95	0.10	4.04	0.11	4.07	0.07
His	1.37	0.04	1.44	0.07	1.49	0.05	1.27	0.03	1.33	0.06	1.42	0.03	1.36	0.06	1.37	0.07	1.37	0.03
Arg	5.34	0.15	5.10	0.10	5.61	0.09	5.57	0.09	5.53	0.12	5.58	0.10	4.92	0.33	5.37	0.07	5.65	0.14
Non-essential																		
Asp	12.62	0.38	12.43	0.23	13.03	0.19	11.90	0.08	11.82	0.20	11.98	0.11	12.59	0.11	12.07	0.21	12.51	0.18
Ser	11.24	0.17	11.26	0.41	10.88	0.15	12.39	0.21	12.02	0.20	12.31	0.14	10.70	0,14	10.40	0.07	10.76	0.11
Glu	13.04	0.14	13.87	0.09	13.63	0.05	13.56	0.39	13.65	0.13	13.69	0.12	13.45	0.19	13.56	0.15	13.48	0.14
Pro	10.35	0.08	10.79	0.33	11.39	0.19	10.59	0.09	11.47	0.08	11.22	0.21	10.55	0.20	11.55	0.18	11.88	0.13
Ala	4.25	0.10	4.24	0.06	4.34	0.04	4.42	0.07	4.53	0.08	4.70	0.06	4.06	0.05	4.09	0.03	4.38	0.06

1) Data are expressed in percent of the sum of the amino acids.
2) Mean and standard errors of seven observations.

proportion of AA appearing in the poultry excreta. The AA composition of excreta revealed small differences between the excreta derived from the different experimental groups.

Figure 3.1 illustrates the comparison of amino acid composition of dietary feather meal, endogenous excreta and feather meal excreta of the FM_{30} test group. Comparison within all other test groups showed the same clear trend. In Figure 3.1 we can see that the AA composition of feather meal excreta and that of excreta voided by NFD fed birds were not similar. This suggests that dietary feather meal protein had a considerable influence upon the amino acid composition of the excreta. Furthermore the AA composition of excreta from chicks fed processed feather meal was also different from that of the dietary protein. This would indicate some dilution with endogenous amino acids and/or different true digestibility of individual amino acids.

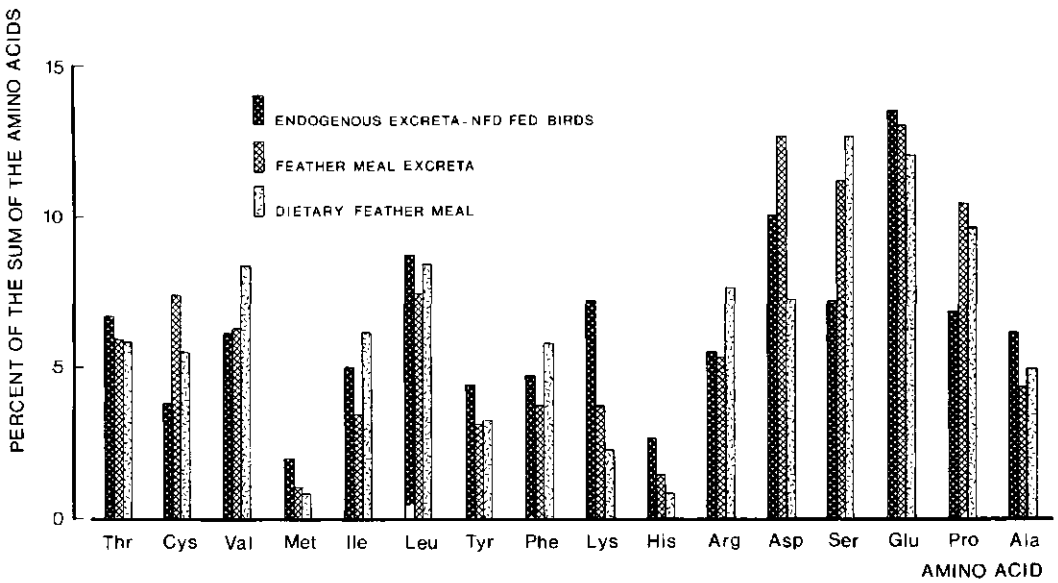


Fig. 3.1. Comparison of amino acid compositions of feather meals, endogenous excreta by NFD fed chicks and excreta voided by chicks during the 36-hr experimental period.

The lanthionine content obtained by hydrolysis of the excreta of chicks fed the different feather meals was compared with that of the dietary feather meal protein and found to be related (Tables 3.2 and 3.5). The lanthionine content increased with increasing processing time in feather meals without additives and with enzymes, whereas the inverse relationship was found with the chemi-

Table 3.5. Metabolic plus fecal excretion of lanthionine in chicks fed on differently treated feather meals[1].

Item	Feather meals								
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$
Lan, %	1.77	1.88	1.92	2.78	2.67	2.56	1.78	1.97	2.16
Cys, %	3.15	2.88	2.41	2.47	2.18	1.94	3.03	2.69	2.46
Lan (%) of ingest. lan.	71.76	67.59	68.63	71.09	69.17	76.55	65.02	77.30	79.55

[1] Duplicate observations with < 5% variation, on pooled samples.
Lan: Lanthionine

cally-treated samples (Fig. 3.2.).

The amount of nitrogen excreted by the test groups is given in Table 3.6.

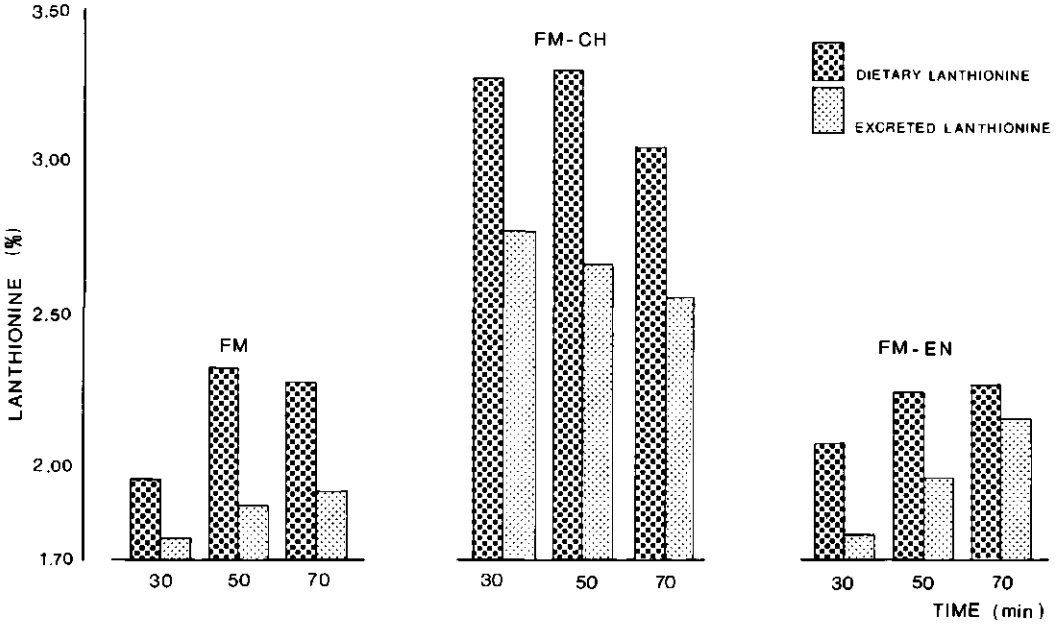


Fig. 3.2. Comparison of lanthionine compositions of feather meals and excreta voided by chicks during the 36-hr experimental period.
FM: feather meals treated without additives; FM-CH: feather meals treated with chemical (NaOH); FM-EN: feather meals treated with enzyme.

Table 3.6. Nitrogen excretion and its digestibility in chicks fed on differently treated feather meals[1].

Feather meals	Nitrogen				
	Intake	Excretion		Digestibility (%)	
		Total[2]	Fecal[3]	Apparent	True[4]
FM_{30}	1853	1837	400	78.4	83.6
FM_{50}	1874	1906	377	79.9	85.0
FM_{70}	1859	1832	360	80.7	85.8
mean		1858	379	79.7	84.8
$FM_{30}CH$	1821	1971	358	80.3	85.6
$FM_{50}CH$	1837	1996	366	80.1	85.3
$FM_{70}CH$	1830	2042	368	79.9	85.1
mean		2003	364	80.1	85.3
$FM_{30}EN$	1857	1780	377	79.7	84.9
$FM_{50}EN$	1973	2000	415	77.9	83.0
$FM_{70}EN$	1859	1940	384	79.3	84.5
mean		1907	392	79.0	84.1
Overall mean		1923	378	79.6	84.7
N-free diet	--	559	96		

1) mg/bird. Triplicate observations on pooled excreta.
2) Fecal plus urinary.
3) The fecal N-values were calculated from precipitable nitrogen (Terpstra and de Hart, 1974).
4) Corrected from fecal-N produced on nitrogen-free diet birds.

3.4.2 Apparent and true amino acid digestibility

The apparent and true amino acid digestibility (AAAD and TAAD) values for all feather meals tested are illustrated in Tables 3.7 and 3.8. From the analysis of variance in Tables 3.9 and 3.10 it is clear that the main significant variable was the processing time. In these tables, components which did not show any significant effect are omitted.

Apparent digestibility of all amino acids (Table 3.9) with the exception of leucine, were strongly influenced by the time-linear effect ($P < 0.001$; only for serine $P < 0.01$). In general the longer the processing time the lower the digestibility (Table 3.7). Only the digestibility of cystine, tyrosine, phenylalanine, serine and alanine showed significant responses to the treatment conditions. Cystine was the only amino acid affected by time x treatment interaction ($P < 0.05$).

The highest apparent digestibility was that of isoleucine (range from

Table 3.7. Average apparent digestibilities of amino acids in differently processed feather meals fed to chicks[1].

Amino acid	Feather meals									
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$	Pooled SE
Thr	63.5^{bc}	61.3^{bc}	59.0^{abc}	60.7^{bc}	59.0^{abc}	52.8^{a}	65.9^{c}	57.5^{ab}	56.2^{ab}	2.26
Cys	52.3^{d}	44.7^{bcd}	51.8^{d}	48.2^{d}	46.1^{cd}	38.1^{abc}	52.4^{d}	34.6^{a}	35.5^{ab}	3.18
Val	74.4^{bcd}	73.6^{abcd}	73.3^{abcd}	74.7^{cd}	71.8^{abcd}	67.3^{a}	77.2^{d}	69.9^{abc}	68.1^{ab}	2.02
Met	55.4^{bc}	47.0^{a}	51.3^{ab}	56.8^{bc}	51.2^{ab}	45.5^{a}	59.1^{c}	48.1^{a}	45.7^{a}	2.32
Ile	79.8^{bc}	79.1^{bc}	78.5^{bc}	79.9^{bc}	77.6^{abc}	73.2^{a}	82.4^{c}	75.1^{ab}	76.2^{ab}	1.54
Leu	70.5^{ab}	70.8^{ab}	72.8^{ab}	71.0^{ab}	70.7^{ab}	67.2^{a}	74.0^{b}	69.4^{ab}	69.6^{ab}	1.86
Tyr	67.6^{c}	64.7^{bc}	64.5^{bc}	63.5^{bc}	64.7^{bc}	57.3^{a}	68.1^{c}	61.6^{ab}	61.4^{ab}	1.84
Phe	76.2^{bcd}	76.5^{cd}	75.1^{bcd}	74.6^{bcd}	73.5^{bc}	67.8^{a}	79.3^{d}	73.0^{bc}	71.4^{ab}	1.52
Lys	42.7^{d}	32.6^{bc}	30.3^{ab}	39.7^{cd}	30.3^{ab}	22.5^{a}	44.2^{d}	29.0^{ab}	26.6^{ab}	2.54
His	41.1^{bcd}	38.6^{bc}	35.6^{bc}	41.9^{cd}	40.2^{bc}	26.7^{a}	47.3^{d}	38.3^{bc}	32.9^{ab}	2.72
Arg	75.9^{bc}	75.3^{bc}	72.7^{ab}	73.7^{b}	72.5^{ab}	67.6^{a}	79.6^{c}	72.0^{ab}	70.9^{ab}	1.78
Essential (mean)	63.6^{de}	60.4^{bcde}	60.5^{bcde}	62.2^{cde}	59.8^{bcd}	53.3^{a}	66.3^{e}	57.1^{abc}	55.9^{ab}	1.98
Asp	40.8^{bcd}	36.4^{abcd}	33.0^{abc}	42.0^{cd}	39.2^{abcd}	29.7^{a}	44.6^{d}	33.6^{abc}	30.0^{ab}	3.37
Ser	70.0^{bc}	68.3^{bc}	68.5^{bc}	65.7^{ab}	65.9^{ab}	61.4^{a}	73.6^{c}	66.9^{ab}	66.5^{ab}	1.94
Glu	63.2^{cd}	58.2^{abcd}	60.2^{abcd}	61.7^{bcd}	59.2^{abcd}	53.9^{a}	64.8^{d}	56.0^{ab}	56.7^{abc}	3.55
Pro	63.6^{cd}	58.4^{bc}	58.5^{bc}	62.1^{cd}	56.5^{abc}	50.6^{ab}	67.3^{d}	53.7^{ab}	50.1^{a}	2.57
Ala	70.3^{bc}	68.6^{bc}	68.6^{bc}	68.5^{bc}	66.0^{ab}	60.5^{a}	73.9^{c}	67.2^{b}	65.3^{ab}	1.86
Non-ess. (mean)	61.6^{cd}	58.0^{abcd}	57.8^{abcd}	60.0^{bcd}	57.4^{abc}	51.2^{a}	64.8^{d}	55.5^{abc}	53.7^{ab}	2.29
Total (mean)	62.9^{cd}	59.6^{bcd}	59.6^{bcd}	61.5^{bcd}	59.0^{bc}	52.6^{a}	65.9^{d}	56.6^{abc}	55.2^{ab}	2.07

[1] Mean of seven observations.

a, b, c, d, e Mean values for individual treatments, followed by different superscripts horizontally are significantly different ($P < 0.05$).

Table 3.8. Average true digestibilities of amino acids in differently processed feather meals fed to chicks[1)].

Amino acid	Feather meals									
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$	Pooled SE
Thr	69.1^{bc}	67.0^{bc}	64.8^{abc}	66.5^{bc}	64.9^{abc}	58.9^{a}	71.6^{c}	63.3^{ab}	62.3^{ab}	2.26
Cys	55.7^{c}	48.5^{abc}	56.1^{c}	52.7^{bc}	51.2^{bc}	43.9^{ab}	55.8^{c}	39.1^{a}	40.8^{a}	3.18
Val	77.7^{bc}	76.9^{abc}	76.7^{abc}	78.1^{bc}	75.2^{abc}	70.8^{a}	80.6^{c}	73.3^{ab}	71.6^{ab}	2.02
Met	67.6^{bc}	59.6^{a}	63.4^{ab}	69.0^{bc}	63.3^{ab}	58.2^{a}	71.8^{c}	59.8^{a}	58.2^{a}	2.32
Ile	83.8^{bc}	83.2^{bc}	82.6^{bc}	84.0^{bc}	81.8^{abc}	77.3^{a}	86.5^{c}	79.2^{ab}	80.4^{ab}	1.54
Leu	75.4^{ab}	75.7^{ab}	77.8^{ab}	75.9^{ab}	75.7^{ab}	72.3^{a}	79.0^{b}	74.4^{ab}	74.7^{ab}	1.86
Tyr	74.0^{c}	71.1^{bc}	70.9^{bc}	69.8^{bc}	71.0^{bc}	64.0^{a}	74.4^{c}	67.9^{ab}	68.0^{ab}	1.84
Phe	80.3^{bc}	80.5^{bc}	79.1^{bc}	78.5^{bc}	77.5^{b}	71.9^{a}	83.3^{c}	77.1^{b}	75.6^{ab}	1.52
Lys	57.9^{d}	47.9^{bc}	45.6^{ab}	55.0^{cd}	45.9^{ab}	38.3^{a}	59.5^{d}	44.3^{ab}	42.2^{ab}	2.54
His	56.0^{bcd}	52.8^{bc}	49.8^{bc}	57.6^{cd}	54.8^{bcd}	41.5^{a}	62.4^{d}	52.3^{bc}	47.9^{ab}	2.72
Arg	79.2^{bc}	78.7^{bc}	76.2^{ab}	77.1^{b}	75.9^{ab}	71.2^{a}	83.0^{c}	75.5^{ab}	74.2^{ab}	1.78
Essential (mean)	70.6^{cd}	67.4^{bcd}	67.5^{bcd}	69.5^{bcd}	67.0^{bc}	60.7^{a}	73.5^{d}	64.2^{ab}	63.3^{ab}	1.98
Asp	47.2^{abc}	42.9^{abc}	39.6^{ab}	48.5^{bc}	45.8^{abc}	36.3^{a}	51.2^{c}	40.3^{ab}	36.7^{a}	3.37
Ser	72.5^{bc}	70.9^{bc}	71.2^{bc}	68.3^{ab}	68.5^{ab}	63.9^{a}	76.2^{c}	69.6^{ab}	69.2^{ab}	1.94
Glu	68.3^{cd}	63.4^{abcd}	65.2^{abcd}	66.8^{bcd}	64.3^{abcd}	58.9^{a}	70.1^{d}	61.2^{ab}	61.9^{abc}	3.55
Pro	66.7^{bc}	61.7^{ab}	61.6^{ab}	65.2^{bc}	59.7^{ab}	53.8^{a}	70.4^{c}	56.9^{a}	53.5^{a}	2.57
Ala	76.1^{bc}	74.4^{abcd}	74.3^{abcd}	74.3^{bcd}	71.8^{b}	66.3^{a}	79.8^{c}	73.1^{ab}	71.1^{ab}	1.86
Non-ess. (mean)	66.2^{cd}	62.7^{abcd}	62.4^{abcd}	64.6^{bcd}	62.0^{abc}	55.9^{a}	69.5^{d}	60.2^{abc}	58.5^{ab}	2.29
Total (mean)	69.2^{cd}	66.0^{bcd}	65.9^{bcd}	68.0^{bcd}	65.4^{abc}	59.2^{a}	72.2^{d}	63.0^{abc}	61.8^{ab}	2.07

[1)] Mean of seven observations.

a, b, c, d Mean values for individual treatments, followed by different superscripts horizontally are significantly different ($P < 0.05$).

Table 3.9. Analysis of variance for apparent amino acid digestibility values (the terms with little overall significance are omitted).

Responses	Source of variation[1)]						
	Time			Treatment			Interaction
	T	TL	TQ	P	C1	C2	
	F-value[2)]						
Thr	8.06^{***}	16.05^{***}					
Cys	8.36^{***}	12.53^{***}	4.19^{*}	5.82^{**}	10.07^{**}		3.12^{*}
Val	6.49^{**}	12.69^{***}					
Met	15.01^{***}	25.33^{***}	4.69^{*}				
Ile	7.52^{**}	14.14					
Leu	0.80			0.89			
Tyr	6.27^{**}	12.54^{***}		3.20^{*}	4.82^{*}		
Phe	9.05^{***}	18.05^{***}		5.33^{**}	6.14^{*}		
Lys	30.85^{***}	57.51^{***}	4.20^{*}				
His	14.10^{***}	27.66^{***}					
Arg	8.40^{***}	16.80^{***}					
Essential	11.13^{***}	21.54^{***}					
Asp	8.79^{*}	17.56^{**}					
Ser	3.69^{***}	7.20^{***}		5.73^{**}		8.73^{**}	
Glu	7.94^{***}	13.46^{***}					
Pro	15.33^{***}	28.81^{***}					
Ala	8.20^{***}	16.21^{***}		4.58^{*}		6.20^{*}	
Non-essential	9.17^{***}	17.76^{***}					
Total	11.13	20.40					

1), 2) For codes and degrees of freedom, respectively, see section 3.3.5.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

73.2 - 82.4%), followed by phenylalanine, arginine and valine, whereas the lowest values for AAAD were found for lysine and aspartic acid (ranges from 22.5 - 44.2% and 29.7 - 44.6%, respectively), followed by histidine and cystine. Although absolute values differed, the observed trend was similar in all feather meal samples for the apparent digestibility of almost all amino acids tested.

It is of importance to notice that the digestibility of the most frequently limiting amino acids in feather meals, namely lysine, histidine and methionine seemed to be particularly sensitive to the treatments imposed.

The overall mean apparent digestibility values of essential, non-essential and total amino acids were higher for $FM_{30}EN$ samples, while the $FM_{70}CH$ samples showed the lowest values. In all cases, mean AAAD for essential AA were higher than AAAD values of non-essential AA.

The true amino acid digestibility (TAAD) values are given in Table 3.8.

Table 3.10. Analysis of variance for true amino acid digestibility values (the terms with little overall significance are omitted).

Responses	Source of variation[1]						
	Time			Treatment			Interaction
	T	TL	TQ	P	C1	C2	
	F-value[2]						
Thr	7.47^{**}	14.83^{***}					
Cys	6.59^{**}	9.06^{**}	4.12^{*}	4.99^{*}	7.56^{**}		2.87^{*}
Val	6.27^{**}	12.24^{***}					
Met	15.31^{***}	25.29^{***}	5.34^{*}				
Ile	7.27^{**}	13.60^{***}					
Leu	0.84			0.68			
Tyr	5.83^{**}	11.64^{**}			4.54^{*}		
Phe	8.81^{***}	17.58^{***}		5.28^{**}	5.87^{*}	4.69^{*}	
Lys	29.76^{***}	55.29^{***}	4.23^{*}				
His	15.26^{***}	30.37^{***}					
Arg	8.25^{***}	16.48^{***}					
Essential	10.69^{***}	20.52^{***}					
Asp	8.61^{*}	17.20^{**}					
Ser	3.70^{***}	7.24^{***}		5.95^{**}		9.17^{**}	
Glu	8.07^{***}	13.83^{***}					
Pro	15.06^{***}	28.30^{***}					
Ala	8.37^{***}	16.58^{***}		4.62^{*}		6.50^{*}	
Non-essential	9.14^{***}	17.71^{***}					
Total	10.24^{***}	19.72^{***}					

[1], [2] See Table 3.9, footnotes 1), 2), respectively.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

From Table 3.10 it is clear that the TAAD values show a great similarity in response to the AAAD values. This similarity is because the AAAD values were not affected by the amount of protein consumed by the birds, since that amount in the present study was kept at an equal level. Some differences between amino acid digestibility (AAD) values in the level of the significant probability were very small.

In general, the essential amino acids showed mean true digestibility (TD)-values of about 7 percentage units higher than their apparent digestibility (AD)-values. The total and non-essential amino acids showed a mean TD of about 6 and 5 percentage units, respectively, higher than their corresponding AD values.

The differences in TAAD of the individual amino acids, as well as those of the essential, non-essential and total amino acids, between the different

processed feather meals, showed a general tendency to be the same as their differences in AAAD values. In the essential amino acids, the highest TD was 86.5% for isoleucine in $FM_{30}EN$ samples and the lowest was 38.3% for lysine in $FM_{70}CH$ samples. The former and latter feather meal samples also gave the highest and lowest TD for the non-essential amino acids which were 79.8% for alanine and 36.3% for aspartic acid, respectively.

The effect of the correction for apparent and true digestibility values on the observed amino acid contents in the test feather meals (Table 3.2), is demonstrated in Appendix 4.

The results in Table 3.5 showed that the ingested lanthionine is not totally recovered in the excreta. The lowest excreta-lanthionine recovery was observed in $FM_{30}EN$ samples (65.0%) and the highest in $FM_{70}EN$ samples (79.7%).

The digestibility of nitrogen (Table 3.6) showed a slight increase with increasing processing time in feather meals treated without additives, whereas the inverse relationship was found with the chemically-treated samples, which also gave the highest mean N-digestibility values. The enzymatically-treated feather meals did not show any clear trend.

3.4.3 Chemical evaluation of feather meal protein

Table 3.11 shows the chemical scores of the different processed feather meals. When the chemical score is based solely on amino acid composition, all the test feather meals showed methionine to be the first limiting amino acid. When digested amino acids were used as the basis for calculating the chemical score (CS) lysine was the first limiting amino acid. This is because the digestibility of lysine is lower than that of methionine. With increasing processing time, the CS (on lysine deficiency) and lysine apparent digestibility (Table 3.7) decreased. When digested amino acids were corrected for endogenous excretions and the values taken for CS calculations, the first limiting amino acid for FM_{30}, FM_{50} and $FM_{30}EN$, was methionine, with lysine for the rest of the test samples.

The results of the laboratory tests on protein evaluation of the test feather meals are given in detail in Chapter 2 (Table 2.9).

3.5 Discussion

In studies reported in the literature, samples of commercially hydrolyzed

Table 3.11. Chemical scores (CS) in differently processed feather meals[1)].

CS	Feather meals								
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$
CS/AAC	31.5 M	30.2 M	31.9 M	32.1 M	32.2 M	30.9 M	30.5 M	32.6 M	30.9 M
CS/DAA_a	16.5 K	12.4 K	11.7 K	15.5 K	11.6 K	8.4 K	16.8 K	11.0 K	9.9 K
CS/DAA_t	21.5 M	17.9 M	17.4 K	21.6 K	17.4 K	14.5 K	21.9 M	16.9 K	15.8 K

1) Chemical score: See section 3.3.3.
AAC, DAA_a, DAA_t: Amino acid composition, digestible (apparent) and digestible (true), respectively.
M: methionine; K: lysine.

feather meals have been found to contain unusually high levels of ash and correspondingly lower levels of crude protein, as well as varying amino acid concentrations. This is probably due to variations in pre-treatment of the source materials in the dressing plants and due to different processing conditions. A more comprehensive evaluation of the chemical characteristics of the different processed feather meals in this study is given in Chapter 2.

3.5.1 The estimate of amino acid digestibility

Methodology

The main criticisms of the fecal analysis method for determining amino acid digestibility are related to the effects of microbial fermentation in the large intestine and the common excretion of feces and urine in poultry. The data on these insignificant influences have been extensively discussed in section 3.2.1.

In this study, the basic methodology of TME procedure (Sibbald, 1976) was used. The recommended excreta collection period in that method is 24 hrs. For several feedstuffs however which are relatively high in crude fiber content and poorly digestible, even a 24-hrs excreta collection period does not allow complete clearance of dietary residue from the digestive tract (Muztar and Slinger, 1980a; Kessler and Thomas, 1981). This might overestimate the actual digestibility of amino acids of these feedstuffs. The latter authors suggested a longer excreta collection period for feather meal. Furthermore, Parsons et al. (1982) reported that feather meal significantly affected amino acid composition of excreta after 24 hrs postfeeding, indicating that more than 24 hrs were required for complete intestinal clearance of undigested amino acids. It is therefore interesting to note a very good agreement between the previous reports and the

corresponding fecal collection period in this study, which was established from our results in preliminary experiments (see material and methods, section 3.3.2).

It may also be suggested that force-feeding leads to some metabolic changes caused by the procedure as such. However, the available data (Cohn, 1963) do not support this argument.

Correction for metabolic and endogenous amino acids

For precise determination of true digestibility of nitrogen and amino accids, valid estimates of fecal metabolic nitrogen (FMN) and amino acids (FMMA) are required. The main sources of FMN and FMAA are gastrointestinal secretions, desquamated cells and intestinal microorganisms. The TME method of Sibbald (1976) includes a correction for these losses by using unfed birds. When applying TAAD calculations it is assumed that metabolic plus endogenous urinary amino acid (M + E AA) losses of the fed birds are similar to those of the unfed birds. However, there is apparently no information available in literature to confirm this assumption. In the present study the estimation of the M + E AA has been based on a group of birds receiving a N-free diet (NFD) on the assumption that the above mentioned excretions are related to the amount of dry matter consumed and that they are not influenced by the presence of protein in the diet. Muztar and Slinger (1980b) reported that the excretion of most of the amino acids examined was significantly increased with the feeding of the NFD as compared with the unfed control birds. In addition Parsons et al. (1983) found that excretion of amino acids by adult cocks fed low- and high-fiber N-free diets was variable. It may be suggested that fasted birds do not provide an accurate estimate of endogenous amino acid excretion in amino acid digestibility trials. Moreover, the fiber content of NFD may also influence the true digestibility estimations. The question, therefore, of which NFD should be used for such estimations, remains.

Amino acid excretion

A comparison of the M + E AA composition in the excreta of chicks in our experiment, with results reported by Slump et al. (1977) using adult cocks, and Skrede et al. (1980) using chicks and colostomised hens, shows similarities. However, there may be some differences in results between laboratories caused by differences in experimental and analytical techniques. The results of total M + E AA reported herein as mg/bird (Table 3.3) are higher than those found by

Muztar and Slinger (1980b) in adult roosters. The discrepancy between the present work and that of these authors can probably be explained in terms of the length of the collection period used and the type of NFD. These workers used a collection period of only 30 hrs (vs 36 hrs in this study) and NFD based on cerelose (vs glucose in this study).

In our studies the amino acids that were excreted to the greatest extent, namely glutamic acid, aspartic acid, leucine, serine and threonine, are those occurring most abundantly in mucin protein (Pigman, 1963; Horowitz, 1067). Mucins are secreted in large amounts by the epithelium of the gastrointestinal tract but they have been found by Hashimoto et al. (1963) to be largely resistant to proteolytic enzymes. Thus, it appears likely that mucin protein is a major source of endogenous amino acids/nitrogen, reflected in the amino acid composition of feces. In addition, the digestion of endogenous proteins seems generally to be less complete than that of dietary proteins (Gitler, 1964; Gebhardt et al., 1978).

The results of the present study showing that the amino acid composition of feces was affected by dietary protein, are in agreement with those of Parsons et al. (1982). In contrast, workers using other species of animals showed very extensive dilution of dietary proteins with endogenous proteins, thus resulting in a relatively constant amino acid composition of feces (Nasset and Ju, 1961; Slump and van Beek, 1975). This study does not confirm an extensive dilution of dietary amino acids with those of endogenous origin in the gastrointestinal tract.

Lanthionine excretion

The results of excretion balance of the unnatural amino acid lanthionine (Table 3.5) show that the total recovery is always less than 100%, which means that lanthionine is either transformed by the intestinal flora, metabolized, or retained in the organism.

There is very little known about the utilization of the cysteine residue of lanthionine. Lanthionine, present in feather meal protein, is released via proteolysis in the gut and then absorbed and transported to the liver where it is acted upon by cystathionase, liberating cysteine, pyruvate and NH_3 (Cavallini et al., 1960). In recent feeding experiments with chicks, an availability of 52% was calculated from L-DL-lanthionine as a source of cysteine, as reported by Robbins et al. (1980) and Baker et al. (1981). The lanthionine formed in heat- and alkali-treated protein probably consists of a mixture of 50% L-lanthionine

and 50% meso-lanthionine (Robbins et al., 1980). If we assume that meso-lanthionine is totally unavailable to the chicks as it is for the rat (Jones et al., 1948), we can indicate from the present results that the retained part in the organism is L-DL-lanthionine. If this is true our observations seem in general to support similar indications from the previously mentioned literature studies. Because there is no information in the literature about lanthionine excretion to serve as comparison and so far the growth assays have been applied in very limited trials, more extensive work is needed before definite conclusions can be drawn about the utilization of lanthionine.

3.5.2 Amino acid digestibility in feather meals

Literature studies have reported considerable variation between feather meals in amino acid digestibility (section 3.2.2). Commercially prepared products may vary widely in digestibility and nutritional quality due to differences in raw materials and/or processing conditions.

In our work even the higher average true digestibility of amino acids in $FM_{30}EN$, 72%, is lower than the 97%, 94% and 82% found by Burgos et al. (1974), Kirby et al. (1978) and Parsons et al. (1982), respectively. The data reported by the several research workers showed small variation between amino acids. According to the present study, the digestibility of individual amino acids in feather meals may vary substantially. Moreover, there are certain consistencies in the relative digestibility of amino acids for all feather meals tested (Table 3.12), the most important of which were the rather high digestibility of isoleucine, phenylalanine, arginine and valine, whereas cystine, histidine, lysine and aspartic acid were generally among the less digestible amino acids. Bielorai et al. (1983) emphasized the same differences for apparent absorption values of individual amino acids from hydrolyzed feather meals, determined in the lower ileum of chicks.

The low digestibility coefficients of some amino acids may be related to the sensitivity of these amino acids to different treatments.

Processing treatments and amino acid digestibility

The present results also showed that processing conditions, namely increasing time and added chemicals, have a substantial negative effect on amino acid digestibility of feather meals (Tables 3.9 and 3.10). This leads us to conclude that autoclaving feather meal may have altered the protein structure in such a

Table 3.12. The relative magnitude of apparent and true digestibilities of amino acids in differently processed feather meals fed to broiler-chicks (feather meals and amino acids are listed in order of increasing digestibility).

Apparent digestibility										True digestibility									
Feather meals[1]									Range[2]	Feather meals[1]									Range[2]
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9	
Essential																			
Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile	73.2-82.4	Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile	Ile	77.3-86.5
Phe	Phe	Phe	Phe	Phe	Phe	Val	Phe	Arg	67.6-79.6	Leu	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Phe	71.9-83.3
Arg	Arg	Arg	Arg	Val	Arg	Phe	Arg	Phe	67.8-79.3	Phe	Leu	Arg	Arg	Val	Arg	Val	Arg	Arg	71.2-83.0
Val	Leu	Val	Val	Leu	Val	Arg	Val	Val	67.3-77.2	Arg	Arg	Leu	Leu	Arg	Val	Arg	Val	Val	70.8-80.6
Leu	Val	Leu	Leu	Arg	Leu	Leu	Leu	Leu	67.2-74.0	Val	Val	Val	Val	Leu	Leu	Leu	Leu	Leu	72.3-79.0
Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	57.3-68.1	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	64.0-74.4
Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	52.8-66.0	Thr	Thr	Thr	Thr	Thr	Thr	Met	Thr	Thr	63.3-71.6
Met	Met	Met	Met	Cys	Met	Met	Met	Met	45.2-59.1	Met	Met	Met	Met	Met	Met	Thr	Met	Met	58.2-71.8
Cys	Cys	His	Cys	Met	Cys	Cys	Cys	Cys	34.6-52.4	Cys	His	His	His	Cys	His	His	Lys	His	41.5-62.4
His	His	Cys	His	His	His	His	Lys	His	26.7-47.3	His	Lys	Lys	Cys	His	Cys	Lys	His	Lys	38.3-59.5
Lys	Lys	Lys	Lys	Lys	Lys	Lys	His	Lys	22.5-44.2	Lys	Cys	Cys	Lys	Lys	Lys	Cys	Cys	Cys	39.1-56.1
Non-essential																			
Ser	Ser	Ala	Ala	Ala	Ala	Ala	Ala	Ala	60.5-73.9	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	66.3-79.8
Ala	Ala	Ser	Ser	Ser	Ser	Ser	Ser	Ser	61.4-73.6	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	63.9-76.2
Glu	Glu	Glu	Glu	Glu	Pro	Pro	Pro	Pro	50.1-67.3	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Pro	53.8-70.4
Pro	Pro	Pro	Pro	Pro	Glu	Glu	Glu	Glu	53.9-64.8	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Glu	58.9-70.1
Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	29.7-44.6	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	Asp	36.3-51.2

[1] 1: $FM_{70}CH$; 2: $FM_{70}EN$; 3: $FM_{50}EN$; 4: $FM_{50}CH$; 5: FM_{70}; 6: FM_{50}; 7: $FM_{30}CH$; 8: FM_{30}; 9: $FM_{30}EN$.
[2] Range of the corresponding amino acid of the last column.

way that the enzymic attack necessarily associated with digestion process is hindered. An indication of these chemical changes is already given with the formation of the unnatural amino acid lanthionine in processed feather meals (Table 3.2).

Possible mechanisms of reduced protein quality caused by heat have been discussed by several authors (Hurrell et al., 1976; Bender, 1977). It is now generally accepted that the effect of heat on proteins in the absences of carbohydrates and fats can impair their nutritive value. It has been suggested that heat causes the formation of new cross-linkages within the protein molecules which lead to the formation of new amino acids (Chapter 2, section 2.5.2). The hypothesis is that cross-link formation reduces the rate of protein digestion possibly due to preventing enzyme penetration or by blocking the sites of enzyme attack. Our results suggest that the amount of lanthionine present in the test samples may be a reasonable index of treatment damage, since feather meals with higher lanthionine content showed lower digestibility values (Fig. 3.3).

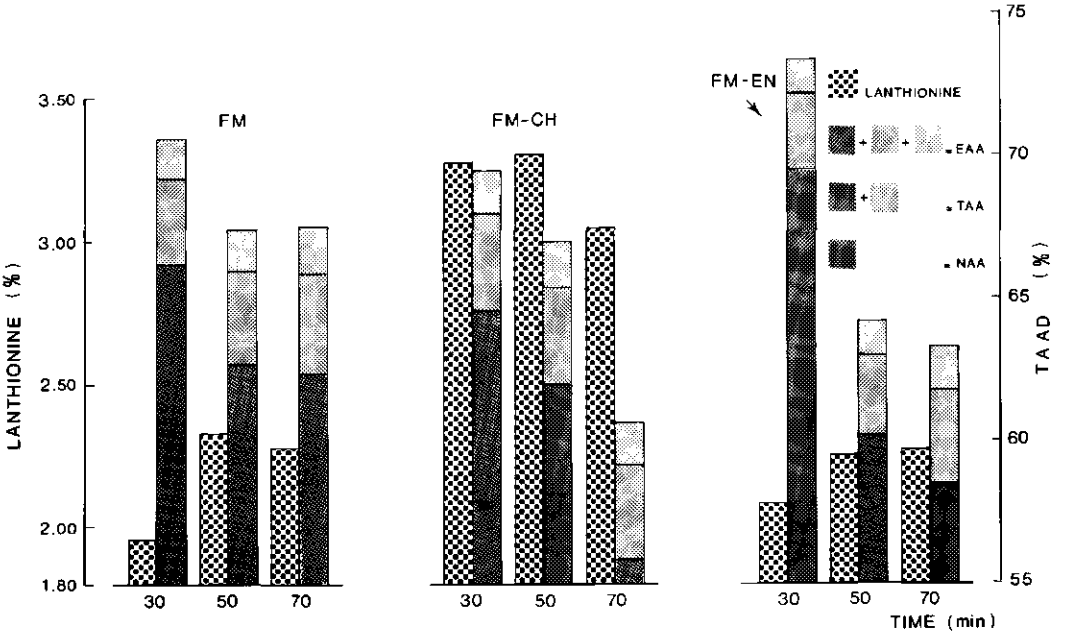


Fig. 3.3. Comparison of dietary lanthionine compositions and true digestibilities of total, essential (total) and non-essential (total) amino acids of feather meals.
For feather meal codes, see Fig. 3.2.

In addition to cross-link considerations discussed above, the exposure of protein to alkali (Provansal et al., 1975; Masters and Friedman, 1980) and also to heat (Sternberg et al., 1975; Liardon and Hurrell, 1983) leads to racemization of amino acid residues.

Even if small amounts of racemization occur in protein during alkali processing, the extended range of the peptide chain around the racemized amino acid residues cannot be used by the substrate binding site of proteases. This causes a major decrease in proteolysis (Hayashi and Kameda, 1980; Masters and Friedman, 1980). It is interesting to notice from our studies that feather meals processed with sodium hydroxide showed reduced digestibility values, compared with the other treatments.

Apparently there is no information in the literature on comparisons between differently heat-treated feather meals. Changes in amino acid digestibility in processed diets were also obtained by other workers (Varnish and Carpenter, 1975b; Ford, 1976; Achinewhu and Hewitt, 1979; Robbins and Ballew, 1982). However, in these studies the heated protein sources were compared with control (untreated) samples, and the variations during processing were not investigated.

Nitrogen digestibility

The mean values of apparent and true digestibility of the total essential and non-essential amino acids, and the digestibility of nitrogen (ND), are graphically represented in Figures 3.4 and 3.5. Comparing these results, it appears that, in all cases, ND values are higher than amino acid digestibility values and that ND revealed differences between the different processed feather meals which were not detectable by the amino acid digestibility determinations.

Comparisons between biological and laboratory tests

In vitro assays have been carried out in an attempt to test additional criteria for feather meal protein evaluation. The relationships between *in vitro* and *in vivo* were not encouraging. These results, as shown by the negative correlation between *in vivo* and *in vitro* tests (Table 3.13), indicate that laboratory evaluations of feather meals are not a reliable index to detect inferior protein/amino acid quality in hydrolyzed feather meals treated under different processing conditions.

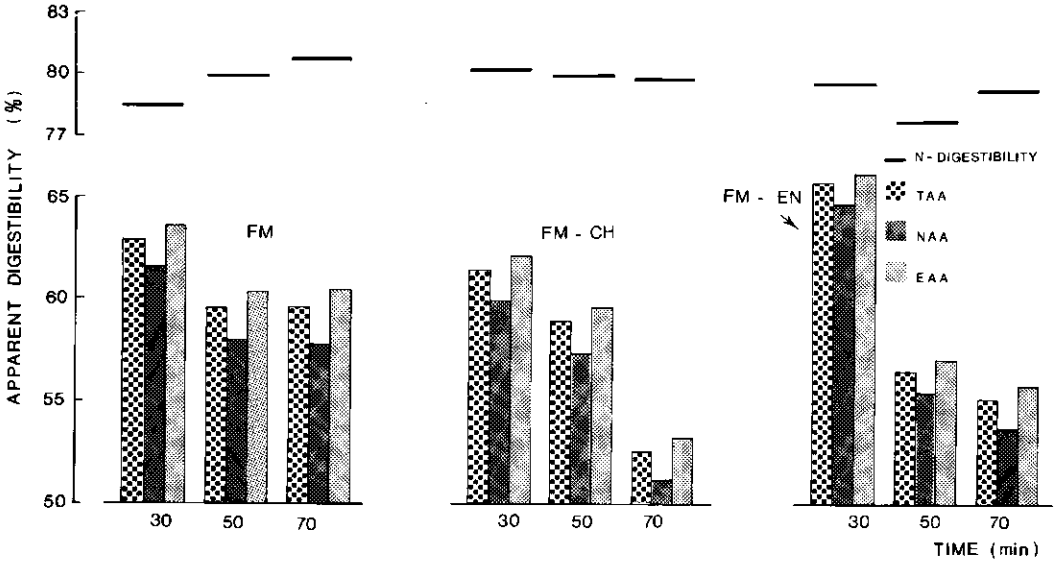


Fig. 3.4. Apparent digestibilities of nitrogen and total, essential (total) and non-essential (total) amino acids.
For feather meal codes, see Fig. 3.2.

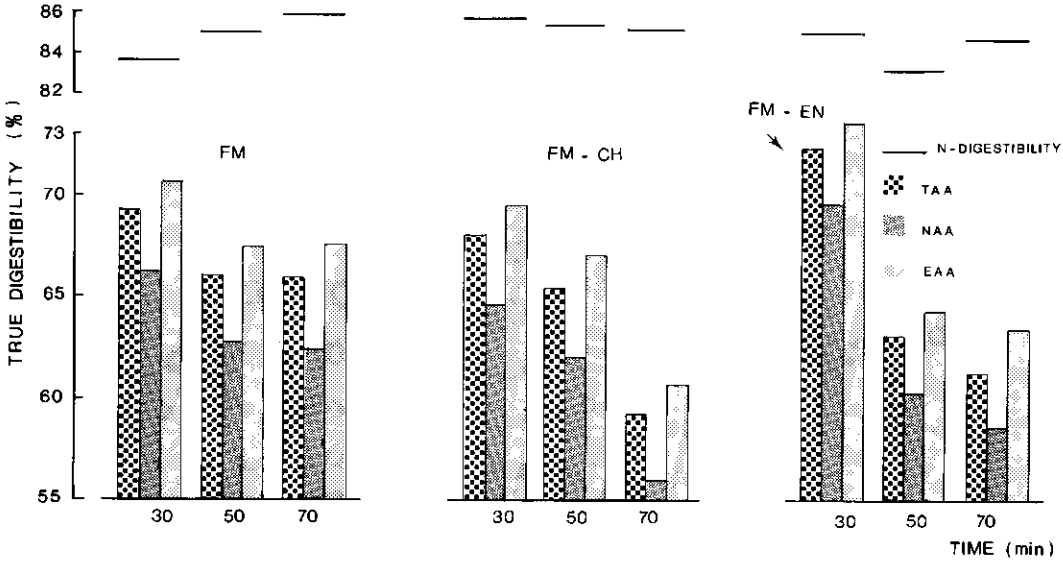


Fig. 3.5. True digestibilities of nitrogen and total, essential (total) and non-essential (total) amino acids.
For feather meal codes, see Fig. 3.2.

Table 3.13. Correlation coefficients between *in vitro* and *in vivo* tests.

In vivo	*In vitro*[1)]							
(True digestibility, %)	PDP_M	PDP_L	NSS	NSH	CI_S	CI_H	CS_{AAC}	CS_{AAD}
Total amino acids	-0.61	-0.60	-0.64	-0.64	-0.87**	-0.83*	-0.10	-0.96***
Cystine	-0.53	-0.70*	-0.64	-0.74*	-0.44	-0.76		
Methionine	-0.44	-0.49					0.53	
Lysine	-0.65	-0.69						0.998***
Nitrogen	0.39	0.03	-0.13	-0.13				

[1)] See Chapter 2, section 2.3.4.
PDP_M, PDP_L : Pepsin digestibility protein with medium and low pepsin concentrations, respectively.
NNS, NSH : Nitrogen solubility in alkali and acid, respectively.
CI_S, CI_H : Chemical index, based on NSS and NSH, respectively.
CS_{AAC}, CS_{AAD}: Chemical score based on dietary amino acid concentrations and digested amino acids, respectively.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3.5.3 Concluding remarks

The conclusion to be drawn from this investigation is that processing conditions do significantly affect the amino acid digestibility of feather meal. The main negative effect for all amino acids (with the exception of leucine) is that with increasing processing time. Under our experimental conditions, 30 min autoclaving hydrolysis is preferred. However, a study on the effect of shorter (than 30 min) processing periods would be of interest in order to find the optimum processing conditions (under the same steam pressure used in this study). Feather meals treated with sodium hydroxide and enzyme were not substantially different from feather meals treated without additions with respect to amino acid digestibility values.

The use of feather meal is limited because of the deficiency of lysine, histidine and methionine. Our studies suggest that the modified amino acid digestibility values due to processing treatments are also of considerable importance. In particular, the poor digestion of lysine, histidine and methionine, seems to be of interest from the nutritional point of view.

The varied digestibilities shown between individual amino acids are sufficiently extensive to be taken into consideration in the formulation of diets for monogastric animals in general and particularly for poultry, and indicate the advantage of balancing the dietary protein on the basis of digestible amino

acids. The question to be asked is whether apparent or true digestibility values should be taken into account. Endogenous amino acid excretion cannot be determined with certainty because it is affected by the dietary carbohydrate level. Until there is a reliable method for measuring endogenous secretion for a given feedstuff, the apparent digestibility of amino acids is a more practical criterion from the point of view of applied nutrition.

chapter 4

AMINO ACID CONCENTRATIONS IN THE INTESTINAL TRACT OF CHICKS AFTER INGESTION OF DIFFERENTLY PROCESSED FEATHER MEALS AND REFERENCE DIETS

4.1 Introduction

For many years considerable attention has been paid by a number of investigators to the gastrointestinal physiology of different species of animals. The digestion and absorption, however, of dietary protein along the intestinal lumen of chicken has not been extensively studied.

Proteolysis of dietary protein precedes its absorption as tri- or dipeptides or as free amino acids in the small intestine by the intestinal mucosa mainly in the proximal small intestine. The small peptides are further hydrolyzed at the brush border, or within the epithelial cells (Kan, 1975; Auricchio, 1981). The final protein digestion products are made available to the chicken, through the portal circulation.

4.2 Literature

4.2.1. The sites of digestion and absorption of protein in the intestinal lumen of chickens

Imondi and Bird (1965) presented data which indicated that, with diets using chromic oxide as a marker, digestion of dietary protein occurred mostly up to the duodenum, whereas most of the nitrogen absorption took place in the upper jejunum. The findings of Hurwitz et al. (1972) using yttrium-91 as a non-absorbed reference, support the previous reports and also added that both processes continued along the entire small intestine.

The role of the various segments of the intestinal lumen in amino acid absorption has been studied by some workers. Bird (1968) concluded that the small intestine plays a central role in amino acid absorption in chickens. Crompton and Nesheim (1969) studied the concentration of amino acids in the intestinal contents of ducks and suggested that the middle portion appeared to be the principal site of amino acid absorption. Wakita et al. (1970) showed in *in vitro*

studies that the upper part of the jejunum accumulated methionine, lysine and glutamic acid more rapidly than the middle and lower parts of the jejunum.

4.2.2 Amino acid concentration in intestinal contents of chickens in relation to dietary protein

Although the available data on the gastrointestinal physiology of chickens have dealt with the different sites within the small intestine for protein digestion/absorption, there is very little information about the amino acid concentrations in intestinal contents in relation to dietary protein.

Crompton and Nesheim (1969) reported that the amino acid composition of the diets based on soybean and corn gluten meals is related to the pattern of 'free' amino acids in the small intestine of young ducks. The results of Bielorai et al. (1972) on the effect of feeding raw and heated soybean meals on the amino acid levels along the digestive tract of chicks are in general accord with those described previously. Skurray and Cumming (1974) studied the digestion of meat meal protein in the small intestine of chicks and showed that the amounts of amino acids were higher in the intestine of chicks fed meat meal compared with those fed the freeze-dried raw materials used for meat meal production.

Scope of the study

These limited studies give very little information about the amino acid profile along the intestinal tract of chickens from dietary proteins subjected to different processing conditions, while there is apparently no information in this respect for feather meals.

The purpose of the present study was to investigate the relationship of the amino acids from differently processed feather meals to the amino acid distribution along the small intestine of young chicks. Also included in this study was an examination of the ileal amino acid composition in comparison with the excreted amino acids of chicks receiving either feather meals or nitrogen-free diets and diets of crystalline amino acid mixture. Such information should provide a basis for evaluating the validity of using either ileal or excreta samples for apparent and/or true amino acid digestibility determinations.

4.3 Materials and methods

4.3.1 Preparation of the test diets and experimental design

The differently processed feather meals which were prepared for amino acid digestibility determinations (Chapter 3, section 3.3.1) were used also in the present study. These nine test feeds constituted a factorial experiment that included two factors, namely processing time (30, 50 and 70 min) and treatment additions (no additives, added sodium hydroxide and added enzyme).

The composition of the nitrogen-free diet (NFD) and the crystalline amino acid mixture (RSD) used, is given in Appendix 5.

4.3.2 Animal experimental procedure

Housing and management of the chicks

Seventy-eight male broiler chicks were used. Details about their management and performance have been described in Chapter 3, section 3.3.2. As soon as the plasma free amino acid procedure was completed (see Chapter 5), the chicks were returned to the ad libitum feeding program with the commercial broiler starter ration with drinking water always accessible. All the chicks were in good health after the previous experiment.

Feeding phase

The feeding procedure applied in this experiment was based on results obtained in preliminary studies. In order to ensure an adequate and controlled feed intake, the force-feeding technique as previously described in detail (Chapter 3, section 3.3.2) was used. In these trials the test feeds were given as often as possible in order to achieve a uniform distribution of digesta within the gastrointestinal tract and to create conditions for uniform protein digestion/absorption. The distribution of the digesta in relation to the quantity of the intestinal contents collected was also taken into account.

This experiment started four days after blood sampling (Chapter 5) and the established feeding schedule was as follows. The test feeds were given to the chicks (after a fasting period of 24 hrs to empty the digestive tract) in 3 equal feeds of 5 g each, at intervals of 3 hrs except for the N-free diet and amino acid mixture. These diets had to be offered at shorter intervals of 2 hrs, in order to get the digesta well distributed and in adequate quantities

for analytical purposes.

With that procedure it was observed that one hour after the second feeding, the first undigested materials were excreted. Examination of the crop and gizzard showed that feed materials were still present 4-5 hours after the last feeding.

Collection of intestinal contents

Because of the time schedule (equal and fixed times for each chick for fasting period, interval of force feeding and sampling of digesta) it was not possible to conduct the entire experiment at one time. Therefore the total experiment was done in three stages.

Each chick was removed from the cage and sacrificed by an overdose of chloroform 2½ hours after the last feed on the day the samples were required. The abdomen was opened and the small intestine of each chick was stretched out and divided into 2 segments consisting of the jejunum and ileum. The jejunum was taken as that portion from the duodenum (the distal attachment of the pancreas after the point of entry of the bile ducts) to Mackel's diverticulum; the ileum from Mackel's diverticulum to a point 1 cm proximal to the ileo-cecal-colic junction. During the operation care was taken to avoid contamination of the samples with blood and other tissue. Hemostats were used to clamp off each segment to prevent the passage of the contents along the intestine during handling.

The contents of each segment were gently stripped by hand into clean and tared plastic beakers which were then immediately reweighed. The contents were frozen directly by immersing the plastic beakers in a mixture of dry ice and alcohol. Such immediate freezing prevents *in vitro* hydrolysis of proteins and peptides by both pancreatic proteolytic enzymes and intraluminal peptidases (Silk et al., 1976). At this point the collected gut samples were stored at -60°C in the well-capped, labelled plastic beakers and kept in the frozen state until amino acid analysis.

The time between sacrificing and emptying of the segments and weighing the samples was kept to less than 5 min for each bird. The replicates differed only in the time interval between sacrifice of the birds and removal of the small intestine.

4.3.3 Analytical methods

Preparation of intestinal contents for amino acid analysis

The stored intestinal samples were thawed, brought to a volume of 100 ml with

distilled water and weighed. The mixture was then homogenized in a glass beaker with an ultrasone homogenizer for 2 min while the sample was kept on ice. One portion of known weight was immediately assayed for total amino acids, the weight depending on the amino acid content of the sample analyzed.

Amino acid analysis

Individual samples from each treatment were analyzed. Caution is required in interpreting cystine and methionine contents. These may be underestimated because of oxidation during acid hydrolysis. However tentative conclusions may be drawn if one assumes that a constant proportion is destroyed when all samples are treated alike, as was the case in the present study. This was tested in preliminary comparative analyses for the sulfur amino acids of the nine differently processed feather meals used in this study. Details of the amino acid analysis have been given in Chapter 2, section 2.3.4.

4.3.4 Statistical methods

The results of the intestinal amino acid concentrations of the feather meal test groups were analyzed statistically by a multiway analysis of variance. The model used is given in Chapter 3, section 3.3.4. When the interaction processing time x method of treatment (with or without addition) was significant the nested model was used.

4.4 Results

4.4.1 Intestinal amino acids from chicks receiving feather meals

The total amounts of intestinal amino acids for the chicks fed on differently processed feather meals are given in Tables 4.1 (jejunal contents) and 4.2 (ileal contents).

Although the statistical analysis did not show a significant ($P > 0.05$) effect of the processing conditions on the total jejunal amino acids (with the exceptions of cystine and valine, Appendix 6) there were some notable differences between the test groups. As shown in Fig. 4.1 higher levels of essential and non-essential amino acids from the chemically treated feather meals were generally found. Lower levels were observed in the contents from the enzymatically treated samples. A similar trend was also found for the individual amino acids, particularly for

Table 4.1. Concentrations of total amino acids in jejunal contents of chicks fed on various processed feather meals[1), 2)].

Amino acid	Feather meals[3)]									Pooled SE
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$	
Thr	50.8	49.8	46.9	57.4	54.9	60.7	21.2	50.2	53.8	9.89
Val	80.1	83.6	68.5	91.2	107.5	127.5	35.4	119.7	159.5	20.96
Cys	42.3	26.8	47.4	41.4	21.6	54.1	13.3	18.5	42.0	7.61
Met	5.7	5.8	5.0	6.8	5.2	5.3	2.0	5.4	5.1	1.16
Ile	46.9	44.0	35.1	52.3	47.6	48.0	19.8	45.8	46.8	8.94
Leu	71.5	67.7	55.1	78.2	72.2	70.2	29.1	67.4	65.4	13.39
Tyr	31.2	30.5	30.2	34.8	33.6	36.8	14.6	33.1	35.9	6.13
Phe	42.1	39.6	31.4	45.5	41.4	39.7	16.9	39.2	42.0	7.71
Lys	20.4	20.6	22.3	25.5	24.4	27.9	11.2	22.9	28.6	4.35
His	7.8	7.5	9.4	9.8	8.9	9.0	4.1	7.7	10.1	1.56
Arg	62.8	59.5	49.8	69.8	64.7	69.7	25.8	60.6	69.3	12.28
Essential	461.7	435.4	401.1	512.8	482.0	548.8	193.4	470.6	558.5	90.75
Asp	72.4	77.9	80.6	84.5	86.7	100.9	35.8	79.8	89.1	15.15
Ser	106.6	107.9	84.5	117.2	116.3	110.5	44.1	116.2	126.4	20.99
Glu	99.9	105.4	96.7	117.5	113.7	127.1	49.0	109.4	145.6	20.42
Pro	93.5	122.7	85.7	107.5	104.3	119.0	38.7	99.1	98.7	22.03
Gly	62.8	65.5	53.1	71.2	76.1	75.5	29.4	75.7	79.0	12.91
Ala	40.4	40.4	32.2	44.8	46.2	44.6	18.4	46.7	50.0	8.04
Non-Essent.	475.6	519.9	432.8	542.7	543.3	577.5	215.4	526.8	588.9	97.11
Total	937.3	955.3	833.9	1055.5	1025.3	1126.3	408.8	997.4	1147.4	187.43

1)
2) mg present in the digesta.
3) Mean of seven observations.
For feather meal codes in this and all subsequent Tables, see Chapter 3, Table 3.1.

methionine, histidine and lysine (Fig. 4.1), the most limiting ones in feather meal.

Differences in essential and non-essential amino acids in jejunal contents related to the processing time were also noticed. Amino acids in digesta derived from feather meals processed for 30 min or 70 min were at the lower and higher levels, respectively. This trend, however, was not clearly evident for all individual amino acids.

The variations between intestinal amino acid levels derived from the differently processed feather meals were more pronounced in ileal digesta. The statistical evaluation of the data is given in Table 4.3. The analysis of variance showed that the main significant differences in amino acid concentrations were due to the effect of processing time tested within the methods of treatment. The lower amino acid levels were observed in the ileal digesta of chicks receiving feather meals treated for 30 min without additions and with enzyme in

Table 4.2. Concentrations of total amino acids in ileal contents of chicks fed on various processed feather meals[1), 2)].

Amino acids	Feather meals									Pooled SE
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$	
Thr	30.0	44.6	46.2	56.7	41.6	40.2	22.8	43.4	40.2	5.17
Val	31.7	47.3	48.6	68.5	46.3	52.5	27.2	52.9	56.6	7.10
Cys	34.3	26.0	57.7	49.1	26.1	29.9	19.8	19.1	31.5	4.91
Met	3.4	5.2	4.7	6.7	4.4	4.2	2.4	4.0	4.0	0.63
Ile	19.2	29.4	22.9	38.7	24.4	27.9	13.7	29.5	29.6	3.92
Leu	35.6	52.2	47.3	63.5	41.0	45.9	23.7	48.5	45.8	6.04
Tyr	14.1	20.7	19.6	25.4	16.9	18.9	11.7	21.7	20.6	2.60
Phe	18.7	28.3	24.1	34.0	22.0	24.7	12.6	26.5	24.4	3.21
Lys	13.7	18.2	21.7	26.3	17.5	18.6	12.4	20.6	19.9	2.52
His	3.9	5.6	6.3	7.5	5.5	5.4	3.4	6.1	5.7	0.72
Arg	27.9	42.6	35.6	53.7	36.4	39.1	21.2	42.1	38.8	5.09
Essential	232.5	320.1	334.6	430.2	282.2	307.3	170.9	314.3	316.9	39.84
Asp	55.4	79.6	90.2	99.4	69.4	73.7	44.1	78.9	73.3	9.88
Ser	57.6	91.8	81.8	104.7	72.6	77.5	42.0	89.2	76.5	10.10
Glu	64.1	92.3	94.8	116.5	79.6	84.1	51.1	92.7	90.4	11.48
Pro	59.0	84.4	86.3	108.4	70.9	79.8	43.5	79.3	78.0	10.33
Gly	29.9	44.7	44.9	56.7	40.7	45.5	25.7	52.6	47.6	5.94
Ala	18.9	30.2	26.8	37.0	24.9	26.8	15.2	31.8	27.8	3.50
Non-Essent.	284.9	423.0	424.7	522.8	358.1	387.3	221.6	424.5	393.6	50.52
Total	517.4	743.1	759.3	953.0	640.3	694.6	392.5	738.8	710.5	89.92

1) mg present in the digesta.
2) Mean of seven observations.

contrast to samples from feather meals treated for longer periods. The significant differences were more pronounced with the enzymatically treated feather meals. The inverse effect of processing time has been found with ileal amino acids derived from chemically treated feather meals.

Differences in ileal amino acids due to the method of treatment, tested within the processing periods, were significant only in chicks receiving feather meals treated for 30 min. The chemically treated samples gave significantly higher amino acid levels.

Comparisons of the amino acid levels, expressed as mg/g digesta from the two different segments of the intestinal tract are shown in Fig. 4.2. There is, in general, a decrease in amino acids from jejunum to ileum. These differences were more pronounced in digesta derived from feather meals processed at the shorter time of 30 min. That these differences were not simply due to changes in water content is ruled out by the findings (in the preliminary trials) that there was no essential difference in dry matter of the digesta from chicks fed

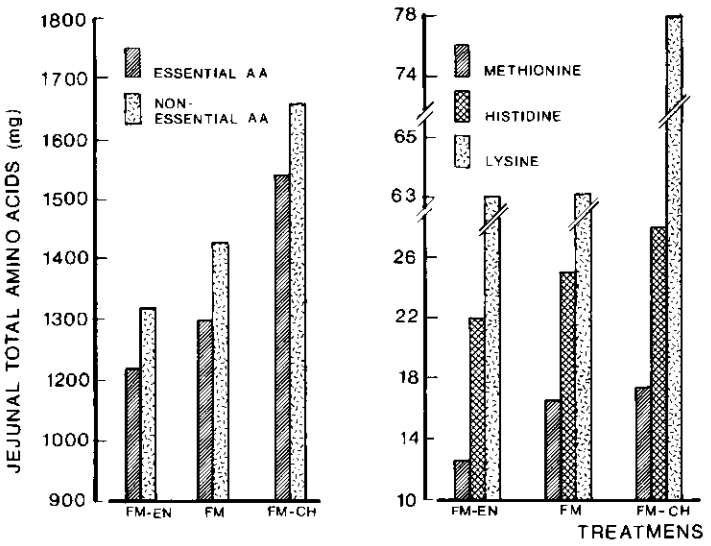


Fig. 4.1. The effect of different methods of processing feather meals on total amino acids present in the jejunal contents. FM-EN, FM and FM-CH are feather meals treated with enzyme, with no additions and with sodium hydroxide respectively.
Each column represents the grand mean of the three processing times (30, 50 and 70) of the corresponding method of treatments. There was only one time x treatment interaction for cystine.

the various test feather meals. On average the ileal contents were 2-3% drier than those of the jejunum.

4.4.2 Intestinal amino acids from chicks receiving reference diets

The total amino acids in jejunal and ileal contents of chicks fed on a nitrogen-free diet (NFD) and a diet of crystalline amino acids (RSD) are given in Table 4.4.

The assumption is that amino acids from the NFD fed chicks reflect the endogenous secretions. The amino acids secreted in the greatest amounts in the jejunum were valine, glutamic acid, aspartic acid, serine and leucine, in order of decreasing abundance, while the amino acids secreted in the lowest quantities were methionine, histidine and proline. The amino acids in ileal contents were lower than those of jejunal contents, but their composition was not comparable for all individual amino acids (Table 4.5). This was the case for methionine, leucine, tyrosine, histidine and proline.

The characteristics described for the NFD fed chicks were also observed for the RSD fed chicks.

Table 4.3. Multiple regression for ileal total amino acids on processing time (T) x method of treatment (P) effects.

Predicted responses ($\hat{Y}$)	Regression equation[1]: $\hat{Y} = b_0 + C_P + C_{TP_1} + C_{TP_2} + C_{TP_3}$								
		C_P		C_{TP_1}		C_{TP_2}		C_{TP_3}	
	b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8
Thr	40.6	-4.8	5.9	16.1*	14.6*	-16.5*	-15.1*	17.4**	20.6**
Val	48.0	3.0	13.2*	16.8*	15.6	-16.0**	-22.2**	29.4**	25.6*
Cys	32.6	-15.9***	-4.3	23.4***	-8.3	-19.2**	-23.0*	11.8	0.7
Met	4.3	-1.0	0.7	1.3	1.7	- 2.5	- 2.2	1.5**	1.6**
Ile	26.1	0.4	6.5*	3.8	10.3	-10.8*	-14.3*	15.9*	15.8*
Leu	44.8	-5.7	5.1	11.7	16.6	-17.6	-22.5*	22.1*	24.8**
Tyr	18.8	-0.1	2.3	5.4	6.5*	- 6.6*	- 8.5**	8.9*	10.0**
Phe	23.9	-2.5	3.2	5.4*	9.6*	- 9.4*	-12.1*	11.8*	13.9*
Lys	18.8	-0.3	2.9	8.1*	4.5	- 7.7*	- 8.8*	7.5*	8.2**
His	5.5	-0.2	0.9	2.4*	1.6*	- 2.0*	- 2.0*	2.3*	2.7**
Arg	37.5	-1.4	7.7	7.7	14.7*	-14.6*	-17.3*	17.6*	20.9**
Asp	73.8	-9.6	5.8	34.8*	24.2*	-25.8	-30.1*	29.2*	34.8**
Ser	77.1	-7.8	7.9	24.2	34.3*	-27.3	-32.2*	34.5*	47.2*
Glu	85.1	-5.6	9.7	30.7	28.2	-32.4	-36.9*	39.1*	41.6*
Pro	76.6	-9.6	9.8	27.3	25.4	-28.6	-37.5*	34.5*	35.9**
Gly	43.2	2.1	7.8	15.0	14.7*	-11.2*	-16.0*	21.9*	26.9***
Ala	26.6	-0.4	4.3	7.9	11.3*	-10.2*	-12.0*	12.6*	16.6*

[1] In the equations $\hat{Y}$ is the predicted response, b_0 is the intercept and C's are contrasts.

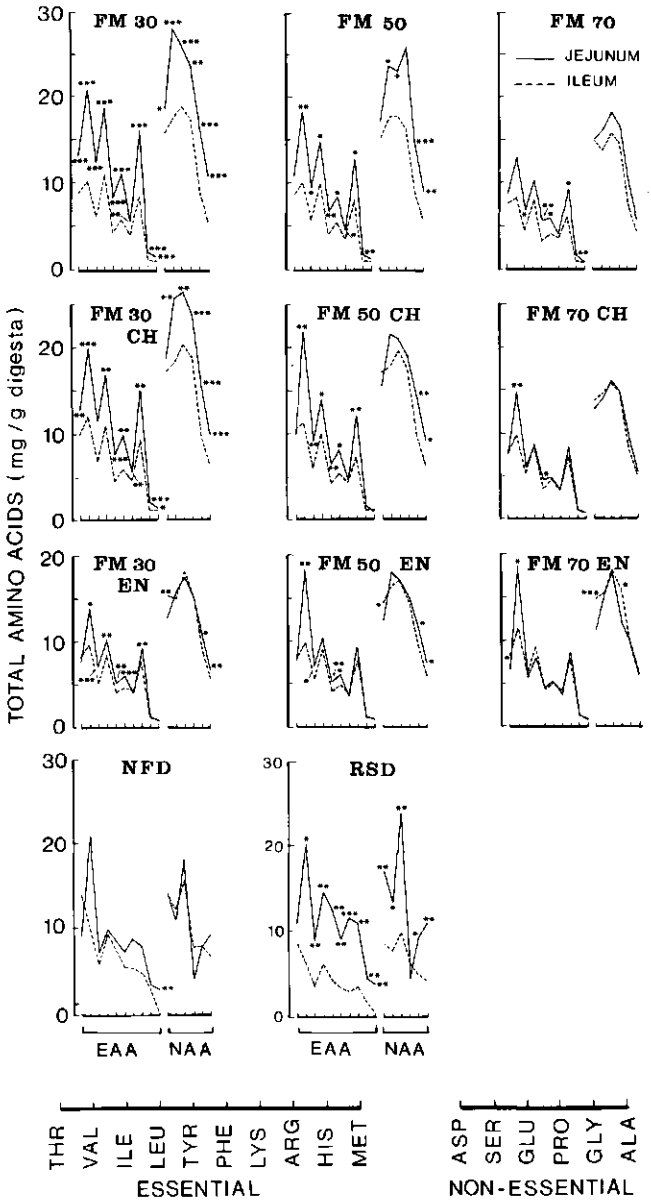
C_P : Contrast of method of treatment (P): NA (no additions); CH (added chemical); EN (added enzyme).
b_1: EN-NA
b_2: CH-NA

$C_{TP_{1,2,3}}$: Contrasts of processing time (T) within the methods of treatment (P)
C_{TP_1} : T within NA — b_3: $T_{70}-T_{30}$; b_4: $T_{50}-T_{30}$
C_{TP_2} : T within CH — b_5: $T_{70}-T_{30}$; b_6: $T_{50}-T_{30}$
C_{TP_3} : T within EN — b_7: $T_{70}-T_{30}$; b_8: $T_{50}-T_{30}$

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

4.4.3 Ileal and excreta amino acid compositions

A comparison between ileal and excreta (Chapter 3, Table 3.4) amino acid compositions of chicks receiving feather meal treated without addition for 30 min is shown in Fig. 4.3. Comparisons within all other test groups of chicks receiving feather meals showed the same clear picture (Appendix 7). These comparisons showed very close similarities. In all of the cases we found highly significant correlation



F.g. 4.2. Comparison of jejunal and ileal total amino acid concentrations of chicks fed the feather meals and reference diets (EAA and NAA: Essential and non-essential amino acids, respectively).
For feather meal codes, see Table 3.1.
Differences between individual amino acids in jejunal and ileal contents significant at ($P < 0.05$), ($P < 0.01$) or ($P < 0.001$) are denoted by *, ** or ***, respectively.

Table 4.4. Concentrations of total amino acids in jejunal and ileal contents of chicks fed on nitrogen-free diet (NFD) and crystalline amino acid mixture (RSD)[1].

Amino acid	NFD		RSD	
	Jejunum	Ileum	Jejunum	Ileum
Thr	3.6 ± 0.48[2]	1.8 ± 0.62	7.0 ± 1.26	3.8 ± 0.42
Val	8.1 ± 2.08	1.3 ± 0.36	13.4 ± 4.30	2.7 ± 0.25
Cys	1.1 ± 0.16	1.0 ± 0.56	4.7 ± 0.77	2.5 ± 0.79
Met	1.2 ± 0.23	0.1 ± 0.02	2.4 ± 0.39	0.3 ± 0.08
Ile	2.7 ± 0.38	0.7 ± 0.19	5.8 ± 0.99	1.5 ± 0.13
Leu	3.7 ± 0.64	0.8 ± 0.18	9.5 ± 1.61	2.6 ± 0.26
Tyr	3.3 ± 0.45	0.9 ± 0.21	7.6 ± 1.46	1.8 ± 0.14
Phe	2.8 ± 0.40	0.7 ± 0.18	5.7 ± 0.95	1.5 ± 0.14
Lys	3.5 ± 0.58	0.6 ± 0.15	7.5 ± 1.40	1.3 ± 0.14
His	1.4 ± 0.21	0.4 ± 0.10	2.9 ± 0.49	0.8 ± 0.08
Arg	3.1 ± 0.51	0.6 ± 0.19	7.0 ± 1.24	1.5 ± 0.17
Essential	34.5 ± 7.09	8.9 ± 2.54	73.5 ± 13.67	20.2 ± 2.44
Asp	5.6 ± 0.82	1.7 ± 0.49	10.9 ± 1.92	3.7 ± 0.36
Ser	4.3 ± 0.46	1.6 ± 0.50	8.3 ± 1.38	3.3 ± 0.32
Glu	7.1 ± 1.08	1.9 ± 0.52	15.1 ± 2.74	4.2 ± 0.35
Pro	1.4 ± 0.29	0.9 ± 0.21	2.7 ± 0.77	2.7 ± 0.26
Gly	3.1 ± 0.40	1.0 ± 0.28	6.1 ± 1.21	2.1 ± 0.19
Ala	3.6 ± 0.66	0.8 ± 0.22	6.9 ± 1.38	1.8 ± 0.16
Non-Essential	25.1 ± 4.22	8.0 ± 2.17	50.0 ± 17.49	17.7 ± 1.60
Total	59.6 ± 11.28	16.9 ± 4.70	123.5 ± 31.05	37.9 ± 4.01

1) mg present in the digesta
2) Mean and standard errors in eight observations, except in RSD-ileum where seven samples were involved.

coefficients ($r > 0.95$, $P < 0.001$) between ileal and excreta amino acid compositions. The sequence of the relationships between individual essential and non-essential amino acids of the different groups is, in general, similar. The amino acids in the greatest amounts were aspartic acid, glutamic acid and serine, while the amino acids in the smallest quantities were methionine, histidine and tyrosine. Cystine was the only amino acid which in some cases was present in different proportions in ileal and excreta samples. A similar statistical evaluation for chicks receiving NFD showed a significant correlation between ileal and excreta amino acid ($r = 0.91$, $P < 0.001$, Fig. 4.3).

4.5 Discussion

4.5.1 Intestinal amino acids from dietary feather meals

The experiment was conducted under *in vivo* conditions on the assumption that

Table 4.5. Mean proportion of individual amino acids, as percent of the sum of the amino acids, in the digesta passing through the jejunum and ileum of chicks fed on nitrogen-free diet (NFD) and crystalline amino acid mixture (RSD).

Amino acid	NFD		RSD	
	Jejunum	Ileum	Jejunum	Ileum
Essential				
Thr	6.15	10.65	5.89	10.03
Val	13.85	7.69	11.28	7.12
Cys	1.88	5.92	3.96	6.60
Met	2.05	0.59	2.02	0.79
Ile	4.62	4.14	4.88	3.96
Leu	6.32	4.73	8.00	6.86
Tyr	5.64	5.33	6.40	4.75
Phe	4.79	4.14	4.80	3.96
Lys	5.98	3.55	6.31	3.43
His	2.39	2.37	2.44	2.11
Arg	5.30	3.55	5.89	3.96
Non-Essential				
Asp	9.57	10.06	9.18	9.76
Ser	7.35	9.47	6.99	8.71
Glu	12.14	11.24	12.71	11.08
Pro	2.39	5.33	2.27	7.12
Gly	5.30	5.92	5.13	5.54
Ala	6.15	4.73	5.81	4.75

anaesthesia and manipulation during the removal of the small intestine and sample collection did not affect the composition of the jejunal and ileal contents. It has been reported by Badawy (1964) that extensive shedding of the mucosa in the gut of sheep may occur. However, there is no experimental proof with poultry to enable an estimate of the extent of such mucosal shedding to be made. Besides that, in this work the intestinal sections were removed and emptied into a sample beaker in less than 5 min. Consequently the time was very short for mucosal shedding to occur.

The amino acid levels in jejunal contents of chicks at 2½ hrs after last feeding were higher when chemically treated feather meals were fed, compared with feather meals treated without additions or with samples from enzymatic treatments. This may be the combined effect of poorer digestibility and delayed digestive release of most of the constituent amino acids under these experimental conditions. The possibility that it may also be a result of different transit times remains to be studied. However, it should be noted that during the preceding feeding period there were no apparent differences in total dietary protein or amino acid intakes with the exception of cystine.

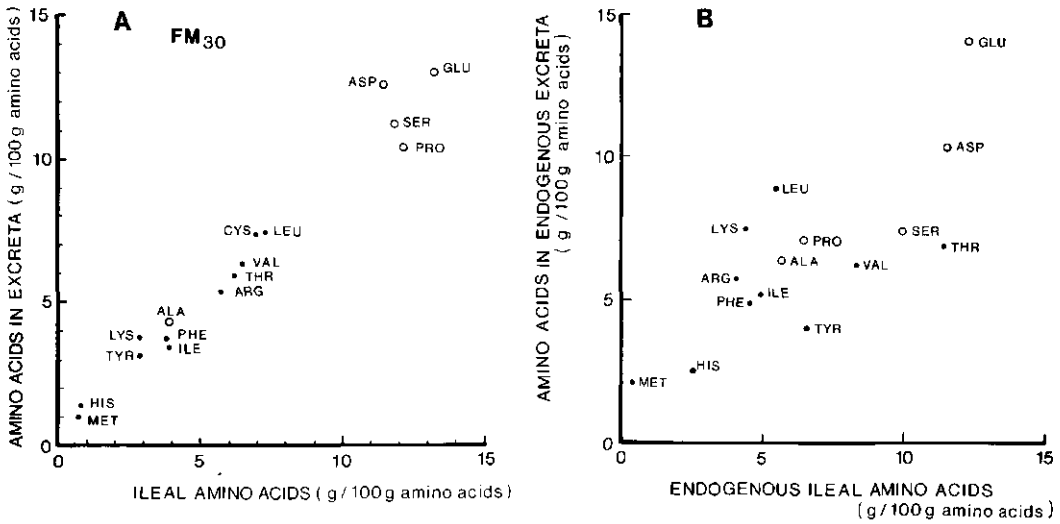


Fig. 4.3. Relationship between the proportions of amino acids in ileal contents and excreta of chicks fed on feather meal processed for 30 min (FM_{30}: A) and nitrogen-free diet (NFD: B).

The bulk of ingested protein appears to be absorbed in the jejunum but small amounts reach the ileum and are absorbed there (Matthews and Adibi, 1976; Bielorai et al., 1977). Differences in total amino acids in the ileum from dietary feather meals may be accounted for by differences in the digestibility of the dietary proteins. The digestibility of the various test feather meals was negatively influenced by increasing the processing time (Chapter 3), therefore amino acids derived from processes of short duration (30 min) appear at lower levels at the ileum. This was the case in the ileal samples of chicks receiving feather meals treated without additions and feather meals treated with enzymes. The opposite trend was found in amino acid levels from chemically treated feather meals obtained ileal digesta. This cannot be explained with certainty from this study. It may be due to the different behaviour during the digestion and absorption processes of these feather meals.

In this experiment, changes in amino acids digesta were determined only in the entire jejunum and ileum. Therefore, the possibility exists that there may be variations in the level of amino acids in the upper and lower parts of the two segments tested. The relationship of amino acid levels in ileal contents derived from feather meals treated with or without enzymes helps to eliminate this possibility. For the chemically treated feather meals, amino acid determinations in

the upper and lower ileum may provide more relevant information.

The higher levels in the jejunum than in the ileum of total amino acid concentrations as found in this study (Fig. 4.2) are in line with previous results. Crompton and Nesheim (1969) reported that the largest quantities of amino acids were found in the middle segment of the intestine of young ducks fed soybean and corn gluten diets. Nitsan and Liener (1976) showed smaller amounts of amino acids in the ileum than in the jejunum of soybean fed rats. The observed differences between individual amino acid concentrations in jejunum and ileum could be attributed to their different rates of absorption from the intestinal segments (Yokota and Tasaki, 1970; Bielorai et al., 1977).

4.5.2 Endogenous digesta

Nitrogenous compounds are secreted along the whole digestive tract of the chicken. The main sources of secreted protein and amino acids are the pancreas, the mucosal glands and desquamated mucosal cells. Although the composition of secreta has been extensively studied in other species, there is very limited information on the quantitative and qualitative aspects of the endogenous nitrogen secretion in the different intestinal segments of poultry.

The composition of endogenous intestinal digesta in this work (Table 4.5) as determined on a nitrogen-free diet is, in general, in agreement with previous estimates made on young ducks (Crompton and Nesheim, 1969) and adult roosters (Parsons et al., 1983). In particular the high contents of valine, leucine, threonine, aspartic acid, serine and glutamic acid found, and the low contents of methionine, tyrosine, phenylalanine and arginine in our study are in agreement with the results reported by these authors. However, alanine was one of the most abundant amino acids in the endogenous protein of adult roosters in contrast with its low value for chicks in the present study. Furthermore, the proportion of threonine in endogenous digesta was lower in the work of Parsons et al. (1983) than in our study. These observations suggest that the secretion of some amino acids by young chicks may vary from that of adult roosters.

In our work, some dissimilarity was observed in the amino acid composition of digesta collected from the jejunum and ileum of chicks fed a nitrogen-free diet. During passage of the digesta there is continuous digestion and absorption of endogenous compounds as well as secretion of the intestinal juice. These processes cause changes in digesta composition along the intestine.

High levels of aspartic acid and glutamic acid and low levels of methionine, histidine and phenylalanine were also observed in the ileal digesta of pigs

(Wünsche et al., 1979; Buraczewski, 1980). However, we found lower contents of proline and glycine and higher content of serine in comparison with those of pigs. Thus, amino acid composition of endogenous protein in the ileum of poultry appears to differ from that of pigs.

The most abundant amino acids found by us, with the exception of leucine and valine, were those that form a major fraction of mucoproteins (Pigman, 1963; Horowitz, 1967). Mucins are secreted in large amounts by the epithelium of the gastrointestinal tract, but they have been found to be largely resistant to proteolytic enzymes (Hashimoto et al., 1963). In addition, Zebrowska et al. (1976) and Gebhardt et al. (1978) have demonstrated that the digestion of endogenous protein is less complete than that of dietary proteins. It is generally believed that digestion and absorption of amino acids are completed in the ileum for single stomached animals. Thus, it appears likely that endogenous secretions with a high proportion of mucins could account for the accumulation of some amino acids in the ileal contents.

Another feature of the present experiment was the dissimilarity in the proportion of some individual amino acids in ileal contents of birds fed NFD and RSD (Table 4.5). On the assumption that the absorption of crystalline amino acid mixture of RSD fed birds is complete in the ileum, the amino acids of ileal digesta would represent endogenous secretions.

The different amounts and composition of endogenous amino acids which are observed in the ileum of the NFD fed birds and in the ileum of RSD fed birds (Tables 4.4 and 4.5) were probably associated with the different composition of the two diets. Wiseman (1974) concluded that the amount of endogenous secretions in small mammals varies with the type of meal given. In addition, the marked differences between our results (Chapter 3) for the total amino acids in endogenous excreta from chicks fed NFD based on glucose and those of Muztar and Slinger (1980b) with NFD based on cerelose, as well as the findings of Parsons et al. (1983) with two different N-free diets of low- and high-fiber content, are in agreement with our suggestions. If this is true, it is important in the consideration of the true amino acid digestibility determinations with ileal contents, where a correction is applied for endogenous amino acids derived from feeding a certain NFD. For such corrections, it is assumed that the endogenous ileal amino acids of the protein fed chicks are similar to those of NFD-fed chicks, but there is no evidence in the literature to confirm this assumtion. Thus the carbohydrate composition of the test diets should be as similar as is practical to that of the NFD to avoid under- or over-estimation of true amino acid digestibility values.

4.5.3 Ileal and excreta amino acid compositions

The magnitude of the effect of microbial action on protein degradation in the large intestine of chickens has been questioned in some studies (see review, Chapter 3, section 3.2.1) because such an effect may influence the amino acid composition of proteins in digesta and thereby their digestibility coefficients as determined by fecal analysis.

The similarity in the composition of the amino acids in the feather meals of the ileal digesta and excreta found in this study indicates little microbial influence on amino acid excretion. Therefore our results suggest that fecal sampling for amino acid digestibility determinations should not be substantially different from the results obtained by ileal analysis applied for the same purpose. This is further supported by the similarity in the amino acid composition of ileal digesta and excreta from chicks fed on NFD. These results are in agreement with the findings of Parsons et al. (1983) in which amino acid excreta from roosters fed nitrogen-free diets were similar to those of digesta from surgically-modified roosters. In contrast the amino acid composition of endogenous ileal protein was considerably different from that of endogenous fecal protein from rats and pigs in which bacterial action influences fecal protein excretions (Mason and Palmer, 1973; Sauer et al., 1977; Taverner et al., 1981). Thus the amino acid composition of endogenous protein secretions of chickens appears to be less affected by microbial fermentation than that of other species.

4.5.4 Concluding remarks

In conclusion, the results of the present experiment suggest that: a) with one exception the jejunal and ileal amino acid distribution reflects the relative digestibility of dietary feather meals as affected by different processing conditions. The notable exception was that of the ileal contents derived from chemically treated feather meals; b) the composition of a nitrogen-free diet may influence the endogenous amino acid pattern in ileal digesta and consequently may result in an inaccurate estimate in true amino acid digestibility trials; c) ileal or fecal analysis may not give very different results for apparent amino acid digestibility determinations of the differently processed feather meals.

It should be noticed, in general, that amino acid determinations in the upper and lower ileum may provide more information about the relative digestibility of amino acids from differently processed feather meals. Furthermore, more work is

needed in order to establish definite data about the endogenous amino acids derived from nitrogen-free diets of different composition, for true digestibility determinations. In order to prove the validity of the very close similarity of ileal and excreta amino acid composition found in this work, comparative studies of ileal and fecal amino acid digestibility may be needed.

chapter 5

CHANGES IN THE PLASMA AMINO ACID LEVELS OF CHICKS AFTER INGESTION OF DIFFERENTLY PROCESSED FEATHER MEALS AND REFERENCE DIETS

5.1 Introduction

It is generally accepted that the final products of protein digestion/absorption, free amino acids and trace amounts of small peptides (which are unusually resistant to hydrolysis in intestinal mucosa), can be detected in blood plasma. Because these products are transported by the blood to supply the nutritional requirements of the tissues, information on amino acid nutrition and metabolism can be obtained by measuring free amino acid concentrations, particularly of the essential amino acids, in tissues and plasma.

Free amino acids are found in all fluids and tissues of the body. The free amino acid pool of the blood plasma is influenced not only by the amino acids absorbed from the intestine but also by the metabolic state of the cells of the body. Munro (1970) concluded that only about 0.5% of the total amount of amino acids in the body of the animal is present as free amino acid. The plasma contains a very small proportion of the total free amino acid pool. However, because of its accessibility, plasma is frequently sampled for free amino acid determination.

5.2 Literature

5.2.1 Free amino acid concentrations in blood plasma in relation to dietary protein

The amino acid content of the blood rises after a protein meal (Charkey et al., 1953; Richardson et al., 1953; Frame, 1958). Therefore, attempts have been made by a number of investigators to study the relationship of dietary amino acid levels to the concentrations of free amino acids in the blood plasma. It has been shown that the free amino acid levels in the plasma of dogs (Longenecker and Hause, 1959) and pigs (Puchal et al., 1962) reflected the composition of the dietary protein. However, this was not confirmed by the work of Frame (1958)

and Yearick and Nadeau (1967) on human subjects.

The influence of the nature and quality of the dietary proteins on plasma amino acid levels has been studied by several workers. Smith and Scott (1965a) showed that fish meal, autoclaved for 2 hrs, increased plasma amino acid levels in chicks, whereas over-processing (12 hours) reduced them. Chicks fed raw and over-heated soybean meal (autoclaved for 4 hours at 121°C) showed lower plasma amino acid concentrations, compared with chicks fed properly heated meal (Smith and Scott, 1965b). A decrease in plasma lysine of chicks fed soybean meal autoclaved for 1 hr compared with control chicks fed commercial soybean meal, was noticed by Hill and Olsen (1967), who also observed that further heating resulted in a continued decrease in the plasma lysine. Bielorai et al. (1972), reported higher free amino acid levels, mainly of the essential ones, in chicks fed commercially processed soybean meal in comparison with those fed raw soybean meal.

These results indicate that free amino acids in plasma may provide some information about the relative digestibility of dietary protein. However, in these studies, heated protein sources were compared with untreated (control) samples. Therefore knowledge of the variations in the treatments is needed in order to evaluate adequately the effect of different treatments of the protein on plasma amino acids.

Some workers have used the free amino acid concentrations in blood plasma of chicks to detect deficiencies and excesses of dietary amino acids. Dean and Scott (1966) concluded that each deficiency or excess could be detected in plasma since the limiting amino acids decreased and the excess amino acids increased. McLaughlan and Morrison (1968) showed the same trend in their work with rats. Ueda and Tasaki (1977) reported that a marked elevation of plasma methionine occurred in chicks fed on diets with excess methionine. In other studies the limiting amino acids in different feedstuffs were determined from the plasma free amino acid levels in chicks (Skurray and Cumming, 1974) and growing turkey poults (Dunkelgod and Winkleman, 1982).

5.2.2 Use of reference diets to relate plasma amino acid concentrations with those in ingested proteins

A number of workers have used the fasting period and/or the feeding of a non-protein diet to study plasma amino acid titers of the test proteins (Charkey et al., 1953; Hill and Olsen, 1963; Zimmerman and Scott, 1967; Boomgaardt and McDonald, 1969; Marrs et al., 1975; Vaughan et al., 1977). However, starvation

increased plasma lysine and threonine, as a result of their resistance to deamination, while protein-free diets resulted in lower concentrations of all amino acids than in starved animals. This may be due to increased insulin secretion and its influence on the movement of amino acids from plasma to muscles (Munro, 1970).

Several attempts have been made to compare the plasma amino acid titers of chicks by feeding a reference protein, such as casein or a diet containing a complete mixture of amino acids in crystalline form (Smith and Scott, 1965a; Skurray and Cumming, 1974; Nasset and Ju, 1975; Chi, 1982). However, casein may not be as standard a product in its nutritional quality as is commonly assumed, and a diet of mixed amino acids may influence plasma amino acid levels due to amino acid imbalance. Although none of the recommended reference diets has been standardized to date, they could be useful as an indicator of protein evaluation by plasma amino acid response if a standard reference could be agreed.

Scope of the study

While most of the data support the conclusion that plasma free amino acids are influenced by the dietary amino acid pattern, systematic studies on the effect of different processing conditions of dietary protein on the plasma free amino acid concentrations in chicks have not yet been reported. Specifically there is an apparent lack of information on the relation of dietary feather meal to plasma amino acid concentrations.

The present study was conducted to establish the relationship of the amino acids provided by differently processed feather meals with the plasma free amino acids of chicks. This experiment also attempted to determine if the plasma amino acid levels could be used to define the limiting amino acids in the various feather meals associated with particular treatments.

5.3 Materials and methods

5.3.1 Preparation of the test diets and experimental design

The nine differently processed feather meals and the reference diets (NFD and RSD) which were prepared for the intestinal amino acid studies (Chapter 4, section 4.3.1) as well as casein were used in the present investigation.

The basic experimental design of this study has been described in Chapter 4, section 4.3.1. In addition, a group of starved chicks was used.

5.3.2 Animal experimental procedure

Housing and management of the chicks

Chicks which had previously been used for amino acid digestibility studies, as well as reserved chicks, and kept under the housing and management conditions described in Chapter 3, section 3.3.2 were also used for the present experiment. The chicks were allowed an adjustment period of four days after excreta collections for amino acid excretion determinations. During the new pre-experimental period the broiler starter diet was supplied ad libitum. The chicks also had free access to water.

Each of the nine test feather meal groups consisted of seven chicks. The remaining chicks were divided into four groups each of nine chicks with the exception of the NFD group which consisted of six chicks.

Feeding phase

All chicks were equilibrated to one feeding pattern. This assured an equal feed intake of all diets under study between experimental groups, prior to the time that blood was taken. The feeding procedure attempted to remove individual variations in plasma free amino acids, due to the metabolic state of individual birds.

On the 33rd day of age the chicks were fasted for 24 hours to empty their alimentary canal of solid matter. At the end of the fasting period each chick in each treatment was force-fed with the appropriate experimental diet in order to ensure an adequate and controlled intake as well as to prevent loss of feed. The force-feeding technique has been described in detail in Chapter 3 (section 3.3.2). In an effort to reach a uniform absorption of amino acids, as far as possible, the test diet was given at 3 hour intervals in 3 equal feeds of 5 g each.

The amount of feed given was based upon preliminary trials which showed that it was approximately the maximum that chicks would consume when fed in that way and still have a reasonably uniform distribution of the passage of feed. The time between ingestion and the excretion of the first residue (appearance time) related to the presence of feed materials in the crop, gizzard and intestine was recorded. Our observations indicated that the force-fed diet was used: no feed remained in the crop 4-5 hours after the third force-feeding nor was there any loss due to regurgitation; while the gizaard still contained some feed materials.

Blood sampling for amino acid estimation

In an attempt to minimize the effect of the time-factor upon the level of plasma amino acids, blood samples were taken at one fixed time after the final feeding. This was at 1 h after the third force-feeding and was based on results obtained in a preliminary experiment designed to determine the optimum conditions relating to the concentration peak of plasma amino acids.

Each chick was restrained by being placed on its back in a holding device. Approximately 5 ml blood were withdrawn from the wing vein of each chick, without anaesthesia, using a syringe equipped with a half-inch, 20 gauge (0.9 x 40 mm) needle. The syringe was previously washed with a heparinized (0.6 mg/ml) 0.93% NaCl solution. The whole blood sample was immediately transferred to a centrifuge tube, kept in a refrigerator at 4°C for a short period (ca 2 h) until all the chicks had been sampled. The samples were then centrifuged directly for 15 min at 3000 rpm (approximately 1200 x g), to separate the blood plasma from the cells. The separated plasma samples were then frozen and stored at -60°C in well-capped, labelled polyethylene bottles and kept in the frozen state until amino acid analyses were performed on the deproteinized supernatants from the plasma.

Time of blood sampling (Preliminary experiment)

This experiment was designed to find the blood sampling time after the last force-feeding, at which the concentration peak of plasma free amino acid (PFAA) was reached.

The chicks were kept under housing and management conditions as described earlier and divided into three lots of 8 chicks each. The feeding phase and force-feeding technique were as described previously. The test feather meal was a mixture of the samples used in section 5.3.1 and was given to a group of chicks at three hourly feeding intervals. The RSD was given to two groups of chicks at two (RSD-2) and three (RSD-3) hourly feeding intervals, in order to study the variations in PFAA between these groups.

Blood samples from the wing vein of each chick of each group, were taken after the last force-feeding at regular intervals of ½, 1, 1½ and 2 hours, respectively. The relative concentrations of the total essential (TEAA), total non-essential (TNAA) and the total amino acids (TAA) in plasma measured after force-feeding are plotted in Fig. 5.1.

The concentrations of PFAA were higher 1 h after the last feeding, in the case of feather meal fed chicks. In the case of RSD-2 fed chicks, the PFAA in

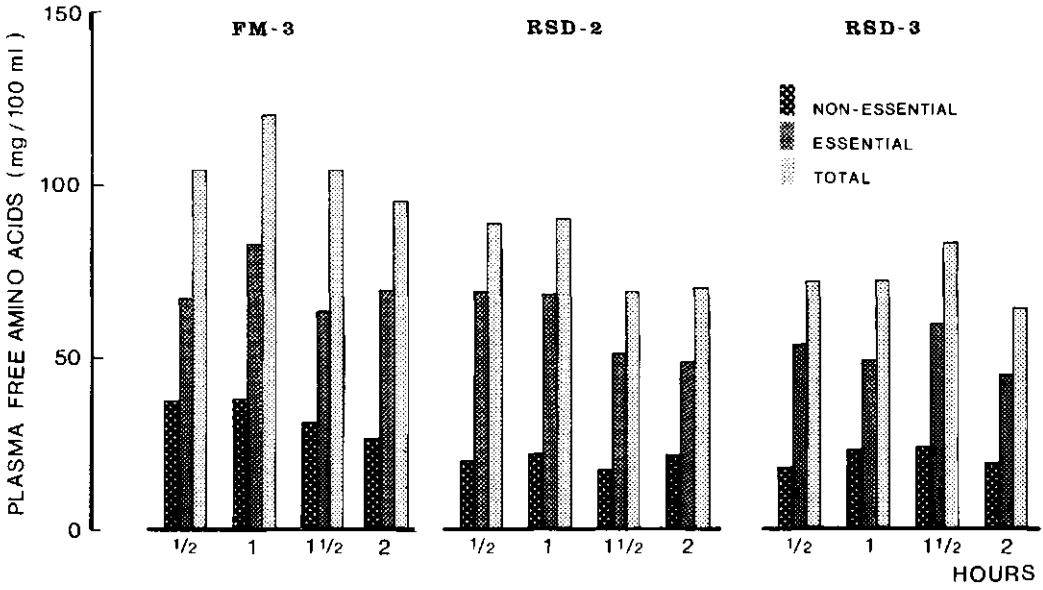


Fig. 5.1. The concentration of amino acids in blood plasma of chicks after feeding feather meal and RSD, as affected by sampling time after the last force-feeding.
FM-3, RSD-3: force-feeding intervals of 3 hours; RSD-2: force-feeding intervals of 2 hours.

½ and 1 h after last feeding were similar and higher than those at 1½ and 2 h. The RSD-3 group showed maximum concentrations of PFAA at 1½ h after the last feeding. This was due to higher levels of threonine, lysine and valine which were reflected in the TAA. The differences in PFAA in the ½ and 1 h blood samples were negligible.

Based on this experiment, one hour after the last force-feeding was chosen as the blood sampling time for all groups in the plasma amino acid studies described here, even though the PFAA showed some differences between chicks on the RSD at 2 and 3 hour feeding intervals. This was considered necessary in order to keep the same experimental conditions.

5.3.3 Analytical Methods

Preparation of Plasma samples for amino acid analysis

At the end of the feeding experiments, the plasma samples were thawed and the plasma proteins precipitated with sulfosalicylic acid (SSA). To 1 volume of plasma, 1 volume of 12.5% SSA with 0.5 μmoles/ml nor-leucine internal standard

solution, pH 2.2, were added, in order to correct for losses during sample preparation and analysis. These samples were shaken and left for approximately 10 min followed by 15-min centrifugation at 3000 rpm of the precipitated proteins. The supernatant fluid was used directly for analysis without further treatment.

Amino acid analysis

150 µl of deproteinized plasma were placed on the column of an automatic amino acid analyzer (Biotronic LC 6000) and the amino acids were assayed quantitatively by ion-exchange chromatography as described by Moore et al. (1958). Individual samples of blood plasma in each treatment were analyzed.

Feather meal samples were analyzed in duplicate. Details were described previously in Chapter 2, section 2.3.4.

5.3.4 Statistical methods

The results of the PFAA analyses from the feather meal test groups were analyzed statistically by a multiway analysis of variance (Steel and Torrie, 1980). The model used for this analysis is given in Chapter 3 (section 3.3.4). Comparisons between differences, where necessary, were carried out by a multiple range test (Duncan, 1955). Correlations were determined between dietary and plasma free amino acids.

5.4 Results

The amino acid composition of the test feather meals is given in Chapter 3, Table 3.2, and the amino acid composition of both the RSD and casein is presented in Appendix 5.

5.4.1 Free amino acids in plasma

Processed feather meals

The average free amino acid concentration, expressed as mg/100 ml blood plasma in the experimental groups fed the test feather meals, is given in Table 5.1. These values are presented graphically in Figure 5.2. The analysis of variance of the data is shown in Table 5.2, where the components which did not show any significant effect are omitted to facilitate the presentation. The time effect

Table 5.1. Concentrations (mg/100 ml) of free amino acids in blood plasma of chicks after feeding the various processed feather meals[1].

Amino acid	Feather meals									Pooled SE
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$	
Thr	15.47^{c}	12.30^{a}	13.67^{abc}	13.33^{abc}	11.54^{a}	11.70^{a}	13.10^{ab}	13.50^{abc}	14.82^{bc}	0.73
Val	$13,67^{bc}$	12.74^{ab}	12.77^{ab}	13.70^{bc}	12.04^{ab}	14.77^{c}	12.32^{ab}	11.47^{a}	15.07^{c}	0.60
Cys	3.64^{d}	2.57^{bc}	1.56^{a}	2.39^{b}	1.58^{a}	1.17^{a}	3.02^{c}	2.34^{b}	1.38^{a}	0.19
Met	1.00^{b}	0.77^{ab}	0.67^{a}	1.01^{b}	0.69^{ab}	0.76^{ab}	0.67^{a}	0.67^{a}	0.64^{a}	0.10
Ile	8.55	7.79	7.14	8.19	6.92	7.83	7.07	6.99	7.22	0.52
Leu	9.24^{d}	8.37^{bcd}	8.45^{abc}	8.76^{ab}	7.55^{a}	9.25^{ab}	7.96^{cd}	7.99^{bcd}	8.70^{ab}	0.56
Tyr	5.16^{c}	4.20^{abc}	3.97^{abc}	3.71^{bc}	3.10^{a}	3.23^{bc}	4.88^{abc}	4.20^{ab}	3.16^{a}	0.34
Phe	2.98^{b}	2.61^{ab}	2.56^{a}	2.80^{ab}	2.18^{ab}	2.72^{ab}	2.52^{ab}	2.41^{ab}	2.20^{ab}	0.17
Lys	8.09^{b}	7.29^{ab}	4.50^{a}	7.57^{ab}	7.92^{ab}	4.88^{ab}	6.68^{ab}	6.25^{ab}	4.75^{ab}	1.07
His	0.85	0.90	0.91	0.93	0.85	0.98	0.71	0.85	0.76	0.11
Arg	13.77	13.14	13.37	13.16	9.82	12.80	12.85	12.10	13.02	0.96
Essential	82.42^{bc}	72.68^{ab}	69.57^{ab}	75.55^{c}	64.19^{a}	70.09^{a}	71.78^{c}	68.77^{a}	71.72^{a}	3.54
Asp	1.38^{cd}	1.00^{ab}	0.98^{abcd}	1.42^{bcd}	0.80^{a}	0.67^{a}	1.54^{d}	0.87^{abc}	0.62^{ab}	0.14
Ser	13.49^{cd}	10.88^{ab}	12.34^{abcd}	12.70^{bcd}	10.14^{a}	10.96^{ab}	13.61^{d}	11.17^{abc}	13.43^{cd}	0.75
Asn	1.29^{b}	1.18^{a}	1.08^{a}	1.07^{b}	1.19	1.21	0.99^{b}	1.42^{a}	1.13^{a}	0.14
Glu	3.13^{b}	1.95^{a}	1.89^{a}	2.85^{b}	1.77	1.65^{a}	3.02^{b}	1.98^{a}	1.44^{a}	0.20
Gln	11.43	11.61	13.24	12.74	10.39	11.89	14.84	13.20	11.54	1.16
Pro	12.32^{cd}	14.07^{ab}	12.22^{bcd}	11.94^{abc}	11.95^{a}	12.29^{bcd}	10.32^{abc}	13.16^{ab}	11.54^{d}	1.23
Gly	10.26^{d}	8.49^{ab}	9.79^{abc}	9.04^{cd}	7.93^{a}	9.54^{bcd}	9.20^{bcd}	8.57^{abc}	10.77^{ab}	0.43
Ala	5.72^{d}	4.18^{ab}	4.65^{abc}	5.14^{cd}	3.80^{a}	3.92^{a}	4.95^{bcd}	4.37^{abc}	4.07^{ab}	0.29
Non-Essent.	59.02	53.36	56.19	56.90	47.17	52.13	58.47	54.74	54.54	3.26
Total	141.44	126.04	125.76	132.45	112.46	122.22	130.25	123.51	126.26	5.97
E:N[3]	1.40	1.36	1.24	1.33	1.34	1.34	1.23	1.26	1.32	0.07
Taurine	1.46_{b}	1.30_{a}	1.92_{a}	1.50_{b}	1.87_{a}	1.52_{a}	1.63_{b}	1.38_{a}	1.52_{a}	0.24
Ammonia	0.70^{b}	0.36^{a}	0.30^{a}	0.65^{b}	0.35^{ab}	0.31^{a}	0.70^{b}	0.33^{a}	0.31^{a}	0.05
Ornithine	4.58^{b}	4.08^{b}	3.48^{ab}	3.04^{ab}	3.25^{ab}	3.84^{b}	2.10^{a}	3.60^{ab}	4.19^{b}	0.48

1) Mean of seven observations.
2) For feather meal codes in this and all subsequent tables see Chapter 3, Table 3.1.
a, b, c, d. Mean values for individual treatments followed by different superscripts horizontally are significantly different ($P < 0.05$).
3) E,N: essential, non-essential, respectively.

Table 5.2. Analysis of variance for plasma free amino acids in chicks fed on various feather meals.
(The terms with little overall significance are omitted).

Responses	Source of variation						
	Time[1]			Treatment[2]			Interaction
	T	T_L	T_Q	P	C_1	C_2	
Essential							
Thr	3.32[*3)]		5.21[*]	4.94[*]		6.72[*]	
Val	9.35[***]		12.96[***]	0.71			3.23[*]
Cys	58.62[***]	112.33[***]		16.78[***]	20.46[***]	11.69[**]	
Met	3.71[*]	5.89[*]					
Tyr	9.02[***]	17.18[***]		8.43[***]	9.49[**]	7.60[**]	
Phe	4.16[*]	4.08[*]		3.29[*]	4.39[*]		
Lys	5.93[**]	10.49[**]					
Non-essential							
Asp	22.19[***]	39.20[***]	5.07[*]				
Ser	8.85[***]		14.52[***]				
Glu	37.62[***]	68.10[***]	9.48[**]				
Gly	12.13[***]		20.46[***]				
Ala	13.44[***]	17.51[***]	8.22[**]				

[1)] Processing time in three levels of 30, 50 and 70 min, respectively.
[2)] T_L, T_Q: Time-linear, -quadratic.
Treatment conditions in three levels: a) only by time (NA), b) by time + chemical (CH), and c) by time + enzyme (EN). C_1 is the contrast between (NA) vs (CH + EN), and C_2 is the contrast between (CH) vs (EN).
[3)] F-value.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

proved to be the main significant variable, whereas the effect of the method of treatment (with or without additions) was significant for only some amino acids. The interaction term (time x treatment) was significant only for valine ($P < 0.05$).

There was a general trend towards decreased essential amino acid levels in the plasma of chicks with increased processing time of the feather meals fed (Fig. 5.2). This trend was quite clear when the processing time was increased from 30 to 50 min, with some exceptions, but was not significant: this applied to histidine in all samples, lysine and methionine in CH samples, threonine and histidine in EN samples. When the processing time of the feather meals was increased from 50 to 70 min the plasma amino acids of two groups of amino acids increased insignificantly. These were threonine, arginine and valine in all test treatments (NA, CH and EN); leucine and isoleucine in CH and EN treatments. The last two treatments also showed significant increases for valine.

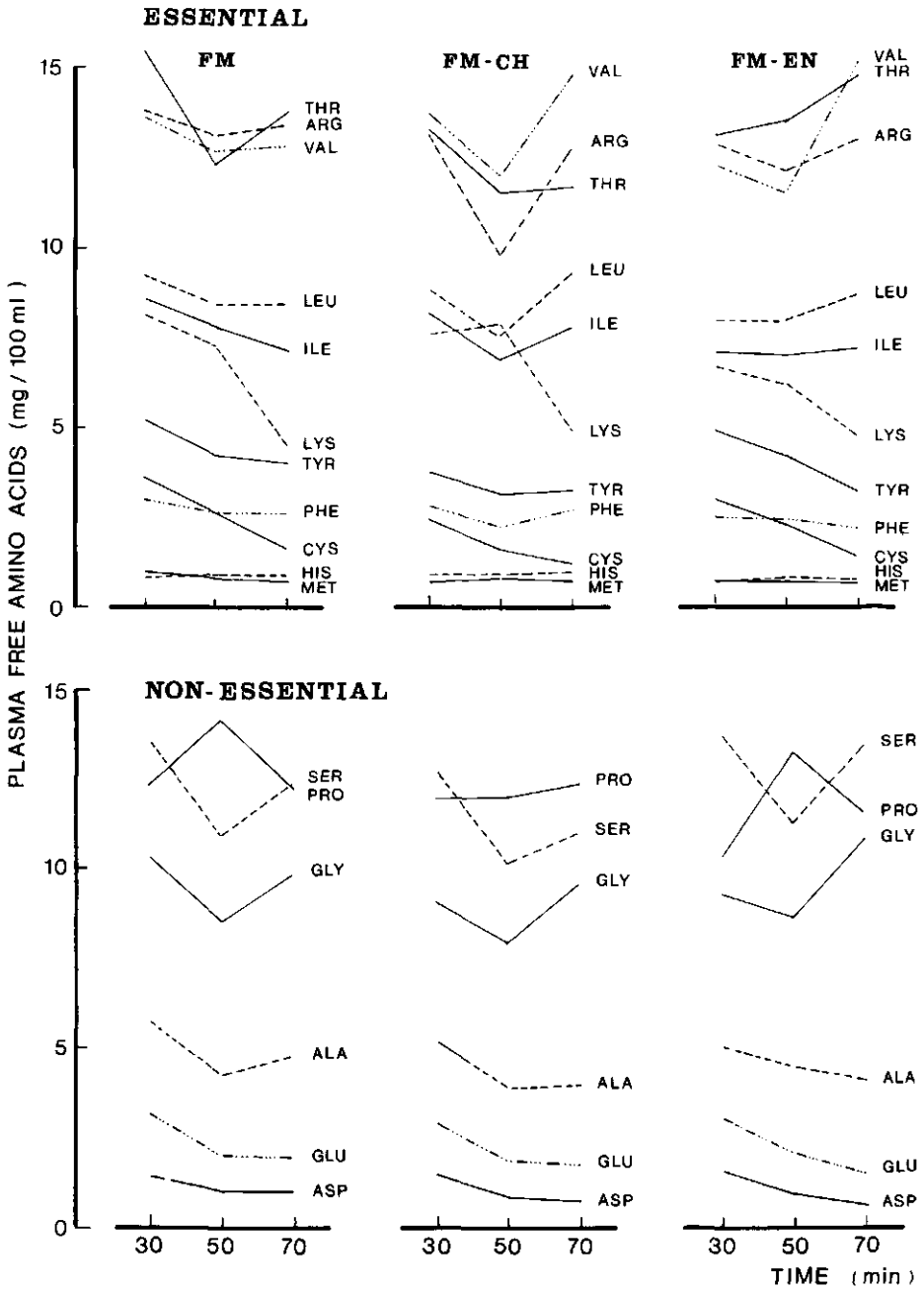


Fig. 5.2. Concentration of essential and non-essential amino acids in blood plasma of chicks after feeding various processed feather meals. FM: feather meals treated without additives; FM-CH: feather meals treated with NaOH; FM-EN: feather meals treated with enzyme.

The characteristics described for the plasma essential amino acids were also observed, in general, with the non-essential amino acids (Fig. 5.2). The plasma amino acid concentrations, with the exception of proline, decreased when the test feather meals were processed at 50 min instead of 30 min. When prolonged (70 min) processed feather meals were fed to the chicks, plasma aspartic acid, glutamic acid and alanine were further decreased, while plasma concentrations of serine, glycine and alanine increased in comparison with those of 50 min processed samples, but only in the case of glycine for CH and EN treatments were the differences significant.

As may be seen from the F-values (Table 5.2), cystine was the most influenced by the treatments, followed by valine and tyrosine, while methionine was less affected in comparison with other essential amino acids. Of the non-essential amino acids, glutamic acid and serine were the most and least influenced respectively by the treatments.

Reference groups

The concentration of plasma amino acids in chicks before (fasting level) and after feeding the reference diets is given in Table 5.3. These values are presented graphically in Figure 5.3. The total concentrations of free amino acids increased after the casein and RSD. In contrast to these diets the NFD failed to elevate the levels of free amino acids in plasma compared with the fasting levels. In general, the essential amino acids showed greater differences in response than the non-essential amino acids.

5.4.2 Relationship between dietary amino acids and plasma free amino acids

Comparisons between the amino acids supplied in the test meals and the levels in the plasma of chicks 1 hr after the last force-feeding, showed varying relationships. The essential amino acids in the plasma were significantly ($P < 0.05$) correlated with the level supplied (dietary and digested) by the differently processed feather meals in 61 of the 63 subjects studied. All the subjects (9) from casein and 8 of the 9 from RSD also showed significant correlations for essential amino acids (Table 5.4). The non-essential amino acids showed low correlations and in no case were significant ($P > 0.05$).

Plasma indices

Plasma amino acid indices calculated from the plasma amino acid results from

Table 5.3. Concentrations (mg/100 ml) of free amino acids in plasma of chicks before (fasting level) and after feeding of reference diets[1)].

Amino acid	Fasting level		NFD		Casein		RSD	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Thr	6.82	0.57	5.31	0.35	15.65	0.84	10.29	0.56
Val	2.55	0.09	1.35	0.12	13.29	0.41	3.55	0.28
Cys	1.26	0.14	1.32	0.11	3.27	0.30	2.65	0.30
Met	0.82	0.05	0.58	0.06	3.88	0.33	1.33	0.17
Ile	1.58	0.11	0.67	0.07	7.86	0.41	1.74	0.19
Leu	2.45	0.12	1.08	0.11	12.37	0.29	2.84	0.32
Tyr	2.78	0.21	2.47	0.20	8.54	0.68	5.29	0.56
Phe	1.34	0.10	1.38	0.12	3.33	0.18	1.61	0.16
Lys	8.38	0.49	5.34	0.18	18.42	0.77	10.68	0.41
His	1.05	0.05	0.86	0.12	3.95	0.34	1.63	0.13
Arg	4.15	0.34	2.72	0.17	6.16	0.37	6.56	0.33
Essential	33.18		23.08		97.72		48.17	
Asp	0.86	0.11	0.50	0.06	1.95	0.29	0.65	0.07
Ser	6.44	0.45	5.98	0.56	9.12	0.53	6.15	0.43
Asn	0.66	0.11	0.56	0.14	3.46	0.32	0.33	0.12
Glu	2.05	0.14	1.42	0.11	3.63	0.28	1.56	0.13
Gln	9.05	0.41	5.96	0.77	18.67	0.78	7.06	0.66
Pro	2.45	0.11	1.85	0.10	32.91	1.81	2.50	0.27
Gly	3.22	0.15	2.77	0.21	5.88	0.32	3.83	0.23
Ala	4.46	0.31	3.86	0.38	6.29	0.32	4.43	0.35
Non-Essent.	29.19		22.90		81.91		26.51	
Total	62.37		45.98		178.63		74.68	
E:N	1.14		1.01		1.19		1.82	
Taurine	1.60	0.19	0.92	0.14	1.95	0.43	1.41	0.28
Ammonia	0.33	0.03	0.20	0.03	0.69	0.12	0.32	0.04
Ornithine	0.35	0.06	0.29	0.04	1.05	0.14	1.11	0.14

[1)] Mean and standard errors in nine observations, except in NFD where six samples were involved.

chicks fed the test diets (Table 5.1 and 5.3), are presented in Table 5.5. The chemical scores presented in the same table were based on the extent of the deficiency of the first three limiting amino acids, taking into account the amino acid composition and the digested amino acids of the feather meals (Chapter 3).

It is evident from the plasma indices that the extent of the increase in concentrations of methionine, lysine and histidine goes in parallel with the amount in the ingested protein, as predicted by the chemical scores, although the limiting order is not the same. The outstanding feature is that when plasma indices take into account the amino acid variations from the fasting level as a part of the supplied (dietary and digested) amino acids or the amino acid

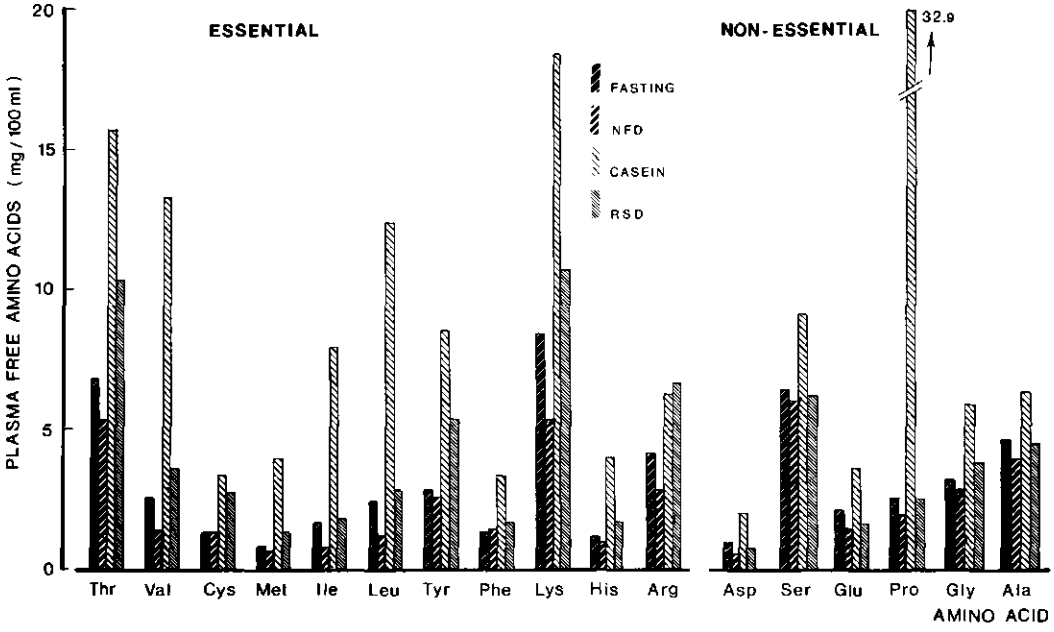


Fig. 5.3. Concentration of amino acids in blood plasma of chicks before (fasting level) and after feeding of reference diets (NFD, Casein, RSD).

requirements, most of the test feather meals showed lysine to be the first limiting amino acid. This was also predicted by the chemical scores based on the digested amino acids.

5.5 Discussion

5.5.1 The effect of dietary proteins on plasma amino acids

The plasma levels of circulating free amino acids after feeding the test feather meals were higher when short-time (30 min) processed feather meals were fed than with long-time processed samples of 50 and 70 min. Our interpretation of these results is that prolonged processing of the test meals reduced digestibility or delayed the digestive release of most of the constituent amino acids under our experimental conditions. The relatively high concentration of some amino acids in the blood plasma after feeding overprocessed feather meals (70 min) suggests that the amino acids of these samples reached a concentration peak at the time that the earlier absorbed amino acids showed a slight decline.

Table 5.4. Correlations between the levels of essential amino acids in the dietary protein and blood plasma of chicks[1)].

Dietary protein	Correlation coefficients[2)]	
	r_c	r_d
FM_{30}	0.69 (0.47 - 0.79)	0.67 (0.41 - 0.79)
FM_{50}	0.73 (0.66 - 0.83)	0.71 (0.62 - 0.82)
FM_{70}	0.75 (0.71 - 0.77)	0.71 (0.66 - 0.74)
$FM_{30}CH$	0.74 (0.60 - 0.83)	0.71 (0.55 - 0.81)
$FM_{50}CH$	0.66 (0.16 - 0.86)	0.60 (0.06 - 0.84)
$FM_{70}CH$	0.82 (0.78 - 0.86)	0.78 (0.72 - 0.83)
$FM_{30}EN$	0.69 (0.53 - 0.76)	0.68 (0.50 - 0.75)
$FM_{50}EN$	0.71 (0.62 - 0.79)	0.67 (0.57 - 0.77)
$FM_{70}EN$	0.76 (0.71 - 0.82)	0.71 (0.64 - 0.80)
Casein	0.73 (0.71 - 0.74)	ND
RSD	0.58 (0.49 - 0.60)	ND

1) Mean of seven and nine observations, respectively, for the feather meals and reference diets. Range in parenthesis.

2) r_c and r_d : for dietary protein, total and digestible amino acids respectively (Chapter 3).

ND : not determined.

The variation in plasma changes as a result of treatment (with or without additives) was less pronounced.

Although there are no directly comparable reports in the literature, our data seem to be in line with the results of some previous studies. Goldberg and Guggenheim (1962) found that meals of properly heated soy flour produced increased concentrations in the portal plasma of rats, while meals of overheated soy flour resulted in little change in plasma amino acid concentration. Smith and Scott (1965a) and Bielorai et al. (1972) reported that the plasma free amino acid pattern in the blood of chicks reflects the availability of amino acids from unheated and heated proteins from fish and soybean meals respectively. Longenecker and Lo (1974) showed that the effect of heat processing on a protein mixture consisting of wheat gluten, milk and egg, caused a reduction in the availability of lysine as determined by plasma techniques in humans. It was reported also by Vaughan et al. (1977) that heated lactalbumin was digested more slowly by humans and had less effect on plasma amino acid concentrations than untreated lactalbumin.

In the present study, changes in the plasma amino acids did not show the same order in decreased or increased digestibility of certain amino acids as was

Table 5.5. A comparison of results obtained in the present experiment for predicting the first three most limiting amino acids in the various processed feather meals.
(Amino acids have been placed in increased observed order of their plasma amino acid indices - PAAI and their chemical scores - CS).

Feather meals	Plasma indices[1]									Chemical scores[2]					
	PAAI-T			PAAI-D			PAAI-R			CS-T			CS-D		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
FM_{30}	-28^{H}	-15^{K}	27^{M}	-86^{H}	-36^{K}	50^{M}	-13^{H}	-6^{K}	-8^{M}	32^{M}	39^{K}	48^{H}	17^{K}	17^{M}	20^{H}
FM_{50}	-57^{K}	-20^{H}	-8^{M}	-175^{K}	-69^{H}	-17^{M}	-21^{K}	-10^{H}	-2^{M}	30^{M}	38^{K}	50^{H}	12^{K}	14^{M}	20^{H}
FM_{70}	-200^{K}	-22^{M}	-19^{H}	-661^{K}	-73^{H}	-44^{M}	-74^{K}	-9^{H}	-7^{M}	32^{M}	38^{K}	50^{H}	12^{K}	16^{M}	18^{H}
$FM_{30}CH$	-42^{K}	-18^{H}	29^{M}	-104^{K}	-61^{H}	50^{M}	-16^{K}	-8^{H}	-9^{M}	32^{M}	39^{K}	46^{H}	16^{K}	19^{M}	19^{H}
$FM_{50}CH$	-24^{K}	-19^{M}	28^{H}	-86^{K}	-86^{H}	-38^{M}	-13^{H}	-9^{K}	-6^{M}	32^{M}	38^{K}	49^{H}	12^{K}	16^{M}	20^{M}
$FM_{70}CH$	-187^{K}	-10^{H}	-9^{M}	-833^{K}	-63^{H}	-21^{M}	-67^{K}	-5^{H}	-3^{M}	31^{M}	38^{K}	49^{H}	9^{K}	13^{H}	14^{H}
$FM_{30}EN$	-88^{K}	-49^{H}	-23^{M}	-200^{K}	-118^{H}	-40^{M}	-33^{K}	-22^{H}	-7^{M}	31^{M}	38^{K}	48^{H}	17^{K}	18^{M}	22^{H}
$FM_{50}EN$	-110^{K}	-27^{H}	-22^{M}	-393^{K}	-86^{H}	-46^{M}	-41^{K}	-13^{H}	-7^{M}	33^{M}	38^{K}	51^{H}	11^{K}	16^{M}	20^{H}
$FM_{70}EN$	-191^{K}	-41^{H}	-23^{M}	-720^{K}	-148^{H}	-60^{M}	-70^{K}	-19^{H}	-8^{M}	31^{M}	38^{K}	48^{H}	10^{K}	14^{M}	16^{H}

[1] $\text{PAAI-T} = \frac{FM - f}{T}$, $\text{PAAI-D} = \frac{FM - f}{D}$, $\text{PAAI-R} = \frac{FM - f}{R}$ where:

FM : Plasma amino acid (PAA) concentration after feeding the test feather meal.
f : fasting PAA concentration.
T, D: Total and digestible amino acids, respectively of the test feather meals (Chapter 3).
R : amino acid requirements for chicks, at 3 weeks old (NRC, 1977).

[2] for CS see Chapter 3, section 3.3.4.
CS-T and CS-D: CS based on total and digestible amino acids, respectively.

H, histidine; K, lysine; M, methionine.

shown in digestibility experiments in which the excretion of amino acids from feather meal was measured quantitatively (Chapter 3). This can be attributed to the dynamic state of the blood. The extent of the variation in the concentration of an amino acid in plasma is not always proportional to the concentration of this amino acid in the dietary protein, because of the balance between input and output in the blood.

5.5.2 Relationship between dietary and plasma amino acids

Our data clearly showed that while there is a rise in plasma levels of most individual free amino acids measured after the protein meals, the nitrogen-free diet did not elevate plasma levels of the same free amino acids above the fasting concentrations (Fig. 5.3). This last observation is in agreement with the reports of Munro (1970), Adibi and Mercer (1973). It has been suggested that protein catabolism was reduced, or reutilization of the amino acids was enhanced, by providing a nitrogen-free diet (energy source).

It has been reported that the pattern in appearance of amino acids in peripheral plasma is not related to the amino acid composition of the ingested protein because of the influence of tissue uptake and release of amino acids (Frame, 1958; Peraino and Harper, 1963; Yearick and Nadeau, 1967), and because ingested protein is mixed with several times its mass of endogenous protein so that an amino acid mixture of relatively constant composition is delivered to the portal blood (Nasset, 1972). In contrast to these findings, it is interesting to note that in the present study highly significant correlations were found between the levels of essential amino acids in plasma and the amounts of the amino acids supplied (dietary and digested). Adibi and Mercer (1973), Marrs et al. (1975) previously reported a similar correlation after ingestion of bovine serum albumin and an amino acid mixture simulating casein and a tryptic hydrolysate of casein in humans.

The absence of a precise relationship between dietary and plasma amino acids, correlation coefficients of varying significance for essential amino acids and small correlations for non-essential ones is understandable. It is due to the complex effect of transport and metabolic steps on the rate of absorption of different amino acids from the gastrointestinal tract into the blood circulation (Wiseman, 1974). The distinct variations in digestibility of individual amino acids in feather meals (Chapter 3) could partly explain the varying relationships between ingested and plasma amino acids. Besides that, the variation in rates of absorption of different amino acids, the extent to which some are metabolized by the intestinal mucosa during absorption, the modification of plasma amino acid

pattern by the liver and the rates of loading of cells with amino acids, are factors which may influence the changes of amino acid levels in the blood, especially in systemic blood.

Literature studies showed that lysine, methionine and histidine are limiting amino acids in hydrolyzed feather meals in poultry rations, determined by growth assays (Moran et al., 1966; Morris and Balloun, 1973a; MacAlpine and Payne, 1977), and recently by plasma amino acid concentrations (Dunkelgod and Winkleman, 1982). This is further confirmed by our results from the chemical scores based on dietary and digestible amino acids. The plasma amino acids indices are in the same line (Table 5.5). In previous studies, plasma indices have been used to identify the first limiting amino acid in animal diets (Longenecker and Hause, 1959; Potter et al., 1972; Bodwell, 1977).

In most of the cases plasma lysine indices were decreased when processing time of the test feather meals was increased. This is clearly related to lysine digestibility which decreased by increasing processing time of the samples, as we found in a previous investigation (Chapter 3). It is of interest to note that histidine and methionine, although they did not show a clear sequence in their limiting order between the differently processed feather meals were, together with lysine, the least digested amino acids determined by the digestibility experiments.

5.5.3 Concluding remarks

These studies demonstrate that the limiting amino acids in feather meal fed to chicks may be identified by a rapid and relatively simple procedure. They suggest further that information on the comparative digestibilities of feather meals, adversely affected by overlong processing, may be obtained from changes in the plasma amino acid concentrations occurring 1 hour after the last force-feeding of the chicks. However, because of the variety of factors to be considered in blood studies, it is difficult in practice to translate these changes into a quantitative determination of amino acid digestibility. It would be useful, therefore, if a specific method for each amino acid could be evolved.

chapter 6

SUMMARY AND CONCLUSIONS

Feather waste at poultry processing plants, has been of interest in nutritional studies because of its high protein content. This material must be hydrolyzed in order to be digested by the animal, because in its natural state it is of no nutritive value. However, this product will be of variable quality because hydrolysis has not only beneficial effects but can also reduce the nutritive value in terms of amino acid content and digestibility. Although feather meal is used in poultry feeding, our present knowledge of its protein-amino acid quality is inadequate. The value of feather meal as a component of animal feeds could be better assessed if more were known about the effect of different processing conditions on the content and digestibility of its amino acids. Therefore, the investigation described in this thesis was conducted in order to study chemical, nutritional and physiological aspects of feather meal treated under different conditions.

The first major part of this study was an evaluation of feather meal protein-amino acid quality by chemical methods (Chapter 2). Crude protein analysis and digestibility determinations *in vitro* are often used in practice as a rapid quality-control method. The amino acid composition in relation to the amino acid requirements of the animal is also taken into account in formulating rations.

The effects of five processing times (30, 40, 50, 60 and 70 min), five moisture contents (50, 55, 60, 65 and 70%), five added levels of sodium hydroxide (0.2, 0.3, 0.4, 0.5 and 0.6%) and proteolytic enzyme (0.2, 0.3, 0.4, 0.5 and 0.6%) upon amino acid concentration and nitrogen solubility of feather meal were studied by multiple regression techniques in three experiments (section 2.3.2). The first experiment examined the combinations of time and moisture. The second and third experiments contained the combinations of time, moisture, sodium hydroxide and of time, moisture, enzyme respectively. All test products were autoclaved at a constant temperature of 146°C.

The results of these experiments showed that the individual amino acids have distinct variations in their response to various processing variables (time, moisture, sodium hydroxide, enzyme). There are losses, in general, of amino acids during the processing of feather meal. These losses were more pronounced in the

chemically treated feather meals, followed by the enzymatically treated ones compared with feather meals treated without additions. In the last treatments some amino acids, valine, leucine, tyrosine and phenylalanine, proved to be rather stable during processing. The protein solubility of the test feather meals in pepsin-HCl solution and alkali or acid solvents was increased as a result of processing. However, the amino acid contents and especially that of cystine were decreased. This suggests that the amount of cystine may be a reliable index of the degree of processing since feather meals with lower cystine content showed higher protein solubility/digestibility values.

The reduction of amino acids can be explained by changes in the protein structure as a result of protein cross-linking reactions and the formation of new amino acids (section 2.5.3). Evidence of the nature of these chemical changes is given in section 2.4.5 in which the formation of the unnatural amino acid lanthionine concurrent with the drastic destruction of cystine is described.

Summarizing the above, it can be concluded that:

a) there is, in general, a negative processing effect on amino acid contents and a positive one on nitrogen solubility;
b) amino acids and nitrogen solubility/digestibility, estimated by chemical methods, have very clear variations in their response to different process variables, leading to the conclusion that it is difficult to find an adequate criterion of optimal processing conditions;
c) there is an inverse relationship between amino acid contents and nitrogen solubility/digestibility, suggesting that, in this stage, it is difficult to evaluate the effect of processing on feather meal protein as a whole from the corresponding effects on the amino acid contents and nitrogen solubility.

The losses of amino acids as demonstrated in Chapter 2 may not be of much nutritional significance unless other structural modifications to the protein affect *in vivo* digestibility of crude protein and amino acids. There is a need for a bioassay of the protein because physico-chemical analyses have a limited value in that they give little or no indication of individual amino acid availability for digestion, absorption and metabolism by the animal.

Therefore, a second major objective of this study was the use of biological methods for estimating the value of the differently processed feather meals (Chapter 3). In this chapter the effect of three processing times (30, 50 and 70 min) and three methods of treatments (without additions, added sodium hydroxide 0.4% and added enzyme 0.4%) were investigated. The digestibility of

the test feather meal protein/amino acids was determined by quantitative excreta collection over a period of 36 hrs. All the test chicks were force-fed with 12 g of feather meal (dry matter) given in 2 doses with a 3 hr interval between feeds. Correction was made for metabolic plus endogenous excretions by using a nitrogen-free diet to distinguish apparent from true digestibility.

The apparent and true digestibility of all individual amino acids were influenced by the processing conditions. The main significant effect was the processing time. There was a negative time-linear effect on the digestibility of all amino acids of the feather meals, with the exception of leucine. Significant differences in digestibility coefficients due to the method of treatment were found for some amino acids, cystine, tyrosine, phenylalanine, serine and alanine. Their values were lower in the chemically treated feather meals. Comparison between the three methods of feather meal treatment revealed higher values for the enzymatically treated samples and lower values for the chemically treated ones, compared with samples treated without additions.

An interesting feature of this experiment was the considerable variation between individual amino acids in their digestibility values. They ranged in apparent digestibility values from 22.5% for lysine to 82.4% for isoleucine and, in true digestibility values, from 36.3% for aspartic acid to 86.5% for isoleucine. The three limiting essential amino acids lysine, histidine and methionine, in increasing order, were particularly low in digestibility.

The reduction in amino acid digestibility can be explained by the fact that autoclaving feather meal may have altered the protein structure in such a way that the enzymic attack, necessarily associated with the digestion process, is hindered. It has been suggested that heat/alkali causes the formation of new cross-linkages within the protein molecules and this leads to the formation of new amino acids (section 2.5.3), as in the case of lanthionine in our experiments. Cross-linkage formation reduces the rate of protein digestion possibly by preventing enzyme penetration or by blocking the sites of enzyme attack (section 3.5.3). Our results showed that feather meals with higher lanthionine content had lower amino acid digestibility values.

Comparing the digestibility values of amino acids with those of crude protein, it appeared that the latter revealed differences between the differently processed feather meals which were not shown by the former. Furthermore, we found negative correlations between *in vivo* and *in vitro* tests.

The following general conclusions may be drawn from this experiment:

a) processing can affect amino acid digestibility of feather meal indicating that

prolonged time (longer than 30 min) and use of NaOH are not desirable; a study on the effect of shorter than 30 min processing periods would be of interest;

b) crude protein digestibility estimations cannot be used as predictors of amino acid digestibility;

c) laboratory (*in vitro*) tests are not reliable in detecting inferior protein/amino acid quality in differently processed feather meals;

d) the variations between individual amino acids in their digestibility values are sufficiently extensive to suggest that, in the formulation of diets for poultry, it is essential that the dietary feather meal protein must be balanced on the basis of digested amino acids;

e) the lanthionine present in test feather meals may be a reasonable indicator of treatment damage since the amino acid digestibility values of processed feather meals are inversely proportional to the lanthionine contents of the test samples.

The digestibility of the individual amino acids in dietary feather meal has also been studied in broiler chicks by their changes in the intestinal contents (Chapter 4) and blood plasma (Chapter 5). In these nutritional-physiological studies the feather meals prepared in Chapter 3 were also used. The test feeds were given to the birds by force-feeding in 3 equal doses at 3 hr intervals, to insure an adequate and controlled intake and to get a uniform distribution of the passage of feed. Nitrogen-free diet, standard amino acid mixture and casein were used as reference diets.

Intestinal amino acid concentrations in jejunal and ileal contents of chicks were measured at 2½ hrs after the last force-feeding. These concentrations reflected the relative digestion of dietary feather meal protein as affected by different processing conditions. Digesta taken from the jejunum showed higher levels of amino acids derived from the chemically treated feather meals and lower levels from the enzymatically treated ones. The variations between intestinal amino acid levels were more pronounced in ileal digesta, where samples derived from feather meals processed for 30 min showed the lower amino acid levels. This was true for feather meals treated with enzymes and for products without additions, while the chemically treated feather meals showed an inverse trend. It should be noticed that feather meals treated for 30 min and the enzymatically treated products had the higher amino acid digestibility values while the chemically treated products were less well digested (Chapter 3).

Comparing the ileal digesta derived from chicks fed on nitrogen-free diet and standard amino acid mixture, there were differences in the proportions of methionine, leucine, tyrosine, histidine and proline. Comparing the ileal digesta with the excreta, there were very close similarities in the proportions of all individual amino acids, with the exception of cystine, indicating an insignificant microbial influence on amino acids in the large intestine.

It can be concluded that:

a) the composition of a nitrogen-free diet may influence the amount of endogenous amino acids. These findings in relation to the small differences between apparent and true amino acid digestibility values found in this study (Chapter 3), suggest that apparent digestibility determinations are reliable measures for practical purposes;

b) the similarity of the proportions of amino acids in the ileal digesta and excreta derived from the test feather meals, as well as from the nitrogen-free diet, indicates that digestibility values for feather meal amino acids based upon fecal analysis are not substantially different from those obtained by using ileal assays. Comparative studies of ileal and fecal amino acid digestibility may be needed in order to prove the validity of the very close similarity of ileal and excreta amino acid composition. It is suggested, however, that fecal analysis is a more practical and reliable criterion for routine amino acid digestibility determinations, from the point of view of applied nutrition.

Plasma amino acid concentrations in broiler chicks were estimated at 1 hr after the last force-feeding. There was a relationship between plasma amino acid levels and the relative digestibility of the feather meal protein subjected to various processing treatments. Comparisons between dietary/digested amino acids and their levels in plasma revealed significant correlations for the essential but not for the non-essential ones. Possible reasons for the lack of a precise relationship between dietary/digested and plasma amino acids are discussed in section 5.5.

It can be concluded that:

- although blood plasma studies may provide useful information on the relative digestion of dietary feather meal, it seems difficult to interpret plasma amino acid changes as a practical quantitative measurement of amino acid digestibility.

The final conclusion, referring to the points of investigation which are mentioned in the introductory chapter is that:

a) Feather meal protein-amino acids are affected in both their contents and digestibility, by different processing conditions, time being the most significant one.
b) Chemical methods (total amino acids, soluble nitrogen) and qualitative assays in blood plasma and intestinal amino acids are not adequate to evaluate feather meal protein quality as affected by different processing conditions.
c) Feather meal must be evaluated by quantitative *in vivo* digestibility measurements of the individual amino acids and should be used in poultry rations on the basis of the digested amino acids it supplies.

SAMENVATTING EN CONCLUSIES

Veren, afkomstig van pluimveeslachterijen, zijn een interessant afvalprodukt voor veevoedkundige doeleinden vanwege hun hoog gehalte aan eiwit. Verenafvallen moeten tevoren worden gehydrolyseerd om door dieren verteerd te kunnen worden, omdat zij van nature geen voederwaarde hebben. Het bewerkte produkt is echter wisselend van kwaliteit omdat deze hydrolyse naast gunstige effecten ook nadelen met zich mee kan brengen in de vorm van een teruggang van het aminozurengehalte en de verteerbaarheid. Hoewel verenmeel reeds als grondstof voor pluimveevoer wordt gebruikt, is de huidige kennis omtrent de kwaliteit van de daarin aanwezige eiwitten en aminozuren nog niet toereikend te noemen. De voederwaarde van dit verenmeel zou op een betere wijze vastgesteld kunnen worden, indien meer bekend zou zijn over het effect van verschillende bewerkings-procédé's op het gehalte en de verteerbaarheid van de aminozuren in het eindprodukt. Het onderzoek, dat in dit proefschrift is beschreven, werd opgezet om de chemische, veevoedkundige en fysiologische aspecten na te gaan van verschillende methoden van voorbewerking.

In Hoofdstuk 2 is de waarde van het verenmeel-eiwit, en de kwaliteit van de aminozuren daarin, bestudeerd aan de hand van chemische methoden. In de praktijk worden vaak ruw eiwit- en *in vitro* verteerbaarheids-bepalingen gebruikt om op een snelle wijze de kwaliteit van het verenmeel vast te stellen. Daarnaast wordt bij het samenstellen van voedermengsels ook rekening gehouden met de aminozuren-samenstelling in relatie tot de aminozuren-behoefte van het dier.

De invloed van vijf verschillende bewerkingstijden (30, 40, 50, 60 en 70 minuten), vijf vochtgehalten (10, 55, 60, 65 en 70%), vijf verschillende hoeveelheden toegevoegd NaOH (0,2, 0,3, 0,4, 0,5 en 0,6%) en eiwitsplitsend enzyme (0,2, 0,3, 0,4, 0,5 en 0,6%) op de aminozuurgehalten en de oplosbaarheid van N-houdende stoffen in verenmeel werd nagegaan met behulp van multiple regressiemethoden in drie experimenten (zie 2.3.2). In het eerste experiment werd de combinatie van tijd- en vochtigheidsgraad bestudeerd; in het tweede en derde experiment kwamen de combinatie: tijd, vochtigheidsgraad, NaOH-toevoeging, en de combinatie: tijd, vochtigheidsgraad en enzyme aan de orde. Alle te onderzoeken produkten waren met behulp van een autoclaaf gehydrolyseerd bij een constante temperatuur van 146°C.

De resultaten van het onderzoek toonden aan dat elk aminozuur afzonderlijk verschillend reageert op de onderscheiden proces-variabelen: tijd, vochtigheidsgraad, NaOH- en enzymtoevoeging. Over het geheel genomen gaan de gehalten aan aminozuren gedurende het bewerkingsproces achteruit. Deze achteruitgang was bij de chemisch en de enzymatisch behandelde monsters, in deze volgorde, groter dan bij het bewerkte verenmeel zonder toevoegingen. In het laatste geval bleken enkele aminozuren, te weten: valine, leucine, tyrosine en phenylalanine, tijdens de bewerking vrij stabiel te blijven. De oplosbaarheid van het eiwit in de onderzochte monsters verenmeel nam toe als gevolg van een behandeling met een pepsine-HCl-oplossing en alkalische of zure oplosmiddelen. Daar stond echter tegenover dat ook de gehalten aan aminozuren, en in het bijzonder dat van cystine, onder invloed van deze behandelingen achteruit ging. Dit zou in kunnen houden dat het cystine-gehalte in verenmeel een betrouwbare aanduiding kan zijn van de mate van inwerking van het bewerkingsproces aangezien verenmeel met een lager cystine-gehalte hogere eiwitoplosbaarheids- c.q. -verteerbaarheidscijfers te zien gaven.

De teruggang in aminozuurgehalte kan verklaard worden aan de hand van veranderingen in de eiwitstructuur als gevolg van wijzigingen in de eiwitmolecuulverbindingen en de vorming van nieuwe aminozuren (zie 2.5.3). Op de aard van deze chemische veranderingen wordt nader ingegaan in 2.4.5, waarin de totstandkoming van het niet in de natuur voorkomende aminozuur lanthionine, tesamen met een gelijktijdig optredende drastische achteruitgang van de hoeveelheid cystine, beschreven wordt.

Het voorgaande samenvattend kunnen we concluderen dat:

a. de bewerking van verenmeel, over het geheel genomen, een negatief effect heeft op het gehalte aan aminozuren en een positief effect op de oplosbaarheid van de N-houdende stoffen,
b. de oplosbaarheid c.q. verteerbaarheid van aminozuren en N-houdende stoffen, aan de hand van schattingen met behulp van chemische methoden, duidelijke variaties vertonen in hun reactie op de verschillende variabelen in het bewerkingsproces, hetgeen leidt tot de conclusie dat het ondoenlijk is om hieruit een toereikend criterium af te leiden voor het vinden van optimale bewerkingscondities,
c. er een omgekeerd verband bestaat tussen de aminozuurgehalten en de oplosbaarheid c.q. verteerbaarheid van de N-houdende stoffen, zodat het effect van bebewerkingsprocessen op verenmeeleiwit moeilijk af te leiden is uit het tegelijkertijd optredend effect op het aminozurengehalte en de oplosbaarheid van N-houdende stoffen.

De verliezen aan aminozuren, vermeld in hoofdstuk 2, zijn uit een veevoedkundig oogpunt wellicht van weinig betekenis, tenzij er wijzigingen optreden in de eiwitstructuur die de *in vivo* vast te stellen verteerbaarheid van ruw eiwit en aminozuren beïnvloeden. Zodoende is een voederwaardebepaling langs biologische weg noodzakelijk, omdat analyses langs fysisch-chemische weg slechts van beperkte waarde zijn, waar zij weinig of geen aanwijzing opleveren omtrent de beschikbaarheid van de afzonderlijke aminozuren voor de vertering, de absorptie en het stofwisselingsproces van het dier.

Daarom werd in een tweede deel van dit onderzoek nagegaan in hoeverre biologische methoden bruikbaar zijn voor de vaststelling van de voedingswaarde van op verschillende wijzen voorbewerkt verenmeel (Hoofdstuk 3). In dit hoofdstuk werd het effect onderzocht van drie bewerkingstijden (30, 50 en 70 minuten) en drie bewerkingsmetoden, nl. zonder toevoegingen en mèt toevoeging van resp. 0,4% NaOH en 0,4% enzyme. De verteerbaarheid van de aminozuren in de onderzochte monsters werd bepaald aan de hand van de hoeveelheid mest die verzameld werd in een periode van 36 uur. De proefkuikens werden geforceerd gevoerd met 12 g verenmeel (op droge stof-basis) die in 2 doses met een tussenpoos van 3 uur werden verstrekt. Er werd een correctie toegepast voor metabolische en endogene exctreties met behulp van een N-vrij rantsoen, waardoor onderscheid gemaakt kon worden tussen schijnbare en werkelijke verteerbaarheid.

Zowel de schijnbare als de werkelijke verteerbaarheid van alle aminozuren afzonderlijk werden beïnvloed door de wijze van bewerking. De belangrijkste invloed ging uit van de procestijd. De tijdsduur van het bewerkingsproces had een negatief lineair effect op de verteerbaarheid van alle aminozuren in de verenmeelmonsters, met uitzondering van leucine. De aard van de toevoeging had een significant verschillend effect op de verteringscoëfficiënten van enkele aminozuren: cystine, tyrosine, phenylalanine, serine en alanine. Hun verteerbaarheid lag lager bij het chemisch behandelde verenmeel. Vergeleken met de monsters zonder toevoeging gaven de enzymatisch behandelde monsters hogere verteerbaarheidscijfers te zien en de chemische behandelde lagere.

Een interessant aspect van dit onderzoek was de grote variatie in verteerbaarheid tussen de afzonderlijke aminozuren. De schijnbare verteerbaarheid liep uiteen van 22,5% voor lysine tot 82,4% voor isoleucine en de werkelijke verteerbaarheid van 36,3% voor asparaginezuur tot 86,5% voor isoleucine. De drie limiterende essentiële aminozuren: lysine, histidine en methionine, waren, in deze volgorde, bijzonder laag in verteerbaarheid.

De teruggang in de verteerbaarheid van aminozuren kan worden verklaard uit de omstandigheid dat het bewerken van veren via een autoclaaf de eiwitstructuur zo-

danig kan hebben gewijzigd dat de inwerking van enzymen bij het verteringsproces belemmerd wordt. In dit verband wordt wel gedacht aan de totstandkoming van nieuwe bindingen binnen de eiwitmoleculen onder invloed van verhitting c.q. alkalische inwerking, wat op zijn beurt weer leidt tot de vorming van nieuwe aminozuren (zie 2.5.3), zoals dat in ons onderzoek het geval was met lanthionine. De totstandkoming van bindingen verlaagt mogelijkerwijze de eiwitverteringsintensiteit omdat hierdoor de enzym-penetratie wordt belemmerd ofwel omdat de plaatsen waar de enzymen moeten inwerken, geblokkeerd worden (3.5.3). Uit dit onderzoek bleek dat verenmeelmonsters met een hoger lanthionine-gehalte een lagere aminozurenverteerbaarheid vertoonden.

Uit de vergelijking van de verteerbaarheidscijfers van de aminozuren met die van het ruw eiwit bleek dat de verschillen als gevolg van uiteenlopende bewerkingsprocédé's bij beide categoriën niet dezelfde waren. Verder werden negatieve correlaties aangetroffen tussen de *in vivo* en de *in vitro* bepalingen.

Uit het onderzoek kunnen de volgende algemeen geldende conclusies worden getrokken:

a. Bewerking van veren beïnvloedt de verteerbaarheid van aminozuren in die zin dat een verhoogde inwerkingsduur, d.w.z. langer dan 30 minuten, en het gebruik van NaOH ongewenst zijn; het zou interessant kunnen zijn het effect van een bewerkingsduur van minder dan 30 minuten nader te onderzoeken.
b. Ruw eiwit-verteerbaarheidscijfers zijn niet bruikbaar als maatstaven om de verteerbaarheid van aminozuren te voorspellen.
c. Laboratorium-methoden (*in vitro* bepalingen) zijn niet bruikbaar voor het aantonen van een minder goede eiwit- of aminozuurkwaliteit als gevolg van verschillen in bewerking van verenafvallen.
d. De verschillen in verteerbaarheid tussen afzonderlijke aminozuren zijn zodanig dat het bij de samenstelling van pluimveevoeders van essentiëel belang is om het verenmeeleiwit daarin op te nemen op basis van verteerbare aminozuren.
e. Het lanthioninegehalte van verenmeel kan een betrouwbare aanwijzing geven omtrent de schadelijke werking van de toegepaste voorbewerking, aangezien de verteerbaarheid van aminozuren in verenmeel omgekeerd evenredig is met het lanthionine-gehalte in de onderzochte monsters.

De verteerbaarheid van de afzonderlijke aminozuren in verenmeel is ook onderzocht aan de hand van wijzigingen in de darminhoud van slachtkuikens (Hoofdstuk 4) en in het bloedplasma (Hoofdstuk 5). In dit voedingsfysiologisch onderzoek werden de verenmeelmonsters uit het onderzoek, beschreven in Hoofdstuk 3, betrokken. Deze monsters werden in 3 gelijke porties, telkens met een tussentijd van 3 uur, geforceerd aan

de kuikens gevoerd, om zeker te zijn van een voldoende en nauwkeurig afgepaste opname en om een uniforme spreiding van de voedselpassage te bewerken. Een N-vrij rantsoen, een standaardmengsel aan aminozuren en caseïne werden als referentierantsoenen gebruikt.

De aminozuurconcentraties in de darm werden afgemeten aan de hand van de aminozuurhoeveelheden in het jejunum en het ileum van de kuikens op een tijdstip van 2½ uur na de laatste geforceerde voeding. Deze concentraties gaven de relatieve verteerbaarheid van het verenmeel-eiwit weer zoals deze tot stand gekomen was onder invloed van de verschillende voorbewerkingen. In het jejunum werden uit chemisch voorbehandeld verenmeel grotere hoeveelheden aminozuren aangetroffen dan uit enzymatisch behandeld verenmeel. De verschillen in hoeveelheden aminozuren in de darminhoud kwamen sterker tot uitdrukking in het ileum, waarin verenmeelmonsters die 30 minuten waren voorbewerkt lagere hoeveelheden aminozuren vertoonden. Dit gold voor verenmeel dat met enzymen behandeld was en voor verenmeel zonder toevoegingen, terwijl de chemisch behandelde produkten een omgekeerde trend vertoonden. In het oog gehouden moet worden dat een voorbewerking gedurende 30 minuten en een enzymbehandeling een hogere verteerbaarheid van aminozuren te zien gaf terwijl de chemische behandelde monsters minder goed verteerbaar waren (Hoofdstuk 3). Uit een vergelijking van de ileum-inhoud van kuikens die een N-vrij rantsoen en een standaard aminozuren-mengsel werd verstrekt, bleek dat er verschillen optraden in de hoeveelheden methionine, leucine, tyrosine, histidine en proline. Bij een vergelijking van de ileum-inhoud met de excreta kwamen de daarin aangetroffen hoeveelheden van alle afzonderlijke aminozuren zeer goed met elkaar overeen, met uitzondering van cystine. Hieraan zou de aanwijzing kunnen worden ontleend dat er geen microbiologisch effect van betekenis optreedt op de aminozuren-samenstelling in de dunne darm.

Geconcludeerd kan worden dat:

a. de samenstelling van een N-vrij rantsoen de hoeveelheid endogene aminozuren kan beïnvloeden. Deze bevinding, tesamen met de kleine verschillen tussen schijnbare en werkelijke aminozuur-verteerbaarheidscijfers in dit onderzoek (Hoofdstuk 3), geven aanleiding tot de stelling dat een bepaling van de schijnbare verteerbaarheid voor practische doeleinden voldoende betrouwbaar is;
b. de overeenkomst in aminozuur-hoeveelheden tussen ileum en excreta, afkomstig van zowel de onderzochte verenmeelmonsters als van het N-vrije rantsoen, de aanwijzing oplevert dat verteerbaarheidscijfers van aminozuren in verenmeel, gebaseerd op faecale analyses, slechts in onbetekenende mate afwijken van die welke verkregen werden met behulp van bepalingen in het ileum. Een vergelijkend onderzoek van aminozuurverteerbaarheidscijfers in het ileum en de excreta kan noodzakelijk zijn om de geldigheid aan te tonen van de zeer grote overeenkomst

in aminozuur-samenstelling in ileum en extreta. Het is echter denkbaar dat, vanuit het gezichtspunt van toegepaste veevoeding, faeces-analyse een practisch beter hanteerbaar en betrouwbaarder criterium oplevert voor routine-bepalingen van de aminozuur-verteerbaarheid.

Aminozuren-concentraties in het bloedplasma van slachtkuikens werden 1 uur na de laatste geforceerde voeding gemeten. Er bestond een verband tussen de plasma-aminozuurgehalten en de relatieve verteerbaarheid van verenmeel-eiwit dat onderworpen was geweest aan verschillende wijzen van voorbehandeling. Een vergelijking van de verteerbaarheidscijfers van aminozuren in de onderscheiden rantsoenen met de hoeveelheden van deze aminozuren in het bloedplasma brachten aan het licht dat hiertussen duidelijke correlaties bestaan voor de essentiële aminozuren maar niet voor de niet-essentiële aminozuren. De mogelijke redenen voor het uitblijven van een duidelijk verband tussen de verteerbaarheidscijfers en de aminozuren in het bloedplasma worden besproken in 5.5.

Geconcludeerd kan worden dat: bloedplasma-onderzoek weliswaar nuttige informaties zou kunnen verschaffen over de relatieve vertering van verenmeel in het rantsoen, maar dat het toch bezwaarlijk lijkt om wijzigingen in plasma-aminozuren als maatstaf te gebruiken bij een kwantitatieve bepaling van de aminozuur-verteerbaarheid.

Uitgaande van de onderzoekdoelstelling, zoals deze in het inleidend hoofdstuk werd geformuleerd, is de slotconclusie dat:

a. aminozuren uit verenmeeleiwit zowel naar hoeveelheid als verteerbaarheid door verschillen in voorbewerking worden beïnvloed, waarbij de tijdsduur de belangrijkste factor is,
b. chemische bepalingsmethoden, zoals van het totale gehalte aan aminozuren en de oplosbare N-houdende stoffen, en kwalitatieve bepalingen in het bloedplasma en de darminhoud, niet toereikend zijn voor de vaststelling van de kwaliteit van verenmeel-eiwit, zoals deze door verschillen in voorbewerking tot stand komt,
c. de waarde van verenmeel moet worden vastgesteld aan de hand van kwantitatieve *in vivo* verteerbaarheids-metingen van elk aminozuur afzonderlijk. Verenmeel dient in pluimveevoeders te worden opgenomen op basis van de daarin aanwezige verteerbare aminozuren.

Appendix 1. Amino acid composition and N-solubility characteristics of hydrolyzed feather meals as affected by processing time and moisture content[1].

Treatment no.	1	2	3	4	5	6	7	8	9
Time (min)	40	40	60	60	50	30	70	50	50
Moisture (%)	55	65	55	65	60	60	60	50	70
Response (%)									
Thr	4.75	4.72	4.86	4.70	4.77	4.73	4.65	4.87	4.60
Cys	4.12	4.32	3.91	3.80	3.91	4.54	3.42	4.00	3.99
Val	7.64	7.68	7.93	7.78	7.77	7.70	7.59	7.99	7.66
Met	0.58	0.60	0.59	0.59	0.59	0.61	0.61	0.56	0.59
Ile	4.95	4.97	5.17	5.03	4.97	4.85	4.99	5.07	4.92
Leu	7.52	7.55	7.63	7.54	7.51	7.53	7.43	7.63	7.56
Tyr	2.33	2.42	2.31	2.47	2.30	2.46	2.35	2.36	2.54
Phe	4.15	4.26	4.39	4.48	4.21	4.31	4.20	4.66	4.71
Lys	1.92	1.93	1.96	1.97	1.95	1.94	1.86	1.91	1.96
His	0.69	0.66	0.70	0.70	0.67	0.67	0.68	0.66	0.67
Arg	6.52	6.46	6.71	6.51	6.53	6.57	6.34	6.46	6.47
Essential AA	45.18	45.57	46.16	45.57	45.18	45.91	44.12	46.17	45.67
Asp	6.50	6.40	6.45	6.29	6.56	6.36	6.47	6.41	6.24
Ser	11.57	11.29	11.27	11.29	11.61	11.46	11.51	11.41	11.03
Glu	10.86	10.71	11.12	10.90	10.93	10.82	10.91	11.19	10.42
Pro	9.00	8.92	8.87	8.64	9.17	8.82	9.22	9.11	8.97
Gly	6.91	6.90	6.99	6.94	7.13	6.96	7.05	6.89	6.82
Ala	4.24	4.26	4.29	4.27	4.34	4.29	4.30	4.41	4.27
Non-Essential AA	49.08	48.48	48.99	48.33	49.74	48.71	49.46	49.42	47.75
Total AA	94.26	94.05	95.15	93.90	94.92	94.62	93.58	95.59	93.42
NH_3	1.61	1.82	1.59	1.68	1.77	1.57	1.78	1.67	1.71
CP[2]	96.48	96.27	97.40	95.85	97.32	96.63	95.75	98.07	96.85
Ash	1.24	1.24	1.40	1.22	1.19	1.21	1.26	1.38	1.17
PDP[3]	94.23	92.06	94.23	93.87	91.80	94.02	93.94	94.08	93.56
NSS[4]	64.77	62.88	78.53	76.03	74.29	56.12	82.42	72.47	66.21
NSH[5]	31.02	32.52	42.49	41.82	37.20	26.95	49.01	34.81	37.60

[1] Treatment means; [2] Crude protein; [3] Pepsin digestibility protein; [4] Nitrogen solubility in 0.02 N NaOH; [5] Nitrogen solubility in 6 N HCl.

Appendix 2. Amino acid composition and N-solubility characteristics of hydrolyzed feather meals as affected by processing time, moisture content and added NaOH[1)].

Treatment no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Time (min)	40	40	40	40	60	60	60	60	50	30	70	50	50	50	50
Moisture (%)	55	55	65	65	55	55	65	65	60	60	60	50	70	60	60
NaOH (%)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.4	0.4	0.4	0.4	0.4	0.2	0.6
Response (%)															
Thr	4.66	4.61	4.66	4.57	4.57	4.38	4.49	4.32	4.39	4.47	4.27	4.39	4.22	4.48	4.26
Cys	3.40	2.98	3.22	2.80	2.93	2.59	3.02	2.52	2.83	3.30	2.64	3.11	2.56	3.14	2.46
Val	7.47	7.74	7.74	7.87	7.59	7.46	7.66	7.59	7.53	7.49	7.53	7.51	7.45	7.41	7.40
Met	0.56	0.54	0.53	0.50	0.50	0.53	0.56	0.52	0.54	0.55	0.56	0.59	0.53	0.57	0.55
Ile	5.01	4.96	5.11	5.02	5.21	5.13	5.11	4.97	5.04	4.97	4.96	4.85	4.99	4.77	4.83
Leu	7.52	7.57	7.62	7.69	7.79	7.73	7.79	7.66	7.71	7.63	7.56	7.42	7.42	7.38	7.36
Tyr	2.64	2.49	2.49	2.69	2.74	2.79	2.76	2.77	2.74	2.73	2.73	2.67	2.56	2.59	2.26
Phe	4.73	4.70	4.65	4.69	4.78	4.79	4.75	4.86	4.72	4.88	4.79	4.73	4.62	4.41	4.09
Lys	1.98	1.96	1.96	1.95	1.94	1.97	1.96	1.94	1.93	1.99	1.99	1.97	1.98	1.98	1.70
His	0.62	0.61	0.59	0.61	0.66	0.64	0.62	0.60	0.58	0.55	0.58	0.62	0.56	0.60	0.61
Arg	6.39	6.28	6.43	6.65	6.59	6.43	6.38	6.39	6.24	6.31	6.34	6.38	6.25	6.40	6.27
Essential AA	44.98	44.44	45.00	45.04	45.30	44.44	45.10	44.14	44.25	44.87	43.95	44.24	43.14	43.73	41.79
Asp	6.24	6.45	6.36	6.38	6.13	5.97	5.94	5.93	5.97	6.11	5.99	6.04	5.94	6.04	6.10
Ser	11.10	11.42	11.50	11.20	11.12	10.90	10.93	10.83	10.57	10.89	10.43	10.42	10.30	10.72	10.34
Glu	11.02	11.08	11.12	11.31	11.09	10.98	11.21	11.09	11.02	11.06	10.93	10.71	10.75	10.83	11.10
Pro	8.84	8.81	9.12	9.45	8.45	8.84	9.09	9.74	9.19	9.53	9.58	9.58	9.15	8.88	9.05
Gly	6.84	7.46	6.94	7.14	6.96	7.83	6.73	6.80	6.79	6.82	6.84	6.70	6.66	6.55	6.81
Ala	4.19	4.37	4.31	4.29	4.26	4.10	4.12	4.05	4.08	4.11	4.15	4.12	4.02	4.05	4.12
Non-Essential AA	48.23	49.59	49.35	49.27	48.01	48.62	48.02	48.44	47.62	48.52	47.92	47.57	46.82	47.07	47.52
Total AA	93.21	94.03	94.35	94.81	93.31	93.06	93.12	92.58	91.87	93.39	91.87	91.81	89.96	90.80	89.31
NH_3	1.55	1.57	1.49	1.34	1.23	1.34	1.35	1.34	1.51	1.34	1.46	1.41	1.38	1.38	1.32
CP[2)]	96.65	95.92	96.33	95.83	95.39	94.33	96.62	95.03	95.81	95.41	96.06	96.18	96.38	97.08	95.21
Ash	2.18	2.71	2.29	3.06	2.10	2.71	2.26	2.86	2.42	2.47	2.46	2.30	2.72	1.92	3.08
PDP[3)]	94.40	95.14	96.20	94.08	93.17	92.57	92.94	96.47	94.33	94.62	94.30	93.02	93.32	93.58	94.16
NSS[4)]	69.83	69.11	57.49	64.14	84.37	83.94	79.43	83.01	73.74	47.85	85.42	78.17	72.47	78.92	78.80
NSH[5)]	42.89	51.44	46.80	58.04	58.01	74.15	62.22	78.51	56.78	40.82	75.62	50.24	64.53	47.64	72.81

[1)] Treatment means; [2), 3), 4), 5)] see Appendix 1, footnotes 2), 3), 4) and 5), respectively.

Appendix 3. Amino acid composition and N-solubility characteristics of hydrolyzed feather meals as affected by processing time, moisture content and added enzyme[1].

Treatment no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Time (min)	40	40	40	40	60	60	60	60	50	30	70	50	50	50	50
Moisture (%)	55	55	65	65	55	55	65	65	60	60	60	50	70	60	60
Maxatase (%)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.4	0.4	0.4	0.4	0.4	0.2	0.6
Response (%)															
Thr	4.34	4.49	4.65	4.44	4.38	4.42	4.55	4.44	4.49	4.42	4.31	4.51	4.51	4.74	4.59
Cys	4.00	3.98	3.75	3.97	3.57	3.54	3.82	3.58	3.78	4.63	2.99	3.79	3.53	3.61	3.52
Val	7.19	7.26	7.30	7.18	7.12	7.09	7.20	7.13	7.15	7.06	7.03	7.24	7.17	7.15	7.32
Met	0.56	0.57	0.48	0.51	0.54	0.53	0.55	0.53	0.56	0.51	0.54	0.54	0.53	0.52	0.54
Ile	4.67	4.84	4.99	4.90	4.73	4.81	4.93	4.77	4.81	4.73	4.63	4.73	4.76	4.81	4.86
Leu	7.22	7.38	7.53	7.33	7.26	7.29	7.40	7.29	7.30	7.25	7.16	7.62	7.15	7.45	7.44
Tyr	2.53	2.67	2.65	2.53	2.53	2.55	2.51	2.67	2.51	2.71	2.56	2.70	2.70	2.78	2.68
Phe	4.43	4.54	4.69	4.45	4.60	4.40	4.49	4.56	4.35	4.44	4.44	4.51	4.56	4.66	4.57
Lys	1.88	1.88	1.96	1.84	1.86	1.84	1.82	1.89	1.83	1.83	1.85	1.94	1.90	1.93	1.92
His	0.59	0.61	0.61	0.60	0.62	0.63	0.63	0.63	0.64	0.59	0.63	0.61	0.64	0.64	0.63
Arg	6.10	6.32	6.53	6.30	6.41	6.20	6.35	6.29	6.31	6.41	6.44	6.38	6.31	6.43	6.35
Essential AA	43.51	44.54	45.14	44.05	43.62	43.30	44.25	43.78	43.73	44.58	42.58	41.57	43.76	44.72	44.42
Asp	5.72	5.99	5.97	5.85	5.85	5.88	5.90	5.84	5.93	5.86	5.84	6.03	5.93	5.90	5.98
Ser	9.94	10.21	10.35	10.19	10.05	10.48	10.71	10.39	10.58	9.60	9.66	10.61	10.68	10.76	10.60
Glu	10.02	10.26	10.59	9.70	10.28	10.37	10.66	10.33	10.35	9.99	10.26	10.46	10.38	10.84	10.57
Pro	8.70	8.94	9.10	8.83	8.76	8.47	8.81	8.93	8.78	8.91	8.85	8.44	8.75	8.94	8.91
Gly	6.20	6.32	6.24	6.16	6.33	6.33	6.30	6.24	6.33	6.07	6.42	6.49	6.30	6.35	6.47
Ala	3.88	3.99	4.04	3.91	4.03	4.01	4.08	3.97	3.98	3.68	4.07	3.37	3.96	3.96	4.08
Non-Essential AA	44.46	45.71	46.29	44.64	45.30	45.54	45.46	45.70	45.95	44.11	45.10	45.10	46.00	46.75	46.61
Total AA	87.97	90.25	91.43	88.69	88.92	88.84	90.71	89.48	89.68	88.69	87.68	86.97	89.76	91.47	91.03
NH_3	2.22	2.40	1.77	2.43	2.50	1.91	2.14	1.84	1.96	1.80	1.91	1.86	1.78	1.68	1.77
CP[2]	97.11	96.99	97.15	97.19	97.56	97.65	96.32	96.98	97.63	96.56	96.31	97.25	97.29	97.75	97.19
Ash[3]	1.35	1.31	1.37	1.40	1.29	1.31	1.27	1.32	1.36	1.27	1.31	1.24	1.19	1.26	1.33
PDP[4]	94.13	95.19	95.65	95.23	95.53	93.91	96.61	96.01	94.98	94.75	96.29	94.70	94.06	94.54	94.42
NSS[5]	85.58	87.71	79.95	83.63	89.71	90.86	90.45	90.21	88.35	79.27	92.89	87.69	85.04	86.19	89.48
NSH[6]	72.32	78.34	68.12	75.43	81.13	86.23	85.38	86.01	76.70	51.65	85.09	77.38	74.41	75.81	82.08
PEC	74.86	84.14	72.58	80.83	95.53	99.13	87.55	97.36	83.59	64.05	96.61	85.19	87.36	83.63	92.70

[1] Treatment means; [2], [3], [4], [5] see Appendix 1, footnotes 2), 3), 4) and 5) respectively; [6] Percent enzymatic conversion.

Appendix 4. Effect of the correction for apparent and true digestibility values on the observed total amino acids in the test feather meals[1)].

Amino acid[2)]	Feather meals								
	FM_{30}	FM_{50}	FM_{70}	$FM_{30}CH$	$FM_{50}CH$	$FM_{70}CH$	$FM_{30}EN$	$FM_{50}EN$	$FM_{70}EN$
Essential									
Thr DAA	3.10	2.95	2.75	2.86	2.75	2.39	3.16	2.75	2.55
DAA_C	3.37	3.22	3.02	3.14	3.03	2.66	3.44	3.03	2.83
Cys DAA	2.42	1.80	1.91	1.66	1.40	1.02	2.44	1.22	1.04
DAA_C	2.58	1.96	2.07	1.81	1.56	1.17	2.59	1.37	1.20
Val DAA	5.59	5.45	5.40	5.54	5.28	4.84	5.75	5.14	4.86
DAA_C	5.84	5.70	5.65	5.79	5.53	5.09	6.00	5.40	5.11
Met DAA	0.36	0.30	0.34	0.38	0.34	0.29	0.38	0.33	0.30
DAA_C	0.45	0.38	0.42	0.46	0.42	0.37	0.46	0.41	0.38
Ile DAA	4.10	4.03	3.99	4.06	3.91	3.62	4.18	3.82	3.76
DAA_C	4.31	4.23	4.20	4.27	4.12	3.83	4.39	4.02	3.96
Leu DAA	5.41	5.40	5.61	5.43	5.36	5.06	5.64	5.25	5.21
DAA_C	5.79	5.79	5.99	5.82	5.74	5.44	6.02	5.63	5.59
Tyr DAA	1.95	1.85	1.85	1.82	1.88	1.56	1.95	1.78	1.70
DAA_C	2.13	2.03	2.03	2.00	2.06	1.74	2.13	1.96	1.88
Phe DAA	3.64	3.66	3.58	3.57	3.52	3.16	3.74	3.46	3.29
DAA_C	3.83	3.85	3.78	3.76	3.71	3.35	3.93	3.65	3.49
Lys DAA	0.83	0.63	0.59	0.77	0.58	0.42	0.85	0.56	0.50
DAA_C	1.13	0.93	0.88	1.07	0.87	0.72	1.15	0.86	0.80
His DAA	0.29	0.29	0.26	0.28	0.29	0.19	0.33	0.29	0.23
DAA_C	0.39	0.39	0.37	0.39	0.40	0.30	0.44	0.39	0.33
Arg DAA	5.13	5.03	4.81	4.85	4.75	4.31	5.26	4.75	4.72
DAA_C	5.35	5.26	5.04	5.08	4.97	4.54	5.49	4.97	4.95
Non-essential									
Asp DAA	2.61	2.28	2.07	2.67	2.48	1.87	2.79	2.09	1.84
DAA_C	3.02	2.69	2.48	3.08	2.89	2.28	3.20	2.50	2.26
Ser DAA	7.95	7.68	7.63	7.38	7.59	7.23	8.12	7.21	7.37
DAA_C	8.25	7.97	7.92	7.68	7.88	7.52	8.51	7.50	7.66
Glu DAA	6.77	6.19	6.65	6.72	6.45	5.89	6.79	5.90	6.06
DAA_C	7.32	6.74	7.20	7.27	7.00	6.44	7.34	6.45	6.61
Pro DAA	5.48	4.91	5.14	5.38	4.86	4.24	5.97	4.59	4.11
DAA_C	5.75	5.18	5.42	5.66	5.13	4.51	6.24	4.87	4.38
Ala DAA	3.06	2.97	3.04	2.97	2.86	2.64	3.15	2.87	2.82
DAA_C	3.12	3.22	3.30	3.23	3.12	2.90	3.41	3.12	3.07

1) Data expressed as percentage of feather meal on dry matter basis.
2) DAA and DAA_C, digestible amino acid calculated from the apparent and corrected (true) amino acid digestibility, respectively.

Appendix 5. Composition (%) of the nitrogen-free diet (NFD) and reference standard diet (RSD) and the amino acid composition of the RSD and casein.

Diets			Amino acids		
Ingredient	NFD[1)]	RSD[2)]	Amino acid	RSD	Casein
Amino acid mixture[3)]	-	20.44	Threonine	0.65	4.36
Corn starch	-	54.99	Valine	0.69	6.66
Glucose	81.02	-	Cystine	0.35	1.36
Cellulose	3.00	3.00	Methionine	0.35	3.19
Corn oil	10.00	15.00	Isoleucine	0.60	5.32
Mineral mixture	5.72	5.37	Leucine	1.00	8.94
Choline chloride	0.20	0.20	Tyrosine	0.45	5.83
$NaHCO_3$	-	1.00	Phenylalanine	0.50	5.13
Vitamin mixture	0.06	+	Lysine	1.14	7.60
Enthoxyquin (125 mg/kg)	-	+	Histidine	0.41	2.83
	100.00	100.00	Arginine	1.15	3.57
			Aspartic acid	-	6.86
			Serine	-	6.32
			Glutamic acid	12.00	22.08
			Proline	0.40	10.45
			Glycine	0.60	1.85
			Alanine	-	2.90
			Tryptophan	0.15	-

1) Bragg et al. (1969).
2) Scott (1972).
3) Sasse and Baker (1973).

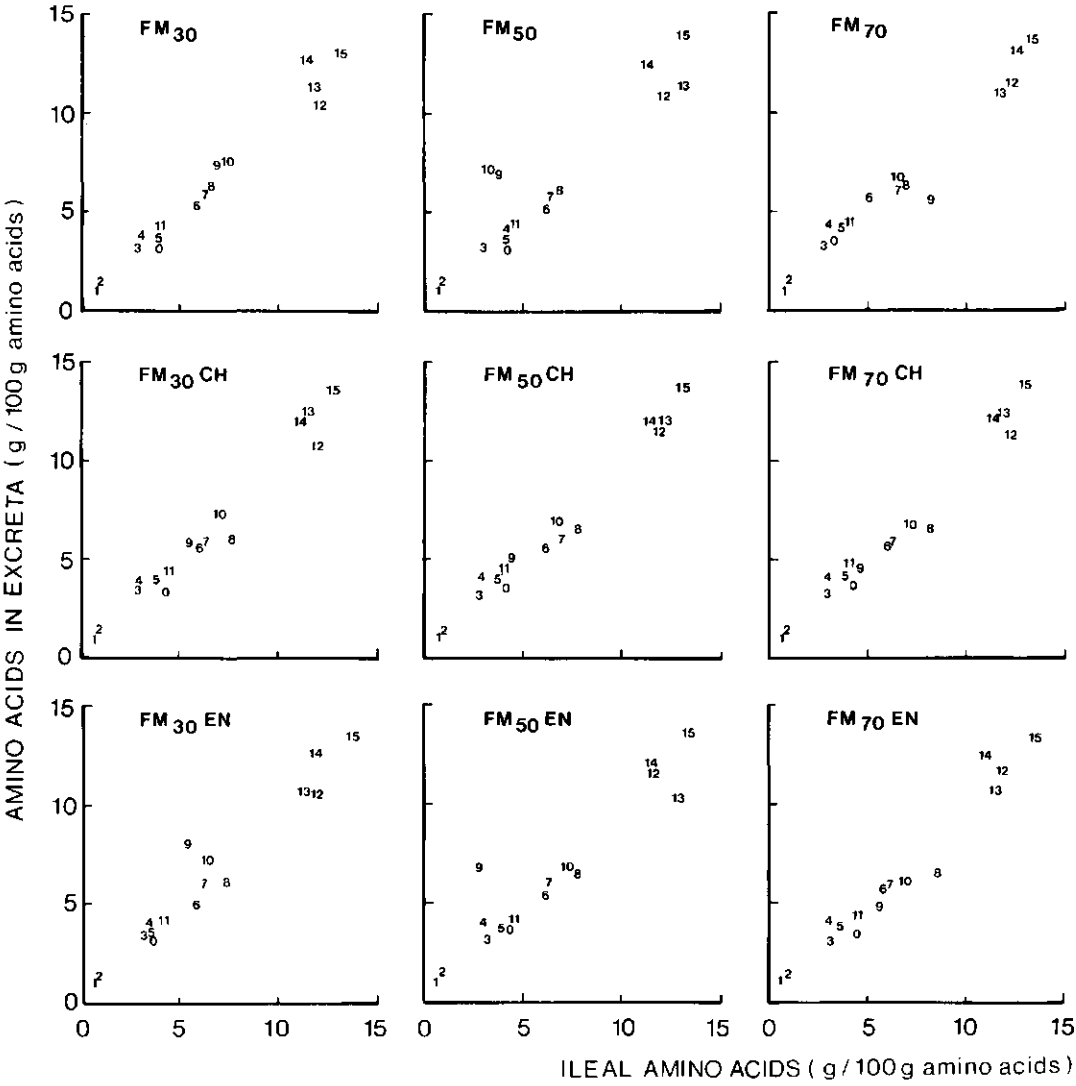
Appendix 6. Analysis of variance for jejunal total amino acids in chicks fed on various feather meals.

Responses	Source of variation[1]		
	Time (T)	Treatment (P)	T x P
Thr	0.97[2]	1.94[2]	1.22[3] *
Val	4.43 **	1.99 *	2.81 *
Cys	8.57 **	3.55 *	1.04
Met	0.23	1.57	1.62
Ile	0.35	1.35	1.57
Leu	0.38	1.61	1.36
Tyr	1.18	1.05	1.23
Phe	0.75	1.13	1.76
Lys	2.07	1.29	1.15
His	1.62	1.17	1.31
Arg	0.61	1.34	1.63
Asp	2.29	1.68	0.84
Ser	1.07	0.69	1.96
Glu	2.15	0.82	1.86
Pro	1.38	1.60	1.01
Gly	1.65	1.07	1.64
Ala	1.26	0.81	1.88
Ess	1.21	1.14	1.73
N-Ess	1.53	1.03	1.48
Total	1.34	1.09	1.60

[1] See section 4.3.4.

[2],[3] F-value with, respectively, 2 and 54, and 4 and 54, degrees of freedom.

* $P < 0.05$, ** $P < 0.01$.



Appendix 7: Relationship between the proportions of amino acids in ileal contents and excreta of chicks fed on differently processed feather meals. For feather meal codes, see Chapter 3, Table 3.1.
1: Met; 2: His; 3: Tyr; 4: Lys; 5: Phe; 6: Arg; 7: Thr; 8: Val; 9: Cys; 10: Leu; 11: Ala; 12: Pro; 13: Ser; 14: Asp; 15: Glu and 0: Ile.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 30 september 1945 te Pogoniani-Ioannina (Griekenland). Na het behalen van het Gymnasium-diploma te Athene, begon hij in november 1964 met de studie aan de Landbouwhogeschool te Athene. In januari 1971 behaalde hij het ingenieursdiploma, richting veeteelt. De daarop volgende militaire dienstplicht werd beëindigd in augustus 1973.

Van september 1973 tot juni 1974 verbleef hij in Nederland op uitnodiging van een Nederlands-Grieks bedrijf om praktische ervaring op te doen in de pluimveesector. Hierna trad hij in dienst als algemeen technisch medewerker bij het vermeerderingspluimveebedrijf "Ptinoparagogiki" N.V. te Ioannina.

Van mei 1978 tot januari 1979 specialiseerde hij zich in pluimveevoeding aan de Landbouwhogeschool te Wageningen hiertoe in staat gesteld door een subsidie van het I.A.C. (Wageningen). Daarna was hij belast met een vooronderzoek. Dit vooronderzoek werd in september 1980 omgezet in een promotie-assistenschap voor de tijd van drie en een half jaar.

The author was born on September 30th 1945 in Pogoniani-Ioannina (Greece). After completion of his studies at the Gymnasium in Athens he commenced his study for a degree at the Athens Graduate School of Agriculture in November 1964. He graduated Ingenieur in January 1971 majoring in animal production. This was followed by military service until August 1973.

From September 1973 until June 1974 he trained in the Netherlands in poultry production at the invitation of a joint Dutch-Greek company. He then joined "Ptinoparagogiki" N.V. Poultry Industry in Ioannina as a member of the technical staff.

From May 1978 to January 1979 he specialized in poultry nutrition at the Agricultural University in Wageningen on a fellowship from the International Agricultural Centre (Wageningen). He was afterwards in charge of a research project leading to the award of a post-graduate fellowship for 3½ years from September 1980.