

AN ABSTRACT OF THE THESIS OF

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Title: LYSINE-ARGININE INTERRELATIONSHIPS AND DRIED WHEY  
UTILIZATION IN MONOGASTRIC RATIONS

Abstract approved: \_\_\_\_\_

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Dr. P. R. Cheeke

A series of eight trials was conducted to determine if supplementation with arginine improved growth of young rats, Japanese quail and swine fed low-lysine diets. In five rat trials, with a variety of basal diets, no evidence of a "sparing effect" of arginine on the lysine requirement was found. A significant ( $P < 0.001$ ) depression in growth was observed in one of the rat trials when the animals were fed added arginine.

Two trials with Japanese quail and one with swine were conducted to examine this interrelationship. There was no response to added arginine in either of these species.

Based on the results of these eight experiments it was concluded that arginine supplementation is of no value in reducing the adverse effects on growth of low lysine diets fed to these monogastric species.

In the second part of this study eight trials were conducted with swine and rats to examine some implications of feeding dried whey to monogastrics. Since dried whey is high in lactose, some nutritional aspects of lactose utilization were also studied.

Because lactose is digested by bacterial action in the large intestine of the post-weaning animal, it was postulated that through stimulation of bacterial growth, fiber digestion might be enhanced in monogastrics fed lactose-containing rations. Inclusion of 10% whey in a swine grower ration containing 20% alfalfa did not influence growth rate. In a digestibility study with rats, substitution of 25% lactose in the diet reduced fiber digestibility.

In feeding trials with swine, levels of 2 to 10% added whey were found to increase average daily gains and feed efficiency.

Starter rations containing dried whey were compared to the currently used Oregon State University starter ration containing dried buttermilk. Results of this trial indicate that dried whey can be used to replace the more expensive dried buttermilk.

Reducing sugars such as lactose may form indigestible complexes with certain amino acids, particularly lysine, by what is referred to as the Maillard or Browning reaction. This reaction is stimulated by heat. Since the pelleting process involves considerable heat, it is conceivable that through the Maillard reaction some of the lysine in pelleted rations containing whey could be unavailable. Three

rat experiments were conducted to examine some factors influencing the Maillard reaction. The substitution of 5 or 10% lactose for starch resulted in a significant ( $P < 0.01$ ) depression in growth, which was largely overcome by supplementation with 0.3% lysine.

The effects of various carbohydrate sources were compared. It was found that the substitution of lactose, dried whey and molasses for starch at the 5 or 10% level all produced a significant decrease in gains. The addition of amino groups in the form of glutamic acid or urea failed to overcome the depression in growth caused by 5% lactose, but  $(\text{NH}_4)_2\text{SO}_4$  did increase gains.

The effects of several feed additives and fat sources on the Maillard reaction were also studied. In a 10% lactose diet vegetable oil increased gains when substituted for lard and alfalfa meal decreased gains. Neither copper sulphate, zinc sulphate nor calcium carbonate had any effect on the growth depression caused by 10% lactose.

It was concluded that dried whey should be utilized in a supplementary capacity in swine feeds, but further work should be conducted on such factors as the effects of feed processing and interrelationships with other ration ingredients.

Lysine-Arginine Interrelationships and  
Dried Whey Utilization in  
Monogastric Rations

by

Donald Earl Stangel

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# LYSINE-ARGININE INTERRELATIONSHIPS AND DRIED WHEY UTILIZATION IN MONOGASTRIC RATINGS

## PART I: LYSINE-ARGININE INTERRELATIONSHIPS IN MONOGASTRIC NUTRITION

### INTRODUCTION

The modern field of amino acid nutrition really began about forty years ago when W. C. Rose of the University of Illinois began his series of studies of amino acid requirements. In one of his early papers, Rose (1938) opened the door to many studies into the nutritive significance of the amino acids. From such works as Rose and Rice (1939), Rose (1952), Rose, Coon and Lambert (1954) and many others came the knowledge of which  $\alpha$ -amino acids can not be formed via transamination reactions at a rate sufficient to meet the body's requirements for these amino acids. The amino acids which must be provided preformed in the diet, either as free amino acids or as constituents of dietary proteins, have been termed essential amino acids.

Although encountering a practical ration totally lacking an essential amino acid is unlikely, a ration will vary greatly in the quantitative relationship of its amino acids. This aspect concerns amino acid balance. The balanced ration will contain all essential amino acids in the proper amounts for maximum utilization, as well as sufficient nitrogen for synthesis of the non-essential amino acids.

In practical monogastric rations the amino acids lysine, methionine and tryptophan will likely be the most limiting amino acids due to the low content of these in most cereal grains. Successful balancing of a ration consists of adding the first limiting amino acid either synthetically or in a natural feedstuff to the ration in such a manner as to achieve a balance with the second limiting amino acid. Any amount added in excess of the balance between these two will be ineffective, and may, in fact, accentuate the severity of the deficiency of the second limiting amino acid.

Besides the detrimental effects of deficiencies of essential amino acids, excesses may also be undesirable. With the increasing use of amino acids as feed additives, to complement or replace protein supplements, it is important to recognize those amino acids which may prove to be potentially toxic and conditions which may enhance toxicities. Daniel and Waisman (1968) in their work with young rats, found that the amino acids could be divided into four groups relative to the concentration required to depress growth at least 50% (Table 1). They considered the catabolic pathway and ultimate fate of the individual amino acid itself the significant factors producing growth depression, rather than the severe amino acid imbalance. In reviewing the effects of high intakes of individual amino acids, Harper, Benevenga and Wohlueter (1970) concluded that the basis for the growth-depressing and toxic effects of an excess amino acid has not been clearly

Table 1. Amino acid toxicity.<sup>1</sup>


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1. Very toxic--less than 7% of diet caused 50% reduction in growth

Methionine  
Cystine

Phenylalanine  
Tryptophan

2. Toxic--7% to 10% of diet caused 50% reduction in growth

Histidine  
Tyrosine

Lysine

3. Slightly toxic--over 10%, but some effect under 10% of diet

Glycine  
Proline

Valine  
Arginine

4. Non-toxic--no effect on growth at levels studied

Serine  
Aspartic acid  
Glutamic acid

Isoleucine  
Leucine  
Alanine

---

established, but is associated with greatly elevated body fluid concentrations of the amino acid fed in excess. There appears to be no common basis for the adverse effects. They appear rather to be due to the specific structures and metabolic relationships of the individual amino acids.

It is evident that a consideration of amino acid nutrition necessitates an appreciation of interactions between amino acids which may influence requirements. In recent years many close relationships have been found to exist between the various amino acids. The scope

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<sup>1</sup>Adapted from R. G. Daniel and H. A. Waisman, Growth, 1968, 32, 255-265.

of this field in itself is so large and in many cases so new that a small portion of a specific interaction provides an opportunity to conduct an intensive study.

Since lysine is usually the first limiting amino acid in most practical monogastric rations it would be of value to find a means of reducing its dietary requirement. This study was prompted by the reports of Abbott et al. (1969 a, b) indicating that in broilers and in replacement pullets, the lysine requirement may be reduced in the presence of high arginine. They concluded that there may be an inter-relationship between lysine and arginine that had not been explored previously. It is well known that there exists an antagonism between these two amino acids, the extent of which has received extensive study (Jones, 1964; O'Dell and Savage, 1966; Jones, Petersburg and Burnett, 1967; Nesheim, 1968 a, b).

In the work of Abbott et al. (1969a) it was found, using a low lysine broiler chick ration, that supplementary arginine did not influence growth on the low lysine diet during the first four weeks, but was effective in maintaining maximum growth from five to eight weeks of age on a low lysine ration. Similar results were obtained with pullets fed a low lysine diet to retard growth and sexual maturity (Abbott et al., 1969b).

This report of a "lysine-sparing" effect of arginine has considerable potential significance in applied animal nutrition. The fact that

lysine is often one of the limiting amino acids in poultry and swine rations may give this relationship a tremendous economic value, especially in areas where ingredients such as sesame seed oil meal and others of high arginine content are available and where a computer is used in formulating least cost rations. Due to the limited amount of investigation of this interrelationship there is ample opportunity for a study that may reveal some of the relationship of lysine and arginine in other species.

In the experimental work reported in Part I of this thesis, an examination of "lysine-sparing" by arginine was made. Eight trials were conducted using rats, Japanese quail and swine as experimental animals.



## LITERATURE REVIEW

Since the first amino acid was discovered by Proust in 1819, the field of amino acid nutrition has grown to include some twenty amino acids. New amino acids of limited distribution may still be undiscovered, but a glance at the volume of work that has been done with proteins since the turn of the century shows that only a few new amino acids have been definitely discovered in this era of modern technology.

Due to the chemical nature of amino acids, having several reactive groups in the same molecule, many reactions take place between the various amino acids. The most important are those involving transamination. This reaction provides a mechanism for the synthesis of those  $\alpha$ -amino acids in cells which can synthesize the corresponding  $\alpha$ -keto acids. An even more important aspect of this is that transamination provides a means for redistributing nitrogen, so that an animal may ingest a mixture of amino acids quite different from that which is required metabolically. Because of these reactions many interrelationships in metabolic function have been demonstrated between amino acids. There are several cases where an animal's requirements for one amino acid can easily be met at least partially by another. Phenylalanine-tyrosine and methionine-cystine have this type of relationship. Some of these interactions are quite straightforward, but many are yet to be completely understood. The relationship of arginine to lysine is one of these.

This review will cover some of the general aspects of the relationship between arginine and lysine and their involvement in the nutrition of monogastrics.

### Lysine

Since lysine is not synthesized by the tissues of higher animals and is not formed by transamination reactions, it is a dietary essential. Being a universal part of an animal's body proteins, this essential amino acid is required in relatively high amounts in the diet. Because lysine must be included in the diet, there is the opportunity for either a deficiency or toxicity state to develop.

Numerous reports have shown that high dietary lysine levels result in growth depression in most species of monogastrics. Anderson et al. (1951) observed such symptoms as anorexia, poor feathering, tremors, general weakness and reduced growth in chicks fed a diet containing 4% lysine. Jones (1964) and O'Dell and Savage (1966) produced similar results to those found by Anderson using varying levels of lysine above the chick's requirement. A growth depression in rats has been seen by Russell, Taylor and Hogan (1952) and Acheampong-Mensah and Hill (1970) at lysine levels of from 3% to 6% depending upon the protein level in the diet.

Daniel and Waisman (1968) reported that the growth depression seen in rats when an excess of an amino acid was included in the diet was caused by a depressed food intake and by a specific toxic effect of the amino acid. They found a direct correlation to exist between the toxicity of the amino acid and its concentration in the

blood, which may explain the reduction in food intake. This reduction in food intake may be a protective response by the animal in order to control the harmful effects of the excess amino acid. In general, the most toxic amino acids in terms of poor growth caused the largest decrease in food intake.

It has been pointed out by Russell et al. (1952) and Harper et al. (1970) that there is probably no common basis for the adverse metabolic effects of excessive amounts of individual amino acids since each one has its own particular role in metabolism. However, Daniel and Waisman (1968) noted that greater toxicity did occur with those amino acids which are essential for rat growth. They also noted that certain of the amino acids such as L-methionine, L-phenylalanine, L-tryptophan and others which were the most toxic also were those whose concentration in the plasma were highest.

The plasma level of lysine has been examined by many workers. Morrison, Middleton and McLoughlan (1961) indicated that the plasma lysine concentration of rats remains low until the dietary lysine level meets the requirement; further increases in dietary lysine result in rapid increases in plasma lysine. Zimmerman and Scott (1965) and Kelly and Scott (1968) noted the same plasma relationship in chicks. In considering the toxic effects of lysine, Sauberlich (1961) in his work with rats found lysine to be less toxic than methionine or tryptophan, two of the other most often supplemented amino acids. Jones, Wolters and Burnett (1966) and Acheampong-Mensah and Hill (1970) concluded that a lysine toxicity for the rat, unlike that for the chick, is mild

even at relatively high levels of supplementation.

Ghadimi and Binnington (1967) and Woody, Ong and Pupene (1967) studied hyperlysinemia in humans and found it to produce severe retardation of mental and physical development much like phenylketonuria would produce. It is not known how excess lysine exerts its effect.

### Arginine

Because of the synthesis of arginine in the Krebs-Henseleit urea cycle (Figure 1) this amino acid is not a dietary essential to some species of animals particularly during their adult stage. The rate of synthesis for young animals is insufficient to permit normal growth without a dietary source. Since birds are uricotelic and lack certain enzymes required to synthesize arginine their requirements have to be met by dietary proteins.

The arginine requirement of chickens has been studied more extensively than for other animals. There are wide variations in the quantitative requirements reported, but this is to be expected due to the variation in diets fed and complications introduced by other dietary components. Almquist, Mecchi and Kratzer (1941) found that creatine, creatinine and guanidoacetic acid stimulated growth in chicks fed an arginine deficient diet. Savage and O'Dell (1960) confirmed the findings that creatine and its precursors spare arginine. They also found glycine to have an important effect on the arginine requirement. They postulated that when free arginine is fed, it is absorbed rapidly and a higher than normal proportion is degraded by kidney arginase and

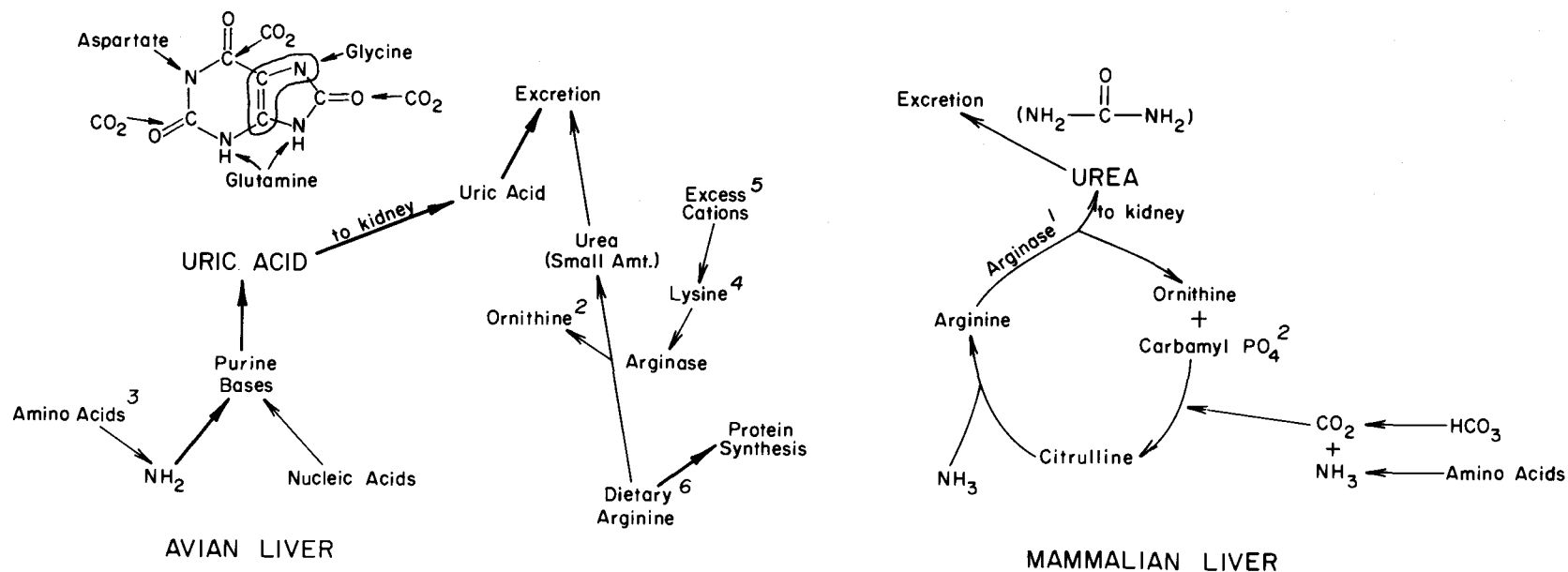


Figure 1. Comparative pathways of nitrogen excretion in birds and mammals.

- <sup>1</sup> The chick lacks arginase in liver, therefore, can not synthesize arginine or ornithine within the body.
- <sup>2</sup> The chick lacks carbamyl phosphate and ornithine which are needed for citrulline formation which can spare arginine requirement of the bird.
- <sup>3</sup> Glycine is required in 1:1 ratio with uric acid formation, therefore, high glycine requirement.
- <sup>4</sup> Excess lysine increases kidney arginase activity, therefore, causes increased arginine degradation.
- <sup>5</sup> Excess cations, i. e., Na and K, reduce lysine transport within the kidney which reduces lysine's effect on kidney arginase activity.
- <sup>6</sup> High dietary arginine requirement due to no synthesis and high level needed for feather proteins.

excreted as urea. This would result in a higher arginine requirement when fed in the free amino acid form. From their work Savage and O'Dell (1961) considered it unlikely that the chick converts any appreciable quantity of creatine to arginine. Essentially creatine spares arginine by eliminating the need for arginine in the biosynthesis of creatine.

Sauberlich (1961) showed that rats on a low protein diet tolerate supplements of arginine better than most of the other essential amino acids. In another low protein trial with rats, Harper, Becker and Stucki (1966) observed a depressed growth rate with 4% arginine, but when an adequate level of protein was fed with the same level of arginine, Jones et al., (1966), saw no adverse effects. From these and other reports it is obvious that protein level plays a very important part when considering an imbalance problem. This fact is probably responsible for the majority of the variations between studies in tolerance levels of individual amino acids. Both rats and chicks when fed an excess of an amino acid demonstrate the imbalance more readily when fed a diet containing a low level of protein (Huston and Scott, 1968).

Gullino et al. (1955) found with rats that mixtures of the ten essential amino acids possessed a toxicity level well below the mean for the individual amino acids. They observed that when arginine was left out of the mixture the toxicity was near that of the individuals. This finding reveals a protective role of arginine and was further

demonstrated by adding arginine to a lethal mixture of the other nine essential amino acids. This addition caused a reduction in mortality from 100% to 24%. Arginine is considered to afford protection against ammonia toxicity, primarily by stimulating urea production from ammonia in the liver. Goldsworthy et al. (1968) in their work with bovine livers clearly demonstrated this function of arginine in maintaining ammonia levels below toxic amounts and enhancing its excretion. Hardin and Hove (1951), however, found that arginine did not alleviate the toxicity of excess methionine in rats. Snetsinger and Scott (1961) observed that arginine does afford protection against the growth depression caused by a high lysine intake in the chicken, where no protective action against ammonia toxicity could be demonstrated due to the lack of the arginase enzyme in the chicken liver. This indicates that there is some lysine-arginine antagonism which is not related to ammonia intoxication.

#### Lysine-Arginine Interactions

Jones (1964) in his work with chicks found that 2% lysine reduced growth and produced bone composition abnormalities, a marked reduction in tissue arginine and an increase of lysine in the tissues. He observed that supplemental arginine eliminated all symptoms, suggesting that excess dietary lysine induces an arginine deficiency by decreasing the availability of arginine. O'Dell and Savage (1966) found

similar results with chicks fed excess lysine, but with excess arginine growth was depressed only when lysine was limiting and not when adequate or near a 1:1 ratio. Recently Jones et al. (1967) in their work with chicks reported that lysine does not alter arginine metabolism at the digestive or absorptive level. They found that excess lysine immediately caused loss of arginine from the tissues and an early decrease in plasma arginine. Kidney arginase levels rose two to four days later. From these findings they postulated that the primary effect of lysine could be increased catabolism of arginine within the chick or competition between lysine and arginine at the renal tubular surface, both of which would result in early plasma arginine decrease. In another chick study Stutz, Savage and O'Dell (1969) observed that supplemental arginine added to an excess lysine diet improved growth rate by lowering plasma lysine and increasing plasma arginine which seems to be beneficial to nitrogen metabolism and growth.

The nutritional antagonism between lysine and arginine is manifest as a reciprocal relationship between their tissue concentrations. Zimmerman and Scott (1965) demonstrated this by varying the lysine content of a chick diet to either side of the optimal growth promoting level. When lysine was low, plasma concentrations of lysine and arginine remained constant, but when lysine was high, it accumulated in the plasma, while arginine disappeared. They also showed the converse relationship to be true. This supported the findings of Jones



(1964) in which, added lysine reduced arginine and increased lysine in the tissues of chicks. Jones et al. (1966) observed that this relationship did not hold for rats except at a temperature of 4°C. Buraczewski et al. (1970) found that intestinal absorption played an important part in regulating levels of these amino acids in swine. A tenfold increase in lysine concentration lowered the rate of absorption of arginine to 54% of its former rate, while increasing the rate of lysine approximately fivefold. Similarly, a tenfold increase in arginine concentration caused lysine absorption to fall to 30% of its former value, while arginine absorption increased almost sixfold. This suggests that arginine may compete more successfully for absorption sites than lysine. This evidence points to the differences between avian and mammalian species when considering this antagonism. Harper et al. (1970) concluded that with chicks, high cellular concentrations of either arginine or lysine induce increases in kidney arginase activity. The response to arginine is a clear instance of a homeostatic response, but the response to lysine is an aberrant response and accounts in part for the lysine-arginine antagonism. It is felt that the response of arginase to lysine is too slow to account for early manifestations of the antagonism. The immediate loss of arginine attributed to competition for tubular reabsorption also seems insufficient by itself. Thus these mechanisms will have to be incorporated into the description of this antagonism.

In looking at the other side of this interrelationship it is quite obvious that the effect of high dietary arginine on the lysine requirement has received less attention. In their work with chicks, O'Dell and Savage (1966) reported that the effect of excess arginine in a low lysine diet was not as dramatic as in the case of excess lysine, but in the presence of excess cations (mainly potassium), growth was depressed by arginine. In recent work Stutz, Savage and O'Dell (1971) found when feeding chicks a diet first limiting in arginine that supplemented cations allowed the chicks to make more efficient use of the arginine consumed. A reasonable hypothesis for the mechanism by which cations make more arginine available for protein synthesis and growth of tissues is that they decrease the rate of arginine catabolism. Since arginine is degraded by the catalytic action of arginase and lysine has been found to be a particularly potent inducer of arginase activity (Sanahuja and Harpster, 1963; Jones et al., 1967), it is postulated that excess cations prevent the induction of arginase by antagonizing lysine transport into kidney cells. This evidence strengthens the case for a true metabolic antagonism between arginine and lysine.

Hill and Shao (1968) found with chicks that excess arginine reduced growth when lysine was the first limiting amino acid, but the effect was not consistent for all their experiments. An unexpected observation was seen when an improvement in growth was obtained when 1.3% arginine was added to a low lysine diet. No explanation for

this increase in weight gain could be found. Similar findings were reported by Abbott et al. (1969 a, b). They observed that arginine supplementation of low lysine rations failed to improve performance in broiler chicks from one to four weeks of age, but did improve growth from five to eight weeks of age. Similar results were seen in broiler replacement pullets fed a low lysine diet to retard growth and sexual development. These workers concluded that when formulating lysine deficient diets for the purpose of retarding growth and sexual development in replacement pullets, the arginine level should be closely considered. If arginine does have a sparing effect on the lysine requirement in a low lysine diet, selecting high arginine protein supplements could become very important economically to the nation's livestock and poultry industry.

## OBJECTIVES

The objective of this experimental work was to examine the suggestion of Abbott et al. (1969 a, b) that supplementary arginine may "spare" lysine. This was to be accomplished by eight separate trials using three different species of monogastrics. These trials were divided specifically into three parts:

1. To determine the influence of supplemental arginine on the growth of young male Long Evans rats<sup>2</sup> receiving low lysine diets.
2. To determine whether or not the findings of Abbott et al. (1969 a, b) could be duplicated with another avian species, the Japanese quail (Coturnix coturnix japonica).
3. To determine the response of swine to arginine supplementation of a low lysine ration.

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<sup>2</sup>Simonsen Laboratory, Gilroy, California.

## EXPERIMENTAL PROCEDURE

### Rat Experiments

The influence of supplemental arginine on the growth of young rats receiving diets low in lysine was examined. The five trials in this section were conducted using male Long-Evans rats, with initial weights of about seventy grams. The rats were housed individually at the Oregon State University Small Animal Laboratory. Feed intake and weight data were obtained throughout the duration of each trial.

Trial 1 was intended as a preliminary trial to examine the findings of Abbott et al. (1969 a, b) using a purified diet (Table 2) with added crystalline amino acids sufficient to meet the rat's requirements for essential amino acids as established by the National Research Council (NRC) (Table 3). The diets were supplemented with L-lysine. HCl and L-arginine to provide various lysine/arginine ratios (Table 4). Four rats were randomly allotted to each experimental diet and were fed for 21 days.

Due to the poor growth response obtained with the zein protein diet in Trial 1, Trial 2 was conducted using soybean meal as the protein source. A low protein diet was supplemented as in Trial 1 with crystalline amino acids to meet the N. R. C. requirements and supplemented with L-lysine · HCl and L-arginine to provide various

Table 2. Composition of basal diets--rat experiments.

Ingredient	<u>Trial 1</u> g/kg diet	<u>Trial 2</u> g/kg diet	<u>Trial 3</u> g/kg diet	<u>Trial 4</u> g/kg diet	<u>Trial 5</u> g/kg diet
Corn oil	100	50	---	50	100
Soybean meal	---	100	---	---	100
Zein	200	---	---	100	40
Sucrose	546	630	12	280	103
Wheat	---	---	925	500	---
Corn	---	---	---	---	500
L-Glutamic acid	---	---	9	10	100
Vitamin mix <sup>1</sup>	10	10	10	10	10
Mineral mix <sup>2</sup>	40	40	---	40	40
Amino acid mix	<sup>3</sup> 4	<sup>4</sup> 70	<sup>5</sup> 17	<sup>6</sup> 10	<sup>7</sup> 7
CaCO <sub>3</sub>	---	---	18	---	---
KH <sub>2</sub> PO <sub>4</sub>	---	---	9	---	---
Cellulose	100	100	---	---	---

<sup>1</sup> Vitamin mix contains 200 IU vitamin A, 6 mg  $\alpha$ -tocopherol, 0.01 mg menadione, 0.25 mg thiamine·HCl, 0.25 mg riboflavin, 0.12 mg pyridoxine·HCl, 1.5 mg niacin, 0.8 mg Ca pantothenate, 1.0 mcg vitamin B<sub>12</sub>, and 75 mg choline Cl, made up to 1 g with sucrose.

<sup>2</sup> Jones and Foster (Journal of Nutrition, 24:245).

<sup>3</sup> 0.3 g DL-histidine, 1.4 g DL-methionine, 1.3 g tryptophan, and 1.0 g valine. Additions of arginine and lysine were made at the expense of sucrose.

<sup>4</sup> 0.33 g L-cystine, 2.1 g DL-serine, 20 g L-glutamic acid, 3.2 g DL-alanine, 0.66 g DL-proline, 2 g DL-histidine, 2.5 g DL-isoleucine, 5 g DL-leucine, 4.5 g DL-valine, 4 g DL-threonine, 5.5 g DL-methionine, 7 g DL-phenylalanine and 1.2 g DL-tryptophan, made up to 70 g with sucrose. Additions of arginine and lysine were made at the expense of sucrose.

<sup>5</sup> 0.57 g DL-histidine, 0.23 g DL-isoleucine, 4.38 g DL-methionine, 3.42 g DL-phenylalanine, 1.76 g DL-threonine, 0.4 g DL-tryptophan, and 2.23 g DL-valine, made up to 17 g with sucrose. Additions of arginine and lysine were made at the expense of glutamic acid.

<sup>6</sup> 3 g DL-methionine, 0.5 g DL-threonine, 1 g DL-tryptophan and 2 g DL-valine made up to 10 g with sucrose. Additions of arginine and lysine were made at the expense of glutamic acid.

<sup>7</sup> 4 g DL-methionine, 1 g DL-tryptophan and 2 g DL-valine.

Table 3. Essential amino acid requirements<sup>1</sup> for growth, given as percent of diet.

	Swine, %	Rat, %	Chick, %
Arginine	0.20	---- <sup>2</sup>	1.2
Histidine	0.18	0.30	0.4
Isoleucine	0.50	0.50	0.75
Leucine	0.60	0.80	1.4
Lysine	0.70	0.90	1.1
Methionine	0.50	0.60	0.4
Threonine	0.45	0.50	0.7
Phenylalanine	0.50	0.90	1.3
Tryptophan	0.13	0.15	0.2
Valine	0.50	0.70	0.85
Glycine	----	----	1.60

<sup>1</sup> Adapted from National Research Council Nutrient Requirements.

<sup>2</sup> Exact amount required is not known.

lysine/arginine ratios (Table 4). This trial was intended to cover a wide range of lysine/arginine ratios to establish ratios in which favorable response might be obtained. Four rats were allotted to each of fifteen experimental diets and were fed for 24 days.

Since the rat trials were intended to precede swine studies, a diet more nearly approaching a typical swine ration was used in Trial 3 (Table 2). These diets based on wheat were also supplemented with amino acids as described above. Analysis of the wheat after the feeding experiment was conducted revealed that the lysine content (1.43%) was much higher than the values listed by the N. R. C. (Table 5) which resulted in a lysine level of 1.32% which exceeds the rat's requirement

Table 4. Summary of rat experiments.

Group	Calculated lysine level (%)	Calculated arginine level (%)	Average daily gain (g)	Average daily feed intake (g)
Trial 1				
1	0.9	0.36	1.14 ± .34	8.6
2	0.4	0.36	0.99 ± .33	9.2
3	0.4	0.61	1.31 ± .55	10.0
4	0.4	0.86	1.42 ± .10	11.0
5	0.4	1.36	1.05 ± .26	8.9
6	0.6	0.36	1.84 ± .36	10.1
7	0.6	0.61	1.86 ± .31	11.0
8	0.6	0.86	1.98 ± .67	10.4
9	0.6	1.36	1.44 ± .25	9.3
Trial 2				
1	0.3	0.32	1.93 ± .29	14.3 ± 2.1
2	0.3	0.57	1.89 ± .27	14.3 ± 0.9
3	0.3	0.82	1.94 ± .15	14.8 ± 0.3
4	0.3	1.07	1.87 ± .29	14.0 ± 1.0
5	0.3	1.32	1.97 ± .28	15.5 ± 1.7
6	0.6	0.32	3.62 ± .35	18.0 ± 0.6
7	0.6	0.57	3.69 ± .55	19.5 ± 1.6
8	0.6	0.82	3.00 ± .22	16.5 ± 1.3
9	0.6	1.07	3.78 ± .76	17.7 ± 5.7
10	0.6	1.32	3.10 ± .33	15.5 ± 0.9
11	0.9	0.32	2.67 ± .57	12.4 ± 1.8
12	0.9	0.57	2.80 ± .51	13.8 ± 2.3
13	0.9	0.82	2.68 ± .52	13.9 ± 1.3
14	0.9	1.07	3.13 ± .34	16.0 ± 1.6
15	0.9	1.32	3.49 ± 1.10	16.9 ± 4.8
Trial 3				
1	1.32	0.60	5.63 <sup>a</sup> ± .59	19.4 ± 1.2
2	1.32	0.85	4.35 <sup>b</sup> ± .35	17.0 ± 1.4
3	1.32	1.10	3.97 <sup>c</sup> ± .61	16.3 ± 1.6
4	1.42	0.60	5.60 ± .46	18.7 ± 1.3
5	1.42	0.85	4.88 ± .51	18.0 ± 1.7
6	1.42	1.10	5.83 ± .48	18.4 ± 0.9
7	1.57	0.60	5.53 ± .34	17.5 ± 0.7
8	1.57	0.85	5.72 ± .76	18.4 ± 1.9
9	1.57	1.10	5.92 ± .69	18.7 ± 2.0
10	1.72	0.60	5.56 ± .42	18.3 ± 1.1
11	1.72	0.85	5.04 ± .71	17.0 ± 1.8
12	1.72	1.10	5.65 ± .68	17.4 ± 2.7

<sup>b</sup> significantly lower than <sup>a</sup> ( $P < 0.01$ ); <sup>c</sup> significantly lower than <sup>a</sup> ( $P < 0.01$ )



Table 4 continued.

Group	Calculated lysine level (%)	Calculated arginine level (%)	Average daily gain (g)	Average daily feed intake (g)
Trial 4				
1	0.71	0.34	$0.27 \pm 0.19$	$9.4 \pm 1.4$
2	1.21	0.34	$3.28 \pm 0.95$	$13.7 \pm 2.4$
3	0.71	0.84	$0.16 \pm 0.20$	$8.5 \pm 0.9$
4	0.71	1.34	$0.24 \pm 0.16$	$8.6 \pm 0.7$
Trial 5				
1	0.38	0.53	$3.00 \pm 0.52$	14.1
2	0.38	1.03	$2.88 \pm 0.37$	13.3
3	0.38	1.53	$3.29 \pm 0.30$	14.8
4	0.58	1.03	$3.29 \pm 0.59$	13.7
5	0.58	1.53	$3.75 \pm 0.54$	14.3
6	0.96	1.18	$3.92 \pm 0.65$	16.1

Table 5. Amino acid values for wheat.

	Wheat, Pacific Coast <sup>1</sup> Dry	Trial 3 Sample <sup>2</sup> Dry
Arginine, %	0.80	0.65
Cystine, %	0.20	0.28
Glycine, %	1.00	0.50
Histidine, %	0.30	0.11
Isoleucine, %	0.60	0.40
Leucine, %	1.00	0.70
Lysine, %	0.51	1.43
Methionine, %	0.20	0.13
Phenylalanine, %	0.70	0.43
Threonine, %	0.40	0.29
Tryptophan, %	0.20	----
Tyrosine, %	0.51	0.25
Valine, %	0.60	0.51

<sup>1</sup> Adapted from National Research Council Nutrient Requirements (1968).

<sup>2</sup> Determined by amino acid autoanalyzer, Agricultural Chemistry Department, Oregon State University.

of 0.9% (Table 3). In this trial five rats were allotted to each of twelve rations and were fed ad libitum for 19 days.

Trial 4 was conducted to clarify results obtained with the high lysine wheat in Trial 3. In this trial zein was included as a protein source, but at a lower level than in Trial 1 and wheat was used as an energy source to make this a more practical type of diet (Table 2). The zein was included to lower the lysine level without reducing total dietary protein. Ten rats were randomly allotted to each of four test diets and each group was fed for 21 days.

In Trial 5, zein and soybean meal were combined to form the protein supplement for a corn based diet (Table 2). Corn was used in place of wheat in order to obtain a basal ration lower in lysine than was the case in the previous experiments. In this trial eight rats were allotted to each of six diets which were supplemented to give various lysine/arginine ratios and to meet the N. R. C. requirements for rats (Table 3). The rats were fed ad libitum for 24 days.

#### Japanese Quail Experiments

The objective of the first of two trials using Coturnix quail was to examine the findings of Abbott et al. (1969 a, b) using another avian species of the same physiological age. The Coturnix are able to reach maturity in six weeks and are comparable to eight-week-old broilers at two weeks of age, at least in terms of sexual maturity (G. H. Arscott, Personal Communication). In the first trial eight groups of ten birds each of the Oregon State University strain of Japanese quail were used. The Coturnix quail respond well under experimental conditions only when environmental temperature is controlled around 100°F. Heat lamps overhead and heating elements under the cages were used to maintain cage temperature.

Light was supplied 24 hours daily during the 14 day experimental period. Water was supplied by automatic waterers and feed was fed free choice from hanging feeders.

The experimental diets (Table 6) based on 50% wheat and from 20 to 22% zein, were fed to seven of the eight groups. The eighth group was fed a soybean meal, glucose monohydrate control ration. Two controls within the seven experimental groups were also used, one was fed the N. R. C. 's chick requirements for arginine and lysine (Table 3) and the other group was fed the N. R. C's turkey requirements for these amino acids. The multiple control system was used because of the lack of established requirements for these amino acids by the Coturnix. The other amino acids were supplied at levels believed to be adequate for these birds. Vitamins and minerals were provided in adequate amounts (Table 6). The Coturnix's high protein requirement was met by the wheat and zein included in these diets. Kjeldahl<sup>3</sup> analysis showed these rations to contain from 28% to 32% crude protein.

The object of the second quail trial was to examine the response of the quail to diets low in lysine and high in arginine at two separate protein levels (Table 6). In this trial nine groups of ten birds each were housed and fed for 18 days in the same manner as in the first trial. Individual bird weights were recorded so statistical difference could be noted between treatments.

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<sup>3</sup>Kjeldahl  $N_2 \times 6.25$ .

Table 6. Composition of basal diets--quail experiments.

Ingredient	OSU Control <sup>1</sup> g/kg diet	Trial 1 <sup>4</sup> g/kg diet	Trial 2 <sup>4</sup>	Trial 2 <sup>4</sup>
			20% protein g/kg diet	27% protein g/kg diet
Soybean oil meal	590	---	150	150
Cerelose	285	190	42	152
Soybean oil	40	40	40	40
Cottonseed meal	---	---	150	150
Zein	---	210	---	100
Corn	---	---	500	300
Wheat	---	500	---	---
Vitamin mix <sup>2</sup>	6	6	6	6
Mineral mix <sup>3</sup>	60	60	60	60
DL-methionine	5	2.7	5	5
Glycine	2	5.5	5	5
Glutamic acid	---	---	30	20
Choline-Cl	12	12	12	12
L-arginine	---	4.5	---	---
L-lysine	---	8.8	---	---
L-isoleucine	---	10.0	---	---
L-tryptophan	---	1.6	---	---

<sup>1</sup> Adapted from R-360 (G.H. Arscott, Unpublished Data).

<sup>2</sup> Gordon's B complex vitamin mixture (Science, 122:1270) supplemented with 928 mg vitamin E, 333 mg vitamin A, 1330 mg vitamin D<sub>3</sub> and 125 mg BHT per kg diet.

<sup>3</sup> Journal of Nutrition, 72:243.

<sup>4</sup> Additions of arginine and lysine were made at the expense of cerelose.

Table 7. Summary of growth response of Japanese quail to various lysine:arginine ratios.

Trial 1				
Group	Treatment	Calculated lysine level(%)	Calculated arginine level(%)	Average 14 day gain (g)
1	OSU control	1.7	1.9	40.6 <sup>1</sup>
2	NRC chick requirement	1.1	1.2	18.8
3	Low arginine-low lysine	0.6	0.7	4.6
4	Normal arginine-low lysine	0.6	1.2	4.2
5	High arginine-low lysine	0.6	1.6	4.6
6	High arginine-normal lysine	1.1	1.6	17.0
7	NRC turkey requirements	1.5	1.6	17.5
8	High arginine, normal lysine	1.5	2.0	14.7
Trial 2				
Group	Treatment	lysine level(%)	arginine level(%)	Average 17 day gain(g)
1	OSU control	1.7	1.9	54.5 ± 6.9
2	20% protein	0.75	1.3	35.3 ± 5.4
3	20% protein	0.75	1.8	35.0 ± 4.6
4	20% protein	0.75	2.3	36.6 ± 8.8
5	20% protein	1.35	1.5	39.6 ± 9.4
6	27% protein	0.71	1.4	31.0 ± 6.8
7	27% protein	0.71	1.9	28.0 ± 6.4
8	27% protein	0.71	2.4	29.4 ± 8.2
9	27% protein	1.31	1.4	46.2 ± 4.0

<sup>1</sup> Preliminary trial run in groups of 10 birds each, individual weights not recorded.

Two protein levels were used because of the poor growth response obtained using zein as the protein source. Four of the diets were based on a ground corn, soybean and cottonseed meal combination which resulted in a lower protein level (Table 6). The other four experimental diets (Table 6) had 10% zein added to them to raise the protein level to meet the quail's requirement. The ninth ration was the soybean meal control diet used in Trial 1.

### Swine Experiment

This trial was designed to test the response of swine to arginine supplementation of a low lysine diet. Three diets were formulated (Table 8) in which a low lysine diet was supplemented with added DL-glutamic acid, L-arginine or L-lysine·HCl to meet the N. R. C. requirement (Table 3) for the growing pig.

Table 8. Composition of rations used in swine experiment.

Ingredient	Ration 1 g/kg	Ration 2 g/kg	Ration 3 g/kg
Corn	510	510	510
Wheat	400	400	400
Alfalfa	50	50	50
Herring meal	15	15	15
Limestone	12	12	12
Methionine hydroxy analogue	4.75	4.75	4.75
Iodized salt	5	5	5
DL-glutamic acid	3	---	---
L-arginine	---	3	---
L-lysine·HCl	---	---	3

Each ration was supplemented with 1320 IU vitamin A, 132 IU vitamin D, 194 mg ZnSO<sub>4</sub>, and 1 g Aureo-SP-250 per kg.

In the trial, 24 Yorkshire x Berkshire barrows averaging about 23 kg. in initial weight were randomly allotted in groups of eight to the three treatment groups from a uniform population of barrows. The pigs were individually housed in half concrete, half slotted floor pens. They were fed ad libitum from individual self-feeders and had free access to water from automatic waterers. Feed consumption and body weight data were recorded weekly for eight to nine weeks.



## RESULTS AND DISCUSSION

### Rat Experiments

No "sparing effect" of arginine when added to low lysine diets was observed in any of the rat experiments. A significant negative response was noted in Trial 3, in which a reduction in growth was observed with arginine supplementation of the lowest lysine level used (Table 4). This is difficult to explain, but it could be construed as evidence that the basal diet was just adequate in lysine and that the added arginine provoked a lysine deficiency that was over come when the lysine level was increased. However, if this explanation were correct, arginine supplementation should have produced even greater adverse effects in the other experiments where the basal lysine levels were calculated to be even lower. For example, in Trial 4, where the lysine deficiency was severe, an antagonistic effect of arginine on lysine utilization would presumably have resulted in more severe growth restriction with added arginine than without, but this did not occur (Table 4). Even though an explanation for the growth depression with added arginine at the lowest lysine level in Trial 3 is not apparent, the results of the rat trials indicate quite conclusively that growth performance on low lysine diets is not improved by increasing the arginine level.

An interpretation of the poor growth response observed in the trials which included zein as part or all of the protein supplement may be difficult, but similar results have been seen by others. Hill, McIndoo and Olsen (1961) reported in their work with chicks that the addition of 15% zein to a diet containing 9.5% soybean protein produced a significant reduction in weight gain and in feed consumption, which is similar to the response seen with the rats used in this study. They also noted a marked decrease in the level of lysine in deproteinized plasma and some smaller decreases of other amino acids and increases of still others. Since zein is almost completely lacking in lysine, this deficiency may put limitations on growth even when lysine is supplemented. This is due to the reduced food consumption created by the addition of this imbalanced protein source to the diet. This was pointed out by the work of Combs et al. (1964). They found that in the chick, the level of certain excess amino acids, or their metabolites which accumulate in the blood when high levels of protein or imbalanced protein mixtures are fed, may reduce the voluntary consumption of energy by the chick which reduces average daily gains and feed efficiency. This type of growth impairment was clearly seen in the rats and dictated the switch from zein to other protein supplements as much as possible.

The problem which occurred in Trial 3 is an obvious reminder to us all that we cannot take a given sample of feed and suppose it is

an average for that type of feed. A sample of high lysine wheat may be encountered infrequently, but this one example does emphasize the benefits of testing a sample of feed before use, to be relatively certain of what you have, especially if a large quantity is being used. Table 5 shows the amino acid composition of the wheat used in Trial 3.

Even though the high lysine wheat in Trial 3 may have modified the results, it did show that growth was reduced with the low lysine diets in the other trials. This can be clearly noted in Table 4.

#### Quail Experiments

Since Abbott et al. (1969 a, b) reported a favorable response to arginine with low lysine diets in chickens older than four weeks of age, a suitable comparison was needed. The Japanese quail are said to be at the same stage of maturity at one week of age as four-week-old chickens (G. H. Arscott, Personal Communication). With this relationship in mind, the quail used in these experiments should have been comparable to the chickens used by Abbott et al. (1969 a, b).

The effect of zein on growth noted by Hill et al. (1961) was obvious in the results obtained with the quail. No significant response to arginine supplementation at various lysine levels was noted (Table 7). The 20% protein basal ration was apparently limiting in some other amino acid, since there was little response to added lysine. A lysine deficiency was definitely produced on the 27% protein basal ration,

since there was a marked response to added lysine (Table 7). A definite growth impairment, poor feathering and a white band appeared on the wings which has been reported to be characteristic of a lysine deficiency in chickens. Klain et al. (1957) observed this achromatosis in the feathers of several breeds of poultry and noted it to be one of a few examples of a specific symptom caused by a deficiency of a single amino acid.

Even though there is a limited amount of work that has been done on the amino acid requirements of the Coturnix quail, the results of these trials fail to give any evidence of the "sparing effect" of arginine on lysine requirements. It was obvious that a low lysine condition did exist and that there was a definite response to lysine, but not to arginine supplementation. Another aspect of interest may be noted in that the high level of arginine added did not apparently interfere with lysine utilization, which suggests that the Coturnix may have a fairly high tolerance level for arginine.

#### Swine Experiment

The economic impact on pork production would be significant if the "sparing effect" of arginine on lysine was found to hold true for swine. Since lysine is one of the most frequently limiting amino acids, any method of reducing the lysine requirement would be very valuable.

The results of the swine experiment can be seen in Table 9. A significant response ( $P < 0.001$ ) to lysine supplementation of the basal ration was noted, indicating that a low lysine situation was indeed present. Arginine supplementation of the low lysine ration did not improve growth; in fact, the only response noted was a slight depression in growth when comparing the arginine supplemented ration to the unsupplemented control ration. This may have been due to a reduction in lysine absorption in the presence of added arginine as was reported by Buraczewski et al. (1970). This effect would have created an even greater lysine deficiency than was present in the low lysine control group.

Table 9. Response of swine to arginine supplementation of a low lysine ration.

Treatment	Days on test	Average daily gain (g)	Kg feed/Kg gain
Basal + 0.3% glutamic acid	59	531 $\pm$ 104	3.5 $\pm$ 0.3
Basal + 0.3% arginine	66	459 $\pm$ 82	3.4 $\pm$ 0.4
Basal + 0.3% lysine	59	817* $\pm$ 118	3.2 $\pm$ 0.4

\*Significantly higher ( $P < 0.001$ ) than means of the other groups.

In conclusion, in this experimental work with the rat, Japanese quail and swine fed low lysine diets, no evidence of a "sparing effect" of arginine on lysine was found. This is in contrast to the published reports of Abbott et al. (1969 a, b) indicating that the four-week-old

chick is capable of normal growth on a low lysine diet when added arginine is present.

There may be several factors to consider before drawing a definite conclusion about the discrepancies between these results. A difference in physiological maturity of the animals may play an important role, but the quail should have been comparable even if the rats and swine were not. The difference in the lysine and arginine requirements of poultry, rats and swine may influence the significance of interrelationships between them in these species. For example, Acheampong-Mensah and Hill (1970) have shown that the toxicity of lysine to rats is much less severe than for chicks and the response to arginine supplementation of high lysine diets is less in rats than in chicks. The fact that the nitrogen metabolism of rats and swine is different from the avian species is a major consideration when trying to compare a given response. These and other differences attributed to the complexities of amino acid interrelationships lead to the conclusion that there will undoubtedly be many contradictory results found by workers in this field.

## SUMMARY

The nutritional and economic potentials of proving that arginine could "spare" the lysine required in a low lysine monogastric diet led to this experimental work. Results obtained from eight separate trials using rats, Japanese quail and swine fed low lysine diets showed no evidence of a "sparing effect" of arginine on lysine. It was concluded that arginine supplementation is of no value in reducing the adverse effects on growth of low lysine diets fed to these species. This is in contrast to published reports indicating that supplemental arginine added to a low lysine diet was able to maintain maximum growth in chicks from four to eight weeks of age. Possible interpretations of these results were discussed.

## PART II: SOME IMPLICATIONS OF FEEDING DRIED WHEY TO SWINE

### INTRODUCTION

Whey, a by-product from the cheese industry, has been utilized by hog producers for centuries. Even in ancient Rome, Cato mentioned that whey was used for pig feeding and noted it to be a valuable supplement when used properly (MacDonald and Redelmeier, 1967).

Today with the technology of modern man whey no longer has to be fed in the liquid form. Several drying processes have been developed to turn this often termed waste product into a usable feedstuff. Besides its use in animal feeding, whey powder is being used as an ingredient for breads, cheese spreads, salad dressings, jams, cakes, cookies, soups and many other foods. Whey derivatives are used in baby foods and some pharmaceuticals. In Europe, a high quality liquid whey is being developed into an acceptable soft drink (MacDonald and Redelmeier, 1967). Whey is changing from a product of negative value in many areas where disposal of surplus whey has created an expensive disposal problem, to a product of increasing worth to the agricultural economy.

The feeding of whey to hogs has diminished in recent years due to changes both on the farm and at the factory. Automation of most feeding equipment and new regulations and procedures for shipping



milk from farm to factory have made it less economical for farmers to use liquid whey. With this decline in farm use, more whey had to be utilized at the factory. New drying installations were built to process the whey into a usable product, but each year the number of cheese factories decreases and the distance between them increases. The cost of drying whey prohibits each plant from having its own dryer, and hauling a product which is 93% water becomes very expensive. This leaves a thin line between profit and loss on this by-product. Most factories would prefer to dump whey as a waste product, but because of its organic nature, all whey has a high biological oxygen demand and is difficult to dispose of by conventional means. Since raw whey is regarded as a serious pollutant, its disposal is receiving increasing attention as pollution becomes an ever increasing problem to today's society (MacDonald and Redelmeier, 1967; Fife and Nilson, 1969).

The study of the use of dried whey in the feeding of hogs has gone on for many years. Hog raisers in the midwest are using dried whey in their creep and starter rations to a much greater extent than their counterparts here on the West Coast. Since the supply of whey products will increase sharply in the near future, as dairy plants process more by-products to limit pollution, new processes and products will need to be developed to widen the use of whey. But immediate promise of absorbing some of the growing supply is through

expansion of present uses (Mathis, 1970). Animal feeding, being one of the major uses of dried whey, should be developed to its fullest extent.

Milk by-products, such as whey, play a very important role in successful swine operations and will in all probability continue to play an increasingly important role in the swine industry of tomorrow.

The study undertaken in Part II of this thesis examines the levels at which dried whey can be best utilized and some implications of the use of dried whey in swine rations.

## LITERATURE REVIEW

Whey is the by-product of the cheese industry. Since cheese consists largely of the casein and butterfat of milk, whey is milk minus these constituents. Whey contains lactalbumin and lactoglobulin, many of the milk minerals, a high percentage of milk sugar, and an excellent collection of the water soluble vitamins present in milk (Table 10). Until quite recently, whey was nearly ignored as a food product. Yet in the past it was a popular social drink and was prescribed by early physicians for many ailments (Weisberg and Goldsmith, 1969). As of now, whey is the largest reservoir of non-fat milk solids that still remains largely outside of human food supply channels. In 1969, about 21 billion pounds of whey were produced; of this, only about one-third was dried. Of the 6 to 7 billion pounds that were dried, 45 percent was processed for human food, the rest for animal feed. Large volumes of dried whey and an undetermined amount of liquid whey continue to be used in the feed industry. Unidentified factors in whey contribute to the efficient growth and good health of livestock, but the feed industry often feels that it can get its protein, vitamins, and minerals from cheaper sources.

Specialty feeds are a more profitable place for whey products to be used. Most milk replacers for calves and pigs are high in whey products. These products produce a better return for whey than when

Table 10. Comparative analyses of dried whey and wheat.<sup>1</sup>

	Dried Whey	Wheat
Digestible energy kcal/kg	3432.0	3618.0
Protein %	14.7	11.1
Lactose %	67.7	---
Ash %	10.3	2.1
Ether Extract %	0.9	2.2
Moisture as fed %	6.0	10.8
Fiber %	---	3.0
Calcium %	0.93	0.14
Phosphorus %	0.84	0.34
Magnesium %	0.14	0.18
Potassium %	2.3	0.58
Sodium %	1.0	0.10
Arginine %	0.40	0.71
Histidine %	0.22	0.27
Lysine %	1.10	0.45
Methionine %	0.38	0.18
Tryptophane %	0.24	0.18
Niacin mg/kg	11.9	66.3
Pantothenic Acid mg/kg	50.8	12.9
Riboflavin mg/kg	31.8	1.2
Thiamine mg/kg	3.9	5.5
Biotin mg/kg	0.4	0.1
Folic Acid mg/kg	0.9	0.4

<sup>1</sup> Adapted from National Academy of Sciences Publication 1599. 1968.

it is marketed as a single feed ingredient.

Weisberg and Goldsmith (1969) feel with increasing centralization of cheese operations into larger units, with growing per capita consumption of cheeses, and with increasing restrictions on stream pollution, we may expect accelerated production of dried whey. The tendency will be toward more whey for human consumption to be produced as more refined drying plants are developed. Until the cost of this type of operation can be minimized, feed grade whey will be plentiful.

The early discovery that whey makes an excellent supplement for feeding swine has led to extensive studies of its utilization by swine of various ages. Becker et al. (1954 a, b) reported a series of tests in which they compared the nutritive value of various carbohydrates with pigs of two different ages. With the baby pig, lactose fed at 56.6% of the diet produced a rate and efficiency of gain superior to either glucose or starch, without any evidence of diarrhea. The finishing pig performed very well at a feeding level of 25% lactose and showed no diarrhea. These workers concluded that finishing swine could be fed a ration containing 35% dried whey without the occurrence of a lactose-induced diarrhea. Krider et al. (1949) had reported the occurrence of diarrhea in young swine fed 4% to 8% dried whey product. They felt that lactose was the causative factor, but this later work seems to lead to the conclusion that if care is taken to avoid sudden feed changes, scours can be avoided with relatively high levels

of dried whey. In a later study (Becker et al., 1957), finishing pigs were fed levels from 0 to 60% dried whey. A level of 60% dried whey produced a depression in growth and a marked diarrhea, but all other levels of whey yielded satisfactory performance, although there was some evidence of diarrhea at the 40% level. These results suggest that dried whey may be fed at levels under 40% satisfactorily. In another early study Callaghan and McDonald (1940) found that when dried whey replaced part or all of the meat meal as a supplement to a wheat based diet, the gains were increased from 1.21 to 1.49 and 1.32 pounds per pig per day respectively. Since the most rapid gains were obtained when the dried whey replaced part of the meat meal, this indicates a supplementary effect of the whey. Krider et al. (1949) reported that the addition of either 2% or 4% fortified dried whey by-product supplied essential nutritional factors which significantly improved growth and feed efficiency of the weanling pig.

Dried whey has received most attention in the area of unidentified growth factors for poultry and swine (Fisher, Scott and Hansen, 1954; Gard et al., 1955). In some early work with chicks, Reed, Atkinson and Couch (1951) carried out four experiments where 3 to 5% dried whey was added to an all-vegetable protein diet. The feeding of the whey produced an increase in weight gain in all instances, even when the birds were given an animal protein factor concentrate with aureomycin or penicillin. This agrees with earlier reports (Berry

et al., 1943; Hill, 1948) that dried whey contains unidentified factors which are necessary for maximum chick growth. The NCR-42 Committee on Swine Nutrition (1970) recently reported in a study of unidentified factors, that the inclusion of 5% dried whey in a standard growing ration fed to pigs at six stations resulted in a significant increase in gains. These results substantiate the hypothesis that dried whey does possess some added factors which are important to animal growth.

Baker et al. (1963) pointed out that there is a wide variation in the nutritive value of dried wheys prepared by either the roller or the spray method of drying. When weanling rats were fed diets which included dried whey as an energy source the processing method had a definite influence on the rate and efficiency of gains. A wide variation in nutritive value was found among spray dried and among roller dried samples. In their work with pigs Lantzsch and Schneider (1969) found that digestibility also varied with types of whey. They found 97.7% protein digestion with sweet dried whey and 82.8% with sour dried whey. In a study carried out by Tomarelli and Bernhart (1962), it was found that whey protein and a mixture of whey and milk protein were superior to milk protein alone when fed to young rats. Their tests of commercial infant foods containing either milk protein alone or the whey-milk protein mixture also demonstrated a nutritional superiority for the mixture. The value of whey protein and whey components is now being realized in both the food and feed industry.

Since whey is approximately 70% lactose, it is a good source of this milk sugar. Lactose is of special interest in nutrition, since it makes up nearly half of the solids of milk, nature's food for the young. The young animal secretes lactase, the enzyme necessary for the digestion of lactose. Lactase production increases gradually in the pig, reaching its maximum between the first and second week of life, and then decreases rapidly (Bailey, Kitts and Wood, 1956). It has been noted by several workers (Whittier, Cory and Ellis, 1934; Riggs and Beaty, 1947; Fischer et al., 1949) that older animals show degrees of adaptation when lactose is fed for an extended period of time.

Since the young pig utilizes lactose efficiently, a considerable quantity is used in starter rations and many workers have studied the effects of high levels of lactose in the diet (French and Cowgill, 1937; Bacigalupo et al., 1950; Lengemann, 1959). In his work with rats, Lengemann (1959) found that lactose enhances mineral absorption within the intestine. Sewell and West (1965) found significant increases in apparent protein digestibility in pigs fed diets containing lactose when compared with the basal diet containing soybean protein without lactose. They also noted an improvement in the digestion of the ether extract fraction of the diet. These results indicate that lactose plays a physiologically important role in nutrient absorption and utilization and accounts for at least a part of the difference in response to proteins



from milk sources as compared to vegetable protein when fed to the young animal.

It appears certain that milk nutrients are very important to young pigs. Besides the fact that lactose increases mineral, fat and protein metabolism, it has been found to be important in establishing the proper flora in the large intestine of the young pig. A well established microflora of lacto bacillus organisms may prevent many disease problems common to the young pig (Lambert, 1970).

It was noted by Lambert (1970) that care should be taken in feeding dried whey due to its high salt content. Dried whey contains about 2% NaCl and since 0.5% NaCl has been found to be the optimum level for most pig starter rations, a ration containing 18% whey product will provide all the NaCl required. If the normal level of salt is added along with whey, palatability will be reduced and scours may result.

Whey and whey products provide nutrients almost as economically as other energy and protein sources. Since whey usually improves the palatability of feeds and increases daily gains and feed efficiency, its addition to livestock feeds can prove very beneficial.

## OBJECTIVES

This experimental work was designed to examine the use of dried whey in swine grower and starter rations. Various levels of dried whey were used and some relationships of whey to other ingredients and feed processing methods were studied in an effort to increase the utilization potential of this surplus feedstuff.

## EXPERIMENTAL PROCEDURE

### Swine Trial 1

The intent of this experiment was to compare the effects of the addition of 10% dried whey to rations with and without 20% alfalfa. Since in the older pig lactose is only slightly digested in the small intestine, because of inadequate lactase secretion, it is digested in the large intestine by bacterial action. It was hypothesized that increased fiber digestion and hence greater utilization of alfalfa might result from the inclusion of whey in a high alfalfa ration.

A group of eight Yorkshire x Berkshire barrows averaging 25 kg. in initial weight were randomly allotted to each of four treatments (Table 11). The pigs on each ration were individually penned and fed ad libitum from self-feeders. Automatic waterers were provided in each pen and all rations were fed in pelleted form. Feed consumption, weight gains, and carcass data were recorded on each pig.

### Swine Trial 2

Trial 2 was essentially a repeat of the swine experiment in Trial 1, except that the pigs were housed in groups of 12 and fed rations 2, 3 and 4 from Trial 1 along with the standard O. S. U. grower ration (Table 11) as a control. Three groups were allotted to the O. S. U. control ration and ration 2. Rations 3 and 4 were fed to two groups

Table 11. Composition of experimental swine rations--trials 1 and 2.

	Ration 1	Ration 2	Ration 3	Ration 4	OSU control
Ingredient	5% alfalfa control lbs./ton	10% dried whey, lbs./ton	20% alfalfa lbs./ton	10% dried whey + 20% alfalfa lbs./ton	
Corn	1017	---	---	---	---
Wheat	800	1629	1449	1248	1540
Dried Whey	---	200	---	200	---
Alfalfa	100	---	400	400	140
Fish Meal	30	40	40	40	---
Soybean Meal	---	---	---	---	100
Cottonseed Meal	---	80	80	80	---
Bone Meal	24	20	---	---	---
Limestone	4	12	---	---	10
Methionine HA	9.5	6.6	6.6	6.6	---
Salt	10	10	10	10	10
Lysine	6	2	2	0.6	---
Molasses	---	---	---	---	100
Dicalcium Phosphate	---	---	22	15	---
Tankage	---	---	---	---	100

Note: Each ration contained 200 gm  $\text{ZnSO}_4$ /ton, 1,200,000 IU vitamin A/ton, 12,000 IU vitamin D/ton, and 2 lbs. Aureo-SP-250/ton (contains per pound: 20 g Aureomycin, 4.4% sulfamethazine and 10 g penicillin).

each. The pigs in each group were fed from an initial average weight of about 20 to 30 kg. to about 80 kg.

### Swine Trial 3

Because of the promising results obtained with the 10% dried whey ration in Trials 1 and 2, Trial 3 was set up to establish the minimum level of dried whey needed to produce the most efficient gains. It is desirable to obtain the lowest level that will give increased performance since high levels of dried whey in a ration are difficult to pellet, whereas a low level serves as a good pellet binder. In this trial, levels of 0, 2, 4, 6, and 8 percent dried whey were used in wheat based rations (Table 12). Eight Yorkshire x Berkshire barrows averaging 25 kg. in initial weight were randomly allotted to each of the five treatments and penned and fed as in Trial 1 at the Oregon State University Swine Center.

### Swine Trial 4

Since dried whey has been used more extensively as an ingredient in swine starter rations, Trial 4 was used to compare dried whey to dried buttermilk in a starter ration. In this trial three treatments were used (Table 13). These were each fed to four litters until the pigs averaged 25 kg. To minimize the sow's influence, only litters of from eight to ten pigs each were used.

Table 12. Composition of experimental swine rations--trial 3.

Ingredient	Ration				
	A1 lbs. /ton	A2 lbs. /ton	A3 lbs. /ton	A4 lbs. /ton	A5 lbs. /ton
Wheat	1778	1738	1698	1658	1618
Alfalfa	60	60	60	60	60
Soybean Meal	80	80	80	80	80
Fish Meal	40	40	40	40	40
Dried Whey	---	40	80	120	160
Bone Meal	16	16	16	16	16
Limestone	16	16	16	16	16
Salt	10	10	10	10	10

Note: Each ration contained 200 gm ZnSO<sub>4</sub>/ton, 1,200,000 IU vitamin A/ton, 12,000 IU vitamin D/ton and 2 lbs. Aureo-SP-250/ton (contains per pound: 20 gm Aureomycin, 4.4% sulfamethazine and 10 gm penicillin).

Table 13. Composition of experimental creep rations--trial 4.

Ingredient	Ration 1 OSU, 6% dried buttermilk control lbs. /ton	Ration 2 10% dried whey lbs. /ton	Ration 3 20% dried whey lbs. /ton
Alfalfa Meal	100	100	100
Soybean Oil Meal	200	200	200
Wheat	1360	1265	1070
Fish Meal	100	120	120
Dried Buttermilk	120	---	---
Dried Whey	---	200	400
Salt	10	5	---
-----			
Proximate Analysis			
Content	Ration 1 %	Ration 2 %	Ration 3 %
Dry Matter	89.74	90.26	90.08
Crude Protein	19.15	19.05	18.85
Ash	4.24	5.02	5.61
Ether Extract	2.76	3.15	3.06
Acid Detergent Fiber	5.76	6.16	5.06

Note: Each ration contained 10 lbs. limestone, 5 lbs. Aureomycin-SP-250 (contains per pound: 20 gm Aureomycin, 4.4% sulfamethazine and 10 gm penicillin), 12 oz. zinc sulphate, and 180,000 IU irradiated yeast.

### Rat Trial 1

A fiber digestion trial was carried out using male Long Evans rats<sup>4</sup> to examine the effects of lactose on fiber digestion. Four rations were designed using 20% casein and 30% starch as a base; to this was added either alfalfa or cellulose (Alphacel) at equivalent fiber levels to test fiber digestion in the presence of 25% lactose compared to 25% sucrose (Table 14). Three rats were allotted to each treatment and fed ad libitum for six days. Fecal collections were taken and the digestibility of acid detergent fiber was determined by the method of Van Soest (1963).

Table 14. Diets used in rat trial 1.

Ingredient	Ration, gm/kg			
	1	2	3	4
Casein	200	200	200	200
Lactose	250	---	250	---
Sucrose	---	250	---	250
Corn Starch	300	300	---	---
Corn Oil	100	100	100	100
Alfalfa	---	---	400	400
Cellulose	100	100	---	---
Vitamin Mix <sup>1</sup>	10	10	10	10
Minearl Mix <sup>2</sup>	40	40	40	40

<sup>1</sup> Vitamin mixture contains 200 IU vitamin A, 6 mg alpha-tocopherol, 0.01 mg menadione, 0.25 mg thiamine HCl, 0.25 mg riboflavin, 0.12 mg pyridoxine HCl, 1.5 mg niacin, 0.8 mg pantothenate, 1.0 mcg vitamin B<sub>12</sub>, and 75 mg choline Cl, made up to 1 gm with sucrose.

<sup>2</sup> Jones and Foster (Journal of Nutrition 24:245).

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<sup>4</sup> Simonsen Laboratory, Gilroy, California.



### Rat Trial 2

Rat trial 2 consisted of a series of three rat experiments. These experiments were designed to examine the significance of interactions occurring between lactose and amino acids that may occur with heating, such as the Browning or Maillard Reaction. Because of the heat used in the pelleting processes, it is conceivable that the formation of unavailable lactose-amino acid complexes could be significant in whey-containing rations.

The basal diets used in this trial can be seen in Table 15.

Trial 2A, consisting of six treatments (Table 16) with four rats each was conducted for 21 days. Each ration was autoclaved for two hours at 116°C to determine the effects of the lactose under heat treatment similar to that which might be encountered during the pelleting process.

Because significant decreases in gains were seen in the treatments in Trial 2A with added lactose, Trial 2B was designed to compare various carbohydrate sources and additives which may have an effect on the Browning Reaction. Thirteen diets (Table 16) were designed and five rats were allotted to each. The animals were fed ad libitum for 13 days.

To further examine this reaction, Trial 2C was conducted to test the effects of various minerals which are commonly added to animal feeds. The effect of the saturation of fats on this reaction was

Table 15. Composition of basal diets--rat trial 2.

Ingredient	Experiment		
	2A g/kg	2B g/kg	2C g/kg
Corn	650	592.5	---
Wheat	---	---	592.5
Starch	50	100	---
Lactose	---	---	100
Fishmeal	70	70	70
Mineral Mix <sup>1</sup>	40	40	40
Vitamin Mix <sup>2</sup>	10	10	10
Cellulose	72.5	80	80
Lard	---	100	100
Vegetable Oil	100	---	---
Amino Acid Mix <sup>3</sup>	7.5	7.5	7.5

<sup>1</sup> Jones and Foster (Journal of Nutrition 24:245).

<sup>2</sup> Vitamin mixture contains 200 IU vitamin A, 6 mg alpha-tocopherol, 0.01 mg menadione, 0.25 mg thiamine HCl, 0.25 mg riboflavin, 0.12 mg pyridoxine HCl, 1.5 mg niacin, 0.8 mg Ca pantothenate, 1.0 mcg vitamin B<sub>12</sub>, and 75 mg choline Cl, made up to 1 g with sucrose.

<sup>3</sup> Two g methionine, 1 g each of phenylalanine, valine, histidine, isoleucine, threonine and 0.5 g tryptophan.

Table 16. Treatments<sup>1</sup> --rat trial 2 showing modifications to basal diets (Table 15).

Group	Experiment		
	2A	2B	2C
1	5% starch	10% starch	10% lactose
2	5% lactose	5% starch 5% sucrose	10% lactose + $\text{CuSO}_4$
3	5% lactose + 0.3% lysine	10% sucrose	10% lactose + $\text{ZnSO}_4$
4	10% lactose	5% starch 5% lactose	10% lactose + $\text{CaCO}_3$
5	10% lactose + 0.3% lysine	10% lactose	10% lactose + vegetable oil <sup>2</sup>
6	10% lactose + 0.3% lysine + 0.2% methionine + 0.1% tryptophan	5% starch 5% whey	10% lactose + 8% alfalfa <sup>3</sup>
7		10% whey	
8		5% starch 5% molasses	
9		10% molasses	
10		5% lactose 4% starch 1% glutamic acid	
11		5% lactose 4% starch 1% urea	
12		5% lactose 4% starch 1% $(\text{NH}_4)_2\text{SO}_4$	
13		5% lactose 5% $\text{NaHCO}_3$	

<sup>1</sup> Each diet autoclaved at 116°C for 2 hours.

<sup>2</sup> 10% vegetable oil replaces 10% lard.

<sup>3</sup> 8% alfalfa replaces 8% cellulose.

tested by substituting vegetable oil for lard in one treatment and the addition of alfalfa in place of cellulose was also examined. Five rats were assigned to each of the six treatments (Table 16) and fed for 12 days.

## RESULTS AND DISCUSSION

### Swine Trial 1

The objective of this trial was to determine if the lactose in dried whey might influence the utilization of high alfalfa rations by growing pigs. The inclusion of lactose might increase fiber digestion through stimulation of bacterial growth in the large intestine; such effects probably would be reflected in improved animal performance. The growth rates and feed conversions observed in both groups fed 20% alfalfa compared favorably with the controls (Table 17). The pigs fed alfalfa had slightly longer and leaner carcasses (Table 17), a result that has been observed by others (Stevenson, Davey and Hiner, 1960; Danielson, Butcher and Street, 1969). These results indicate that high-quality alfalfa can be added to rations in fairly high levels with good results. The added whey apparently did not improve the utilization of alfalfa, and may in fact have had a negative effect, since the lowest gains and feed efficiencies were observed in this group (Table 17). Ration 2, which contained 10% dried whey without alfalfa, produced the best results. A significantly higher feed efficiency ( $P < 0.05$ ) was obtained over both groups fed the 20% alfalfa rations. The excellent feed efficiency and high rate of gain recorded by the pigs fed this 10% dried whey ration might be explained by the favorable effects of whey on protein and fat digestibility as was noted by Sewell

Table 17. Growth performance and carcass data of swine--trial 1.

Ration	Treatment	Average daily gain lbs.	Feed per lb. gain lbs.	Average carcass length in.	Average back fat in.	Average loin eye sq. in.
1	5% alfalfa control	1.95 ± 0.13	2.93 ± 0.26	28.95	1.6	3.4
2	10% whey	2.15 ± 0.24	2.71 ± 0.19	29.34	1.7	3.7
3	20% alfalfa	1.99 ± 0.29	3.04 ± 0.25	29.37	1.5	3.5
4	20% alfalfa + 10% whey	1.77 ± 0.27	3.33 ± 0.22	29.41	1.4	3.6

and West (1965). It was also noted that the pigs on the dried whey rations showed no signs of diarrhea during the trial, but two of the animals on the control diet did show some intestinal disturbance when first introduced to the test ration.

### Swine Trial 2

In this trial the influence of group feeding on the results obtained with the swine rations used in Trial 1 was studied. The results (Table 18) show no significant differences, but the dried whey rations produced the best feed efficiencies, which supports the findings of the NCR-42 Committee on Swine Nutrition (1970) that whey does have some growth factors important to the growing pig. It was also observed again that the addition of 10% dried whey to the 20% alfalfa ration failed to produce better gains than the 20% alfalfa ration without whey.

Table 18. Growth performance of pigs on group feeding--Trial 2.

Ration	Treatment	Average daily gain lbs.	Feed per lb. gain lbs.
1	OSU control	1.97 $\pm$ 0.37	3.13
2	10% whey	2.05 $\pm$ 0.25	3.02
3	20% alfalfa	1.88 $\pm$ 0.27	3.22
4	20% alfalfa + 10% dried whey	1.85 $\pm$ 0.29	2.92

### Swine Trial 3

Since high levels of whey have been fed with good results by Becker et al. (1957) and other workers, this trial was conducted to establish a minimum level. Levels of 0 to 8% dried whey were used. A significantly ( $P < 0.05$ ) better feed efficiency was observed at the 8% dried whey level compared to the 0% control ration (Table 19). All whey rations promoted a faster rate of gain and there was no apparent difference in carcass quality between treatments. From this trial it can be concluded that a low level of whey can promote faster gains and better feed efficiency. The level fed will depend on the cost of dried whey and the processing method used in preparing a ration. It was found that a ration containing below 10% dried whey is fairly easy to pellet and low levels serve as a good binder. If a mash type of diet is fed, the level of whey used is not as critical.

### Swine Trial 4

Dried whey has been used in swine starter rations quite extensively. Trial 4 was designed to compare dried whey and dried buttermilk in starter rations (Table 13). No significant differences in feed conversion or average daily gains were observed between treatments (Table 20), but the litters on the 10% whey ration tended to gain faster and were more efficient during the post-weaning feeding period.



Table 19. Growth performance and carcass data--trial 3.

Ration	Treatment	Average daily gain lbs.	Feed per lb. gain lbs.	Average carcass length in.	Average back fat in.	Average loin eye sq. in.
A1	Control	1.90 ± 0.28	3.52 ± 0.48	28.56	1.55	3.52
A2	2% whey	2.21 ± 0.22	3.24 ± 0.28	29.47	1.46	3.76
A3	4% whey	2.10 ± 0.22	3.20 ± 0.18	29.28	1.43	4.10
A4	6% whey	2.17 ± 0.23	3.24 ± 0.22	28.65	1.55	3.55
A5	8% whey	2.19 ± 0.27	3.08 ± 0.14	28.59	1.60	3.47

Table 20. Growth performance of creep rations--trial 4.

Ration	Treatment	Average daily gain preweaning	Average daily gain postweaning	Feed per lb. gain preweaning	Feed per lb. gain postweaning
1	6% dried buttermilk	0.60 ± 0.11	1.13 ± 0.18	0.46 ± 0.15	2.06 ± 0.23
2	10% dried whey	0.64 ± 0.07	1.29 ± 0.30	0.50 ± 0.23	1.80 ± 0.26
3	20% dried whey	0.56 ± 0.09	1.10 ± 0.29	0.56 ± 0.27	1.88 ± 0.26

Because of the limited number of litters tested these results can not be considered significant, but they do indicate that dried whey can be substituted successfully for more expensive milk products.

#### Rat Trial 1

This experiment using young rats was conducted to determine if lactose influences fiber digestion. The digestibility of fiber was lower in those groups receiving lactose (Table 21); due to the limited number of animals used and the large variations in results obtained for each diet the results were not statistically significant. Since lactose stimulates the propagation of lactobacilli organisms and these in general are noncellulolytic (Hungate, 1966), these results indicate that lactose will not enhance fiber digestion. Diarrhea was common in the rats fed the lactose containing diets. This could also affect fiber digestion adversely and at least partially explain the reduction in gains observed with these rats (Table 21). Since scouring was more prevalent with the lactose plus cellulose diet than the lactose plus alfalfa, there may be an interaction between lactose and alfalfa not concerned with the cellulose portion of alfalfa.

#### Rat Trial 2

The three rat experiments conducted in Trial 2 were designed to determine the effects of heat treatment on the utilization of lactose-

Table 21. Fiber digestion by rats on lactose vs. sucrose diets--trial 1.

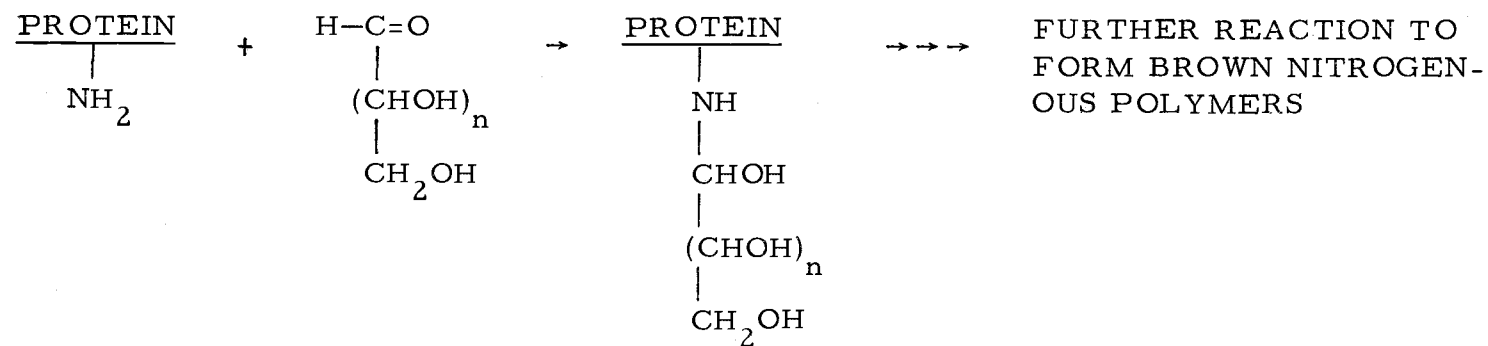
Group	Treatment	Average daily gain gm	Average fiber digestion %	Individual digestibilities %
1	Lactose + cellulose	-0.48	20.03 ± 3.9	22.8, 22.8, 14.5
2	Sucrose + cellulose	3.08	32.56 ± 0.65	32.9, 31.6, 33.1
3	Lactose + alfalfa	2.81	19.69 ± 6.7	10.5, 26.6, 22.0
4	Sucrose + alfalfa	5.48	28.56 ± 8.3	16.9, 35.9, 32.9

containing rations. High temperatures enhance reactions between reducing sugars and amino acids (the Maillard or Browning reaction). Since pelleting is a widely used feed processing procedure and heat is involved in this process, the Maillard reaction may play an important role in the utilization of dried whey in a pelleted ration.

The Maillard reaction involves the decomposition or polymerization of the carbohydrate by the reaction of the free carbonyl group of the sugar with a free amino group (Figure 2). Because L-lysine contains a free epsilon amino group, it is probably the most reactive amino acid toward aldoses and pentoses, so it causes the most browning and is therefore the most likely amino acid to become tied up by this reaction (Ellis, 1959). The Browning reaction is catalyzed by many things such as heat, high pH, phosphates, and some metals. It may be inhibited by low pH, and such compounds as sulfur dioxide and calcium chloride which act to depress pH. These may also block free amino groups and take up excess water (Ellis, 1959).

In Trial 2A, the substitution of lactose at the 5 and 10% level for the starch in the control diet produced a significant ( $P < 0.01$ ) depression in growth (Table 22). The addition of 0.3% lysine to the 5 and 10% lactose containing diets produced a significant ( $P < 0.01$ ) increase in growth, indicating that the lysine was in fact rendered unavailable.

Trial 2B was conducted to examine the relationship which other carbohydrate sources and several additives may have on this reaction.



Protein with  
epsilon amino  
group (lysine)

Aldol sugar  
(reducing)

Figure 2. Outline of Maillard reaction (protein and carbohydrate polymerization).<sup>1</sup>

<sup>1</sup> Adapted from Scott, Nesheim and Young, 1969.

Table 22. Growth performance of rats--trial 2.

Group	Treatment	Average daily gain (g)	Average daily gain as % of control (%)	Feed per gram gain (g)
Experiment 2A				
1	5% starch	4.53 ± 0.84	100	5.3
2	5% lactose	1.08 ± 0.17	24	20.0
3	5% lactose + lysine	4.14 ± 0.32	91	5.9
4	10% lactose	0.59 ± 0.36	14	26.0
5	10% lactose + lysine	2.25 ± 0.62	49	10.3
6	10% lactose + methionine tryptophan lysine	3.04 ± 0.39	67	7.8
Experiment 2B				
1	10% starch	4.72 ± 0.68	100	3.14
2	5% starch, 5% sucrose	4.50 ± 0.30	95	3.50
3	10% sucrose	4.29 ± 0.45	91	3.55
4	5% starch, 5% lactose	2.22 ± 0.35	47	5.03
5	10% lactose	0.98 ± 0.26	21	10.22
6	5% starch, 5% whey	2.87 ± 0.37	61	4.44
7	10% whey	2.60 ± 0.20	55	5.00
8	5% starch, 5% molasses	3.38 ± 0.58	72	4.19
9	10% molasses	2.06 ± 0.44	45	6.00
10	5% lactose, 4% starch, 1% glutamic acid	1.06 ± 0.29	23	9.69
11	5% lactose, 4% starch, 1% urea	2.33 ± 0.60	49	5.33
12	5% lactose, 4% starch, 1% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3.50 ± 0.10	74	4.28
13	5% lactose, 5% NaHCO <sub>3</sub>	1.27 ± 0.27	27	8.75
Experiment 2C				
1	10% lactose	1.13 ± 0.46	100	6.64
2	10% lactose + CuSO <sub>4</sub>	1.36 ± 0.41	120	4.58
3	10% lactose + ZnSO <sub>4</sub>	1.25 ± 0.51	110	5.17
4	10% lactose + CaCO <sub>3</sub>	1.16 ± 0.59	102	5.80
5	10% lactose + vegetable oil	2.13 ± 0.42	188	3.03
6	10% lactose + 8% alfalfa	0.63 ± 0.30	56	9.29

The results (Table 22) indicate that a lactose-containing ration is generally affected more severely than a ration containing other carbohydrate sources, but that this reaction is not limited to just lactose. The substitution of sucrose for starch did not significantly reduce gains, which supports the action of reducing sugars in this reaction since sucrose is a non-reducing sugar. The substitution of lactose for the starch at either the 5 or 10% level again significantly ( $P < 0.01$ ) reduced growth. The gains at the 10% lactose level were significantly less ( $P < 0.01$ ) than at the 5% lactose level.

Substituting dried whey for the starch at the 5 and 10% levels also caused a significant ( $P < 0.01$ ) depression in gains, but there was not a significant difference between the 5 and 10% whey levels, in contrast to the results with lactose. Perhaps a portion of the lactose in whey is already bound to its own protein fractions.

Since molasses is a common sugar-containing energy supplement in many livestock feeding programs, it was substituted for the starch at levels of 5 and 10%. Both levels resulted in a significant ( $P < 0.01$ ) decrease in gains. There was also a significant ( $P < 0.01$ ) decrease in gains with 10% molasses compared to the 5% level. This indicates that Browning reactions may occur involving the carbohydrate fraction of molasses, which is about one-half reducing sugars (Brinkley and Wolfrom, 1953). As was noted by Ellis (1959), the mineral content and humidity of a ration may catalyze the Maillard reaction. Since

molasses is high in minerals and moisture (Kondo and Ross, 1962a), these factors would also enhance this reaction, particularly in the presence of heat.

Since the Maillard reaction involves free amino groups, various additives with an amino or amino-like structure were added to determine if the lactose would preferentially react with these materials, leaving the lysine unaffected. Contrary to expectations, the inclusion of 1% glutamic acid or 1% urea to a 5% lactose diet significantly ( $P < 0.01$ ) reduced gains. The addition of 1%  $(\text{NH}_4)_2\text{SO}_4$  did significantly increase the growth of rats on a 5% lactose diet. The beneficial effects of ammonium sulfate may be due to its effects on pH, rather than through direct reaction with lactose.

The inclusion of a buffer in the form of 5%  $\text{NaHCO}_3$  resulted in a significant ( $P < 0.01$ ) depression in growth. Ellis (1959) reported that the Maillard reaction is increased with increases in pH; the results of this experiment support this conclusion.

Trial 2C was designed to examine the effects of several feed supplements commonly used in swine rations and the type of dietary fat on the Maillard reaction in autoclaved rations containing 10% lactose. The addition of  $\text{CuSO}_4$ ,  $\text{ZnSO}_4$  or  $\text{CaCO}_3$  had no significant effect upon the growth depression, but the substitution of vegetable oil for lard produced a significant ( $P < 0.05$ ) increase in gain. This indicates that there may be some relationship between the saturation of fats and



the Maillard reaction. It has been noted (Frobish et al., 1970; Miller, Conrad and Harrington, 1971) that young pigs can utilize low levels of fat from either animal or vegetable origin and fats are commonly included in swine rations, especially creep and starter rations. Since it is these rations which generally contain lactose from milk by-products, further investigation of the interrelationship between fats and the Browning reaction is needed.

The addition of 8% alfalfa produced the most marked growth depression (Table 22). This is an interesting observation with potential significance, because alfalfa is often added to swine rations. Since pentoses are very reactive in the Browning reaction, the effect of alfalfa may be due in part to its content of these sugars. Goering and Van Soest (1967) also reported that alfalfa is susceptible to browning over a wide range of moisture contents. These results indicate that adding alfalfa to a lactose-containing ration may have adverse effects. This may explain the lower rate of gain seen in Trials 1 and 2 where 10% dried whey was added to a 20% alfalfa containing ration. The heat involved in pelleting this ration may have caused an availability problem with the amino acids in this ration.

Due to the complexities of the Maillard reaction, the effects which it may have on a given ration will vary. The many interactions involved with this type of reaction leave ample opportunity for further research.

## SUMMARY

Increased utilization of dried whey in animal feeding would be desirable. The nutritional value of whey is well known and is being increased by better processing methods and knowledge of how to use this by-product to its fullest potential in both human and animal nutrition. Increased use of whey in animal feeding programs would be of value not only because of its favorable effects on animal performance, but also because it offers a means of avoiding environmental pollution associated with the traditional methods of whey disposal.

The studies carried out in Part II of this thesis give support to other work indicating the value of whey as it is used in swine feeding. Eight separate trials were conducted with rats and swine to evaluate the effects of using dried whey in various levels in both creep and grower rations. Favorable results were seen in the form of increased daily gains and feed efficiency.

Rats were used to examine the effects of the Maillard reaction on a lactose-containing ration undergoing heat processing, such as might occur during the pelleting of a ration. It was concluded that the lactose complexes formed by this reaction can have a detrimental effect, but many variables must be taken into consideration, since other carbohydrate sources can apparently have similar effects. The interpretations and results of these trials were discussed.

## FUTURE WORK

The use of dried whey in animal feeds is an expanding facet of the feed industry. If whey is to be used extensively, any possible unfavorable characteristics, such as the involvement of the lactose in the Maillard reaction, must be thoroughly examined. This study has demonstrated potential detrimental effects of this reaction on protein quality, and some ration characteristics which may influence the magnitude of this effect. Further investigation of inhibitors of the Maillard reaction would be valuable. The suggestion that the nature of the dietary fat might be important should be fully explored. Another area which should be examined closely is the relationship of alfalfa to lactose. Why does alfalfa react with lactose and how do other fibrous feedstuffs compare? The susceptibility of various proteins to the Maillard reaction should also be examined. The apparently increased digestibility of protein and fat in whey-containing rations merits further attention, both as to its extent and mechanisms of action. An area that is relatively unexplored concerns the effects of whey and lactose on the bacterial flora of the digestive tract of the pig. Possibilities include use of whey in feeding programs to maintain a vigorous intestinal population of lactobacilli to inhibit proliferation of pathogens.

The possibilities for research in the area of whey utilization are numerous. I feel that if dried whey is to be used to its fullest extent some of these questions should be answered.

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