WHEY-GROWN YEAST AS A PROTEIN SOURCE FOR EARLY-WEANED PIGS AND SHEEP

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Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1979

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ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Charles V. Maxwell, my major adviser, whose willing and sincere academic guidance and unreserved moral support during the course of this study exceeded the limits of duty.

Special gratitude is expressed to Dr. Fredric N. Owens, Dr. Linville J. Bush, Dr. Donald Holbert and Dr. Eldon C. Nelson for their extremely helpful suggestions and critical evaluation while serving on the advisory committee.

Sincere appreciation is expressed to the African-American Institute (AFGRAD), the people of the Republic of Uganda and to the Oklahoma State University, Stillwater, Oklahoma, for funding this study.

I also wish to express my sincere appreciation and state of indebtedness to Dr. Robert Totusek, Head of the Department of Animal Science, Oklahoma State University, for his excellent leadership; Dr. Joe Whiteman, Graduate Coordinator, Department of Animal Science, Oklahoma State University, whose love for humanity and academics has helped transform me into a more purposeful member of the human society than I would ever be; and Dr. Margaret K. Essenberg, Department of Biochemistry, Oklahoma State University, and her family, without whose sincere moral and financial support at a time of hardship, this study might not have become a reality.

My sincere appreciation is extended to the faculty, staff and graduate students of the Department of Animal Science at Oklahoma State

University for the excellent academic and moral environment they provided during the course of this study. Special thanks are expressed to Mr. Kenneth B. Poling and Mr. James D. Schooley for assisting me in caring for the experimental animals; Mrs. Beverly J. Shockey who assisted me with the chemical analyses; and Mrs. Janet Sallee, who did highly commendable secretarial work in preparing this manuscript.

Last but not least, I am particularly thankful to my wife, Mildred, for typing the draft of this manuscript and for faithfully encouraging me and caring for my children, Joyce, Judith, Jennifer, and Jacquiline, throughout the course of this study.

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CHAPTER I

INTRODUCTION

The future of the world's food production depends on four main factors: (1) Increasing urbanization to accommodate the fast growing non-agricultural industry, increasing use of land for housing the ever-expanding human population, and the expansion and extension of communication lines (roads, rail lines, air-strips) are reducing the land available for agricultural production. (2) Much of the modern industrial technology poses the danger of environmental pollution which may lead to the ban of some chemical aids to agriculture (pesticides, herbicides, antibiotics and growth promotants, fertilizers), or may make the environment unfit for human dwelling and unsuitable for agricultural production. (3) Most of the useful modern agricultural technology is energy intensive. As the world faces a shortage of low-cost energy, agricultural crises worsen. (4) In the developing countries, where an appreciable proportion of the world's present-day available agricultural land is found, modern technology for a high rate of agricultural production is lacking. Attempts to import such technology from developed countries are often resisted politically.

The four factors mentioned above are interrelated so that none can be ignored when considering ways and means to promote world food production. Socially, it is difficult to control the world's human population. Therefore, it appears that increased production of food for

an ever increasing population is essential.

Despite all these apparent inconsistencies, the principal goal of all agricultural scientists still remains to search for better means to adequately and comfortably feed the world's human population. Attempts to achieve this goal are complicated not only by the problems listed above, but also by skyrocketing costs of animal feedstuffs. Rising feed costs depend on two factors: (1) low amounts of feeds are produced, and (2) human and animal populations often compete for the same products.

The search for alternative food sources for mankind to promote supply and lessen competition is frequently resisted by social and psychological customs. It, therefore, seems appropriate to exploit the potential of substitute materials for animals. In this connection, single-cell protein (SCP) is receiving increasing research attention. In his introduction to the "International Conference on Single-Cell Protein", Scrimshaw (1967) stressed the importance of considering SCP as a useful lead in the search for alternative sources of food and feed.

The term single-cell protein applies mainly to proteins of algae, bacteria, fungi and yeast origin. According to Braude (1976), species of algae such as Spirulina, Chlorella and Scenedesmus have been grown commercially and fed to pigs with varying degrees of success. Although still rudimentary, development of bacterial protein is gaining research momentum as a source of dietary protein for animals. In pelleted diets, growing broiler chicks tolerated up to 10% bacterial cell protein (BCP) in a corn-soybean meal ration (Waldroup and Payne, 1974). One demerit of BCP is its high nucleic acid content (Braude, 1976). This could limit its use to species which lack the ability to convert nucleic acid into the readily excretable allantoin. Most mammals have this ability

(Edozien et al., 1970). Some fungi such as Paecilomyces variotii, Aspergillus oryzae, and Rhizopus orrhizus have been cultured and evaluated with varying success as sources of protein for pigs (Forss, 1973). Fungi may present a problem as they produce mycotoxins in a wide variety of feedstuffs (Liener, 1969).

Of the four possible sources of SCP (algae, bacteria, fungi, yeasts), yeasts appear promising for a number of reasons: (1) Yeasts grow on a wider range of substrates than do algae, bacteria or fungi (Braude, 1976). This enlarges the alternatives for substrate for commercial production of yeast cells. (2) Yeasts can reduce total solids and chemical oxygen demand (COD) in cheese whey (Knight, 1972; Knight et al., 1972; Mickle et al., 1974) and reduce the cost and danger of waste disposal from cheese plants. If nutritionally useful, yeast cells could be produced for this dual purpose which would justify higher production costs. (3) Yeasts are low in nucleic acids (Braude, 1976) and other anti-nutritive factors (Shacklady, 1970). (4) Yeasts have relatively high levels of indispensable amino acids, being equal or superior to soybean meal or fish meal in most of the dietary essential amino acids (Shacklady, 1970; Braude, 1976; Mickle et al., 1976).

Criticisms about SCP production center on the cost of production, the possibility of toxic contents and inattractiveness as food. Such arguments seem unrealistic for a number of reasons. The space, time and labor to produce a given quantity of protein often favors SCP over soy protein, fish protein or any other type of plant or animal protein. The chemical hazards from SCP are the nucleic acids and probably tar residues in SCP grown on oil. Methods are now available to reduce the nucleic acid contents of SCP to acceptable levels (Oser, 1975).

Commercial production of SCP could save space and time, reduce the rate of environmental pollution by improving waste disposal, aid in the conservation of energy by way of reusing what would otherwise be disposed of as waste and provide alternate sources of food for man and animals (Davis, 1974; Tannenbaum and Wang, 1975).

Work to date at Oklahoma State University (OSU) has shown promise in the use of yeast (Kluyveromyces fragilis--formerly Saccharomyces fragilis) to reduce total solids and chemical oxygen demand (COD), a measure of biological oxygen demand (BOD), in cheese whey. In the process a high crude protein (72%) by-product is produced in large amounts. Its evaluation as a protein source for livestock production is warranted. The experiments reported in this dissertation were designed to evaluate the potential of K. fragilis as a protein source for non-ruminant and ruminant animals. The animals used were early-weaned pigs, as a typical non-ruminant, and lambs as a typical ruminant animal.

CHAPTER II

REVIEW OF LITERATURE

Evaluation of yeast as a source of food and feed has been limited. Man, rats, poultry and swine have been used, but other classes of animals have not. This review of literature is therefore, divided into sections by species where research information is available. Sections on the chemical composition of yeast and nitrogen metabolism in the ruminant animal are added for clarity.

Yeast in Human Nutrition

Yeast is not a new food for man. The Old Testament repeatedly mentions the use of leaven (yeast) for bread making and brewing by the Jews and the Ancient Egyptians. Large quantities of yeast were consumed by man of the Middle Ages via bread and beer (Thaysen, 1943). No records attest to nutritional benefits and/or hazards of the yeast consumed. In the form of bread and unfiltered beer, the members of some less developed societies of the world still consume appreciable amounts of yeast. Among modern societies, yeast is mainly limited to the bakery and the brewery industries. However, ancient physicians also recommended yeast for medical purposes. In 1552 B.C., the Ebers' Papyrus (cited by Pierce, 1932) recommended a remedy including milk, yeast and honey. Many authors have advocated yeast for correction of intestinal disorders. Yeast has been used for the treatment of constipation (Hawk et

al., 1917). According to Murlin and Mattill (1923) many French scientists used yeast for the correction of constipation and diarrhea. The biological role of yeast in this case is not known. However, improved regularity and ease of passage of indigesta through the alimentary canal when yeast was ingested by human subjects has been observed (Still and Koch, 1928).

Scientific investigation of yeast as a source of food for man did not begin until severe food shortages were experienced during the World War I. Although the original work started in Germany (Hawk et al., 1919), independent investigations followed in Britain, France, and in the U.S.A. One of these pioneer works was conducted by Funk and associates (1916) in New York. When yeast was fed as the sole source of food protein to four men for a period of three weeks, blood uric acid levels were evaluated and appreciable amounts of undigested yeast cells appeared in feces. They concluded that the yeast nitrogen had little food value and was poorly assimilated. Yet they stated that "our studies have by no means enabled us to pronounce a verdict that yeast possesses no value in dietetics". Although these findings agreed with some of the reports from the German laboratories (cited by Hawk and associates, 1919), other workers (cited by the same authors), were convinced that yeast was a source of usable protein and of B vitamins for man.

After studying the work of Funk and associates (1916), Hawk and co-workers (1919) conducted experiments which avoided some of the problems encountered by Funk et al. In their work, Hawk et al. (1919) substituted yeast for 9 to 29 per cent of the food nitrogen in the diets of six men. They found an average of 0.4 grams more nitrogen was retained daily when yeast was fed. They concluded that compressed

bakers' yeast was a satisfactory component of diet for man. In a series of experiments with humans, Pierce (1932) confirmed the laxative effect of yeast and found that yeast nitrogen was readily retained by man.

After the work of Pierce (1932), time elapsed before interest resumed in the evaluation of yeast for man. With men in a closed metabolic unit, Waslien and associates (1968) studied the metabolism of yeast nucleic acid in man. Their results confirmed the earlier reports that increased intake of yeast nucleic acid elevated plasma and urinary levels of uric acid. However, they recommended that microbial protein could advantageously be added up to 20 g to diets of low protein content.

One of the major concerns about feeding products high in nucleic acid to man is that purines derived from nucleic acids are degraded into uric acid (Condon, 1971). The oxidation of uric acid into the more soluble allantoin is catalyzed by the enzyme uricase. This enzyme is absent in man and other primates (Edozien et al., 1970). Therefore, man is unable to metabolize uric acid. The latter compound is not readily soluble at the pH of blood, and it is poorly excreted by the kidneys (Waslien et al., 1968). High levels of dietary purines subsequently elevate plasma uric acid in man (Funk et al., 1916; Waslien et al., 1968; Edozien et al., 1970). This may deposit as uric acid crystals in joints and soft tissues and as stones in the urinary tract (Edozien et al., 1970). Although these conditions are more prevalent in persons with a genetic potential for primary gout, high levels of uric acid in the serum (above 7 mg/100 ml) may precipitate gout in otherwise genetically normal persons (Edozien et al., 1970; Scrimshaw, 1975).

Unless methods are devised to reduce the nucleic acid content of yeast, the use of this SCP source for man may be limited to its supplementation of low protein diets. Methods to decrease the nucleic acid levels in yeast to make it usable as the sole dietary protein source for man have been described by Maul and associates (1970) and by Oser (1975).

Yeast in Rat Diets

Most of the experimental work of feeding yeast to rats started after World War I. Osborne and Mendel (1919) used yeast as the sole source of dietary nitrogen and of water-soluble vitamins for rats. The rats were fed these diets over a year, including the growth period. Although cases of sterility were noted with male rats, no abnormalities were associated with the yeast component of the diet. Some 74 to 83 per cent of the yeast nitrogen was utilized by rats. The use of yeast as a source of water-soluble vitamins was evaluated by Hawk et al. (1919). These authors fed casein, casein plus yeast, lean meat and lean meat plus yeast to young rats and concluded that "addition of compressed yeast to a diet lacking water-soluble vitamins caused an immediate and pronounced increase in body weight". They also noted that compressed yeast could be heated to 105 C without damaging its growth promoting properties. In a later experiment with rats, Hawk and associates (1921) found yeast to be a rich source of vitamin B complex and protein for rats and suggested that increased amounts of yeast be added to bread to increase its food value.

Sterility in rats fed yeast was further investigated by Nelson and co-workers (1923). Saccharomyces cerevisiae proved to be a good

source of nitrogen and of vitamin B complex for normal growth and reproduction in rats. Third generation rats were normal when yeast provided 30, 35, 40 or 45 per cent of the diet and was the sole source of protein. However, when fed at 50 per cent of the diet, yeast was toxic for rats. Addition of 5 per cent of a salt (NaCl) mixture to the diet caused sterility. The role of the salt mixture in the development of sterility was not established. That NaCl has adverse effects when fed at high levels was noticed by Still and Koch (1928) when they fed washed and unwashed raw yeast to experimental rats. The unwashed raw yeast had a higher salt concentration than washed raw yeast and produced severe diarrhea in rats. In a series of experiments with rats they concluded that yeast had poor digestibility, was a poor source of phosphorus and led to poor calcium retention. Since about 2 g of yeast are equivalent to one gram of casein, starch was reduced in the yeast diets to keep the test nutrients in equal proportions. Problems with the yeast diets could have been due to depressed palatability. In the experiments of Still and Koch (1928) the digestibilities of dried raw yeast and coagulated yeast were 72 and 62 per cent and the biological values for the two yeast products were 45 and 38 respectively. These figures are low as compared with the reports of Goyco and Asenjo (1947) in which digestibilities of 88, 87 and 86 per cent and biological values of 49, 45 and 69 were found for two samples of Torula utilis yeast and one of brewers' yeast.

From the results of 9 experiments in which diets complete in their amino acid composition for rats were compared with diets containing 5% of Torula yeast or brewers' yeast, growth rates were improved 30% when yeast was added (Bunyan et al., 1974). The authors attributed part of

the improved growth of rats fed yeast diets to the minerals (zinc, iodine, iron, copper and manganese) present in yeast. An unidentified factor (factor G), was also implicated as a possible beneficial contribution from yeast. Such a factor (which may be galactoflavin) was first mentioned by Schwarz and associates (1966). Variability in results by different investigators is possibly due to experimental methods, strain of yeast and the processing or handling of the yeast. Goulet and coworkers (1976) found that a treatment which produced the best digestibility of yeast did not necessarily produce the best overall result in rat performance. This could be due to poor retention of yeast nitrogen by the rats. They reported that irradiation was superior to freeze drying, autolysis or autoclaving as a treatment method for Rhodotorula utilis and Candida utilis. Vasconcellos and associates (1977) also found differences in performance of rats fed Saccharomyces cerevisiae diets depending upon method of drying. Heat-drying of yeast produced faster growth rates in rats than freeze-drying. Nucleic acid contents of the final products did not differ. Treatments might have differentially affected the amino acid profile of the yeast protein. According to Miller and associates (1965) and Moran and Summers (1968) heating decreases the availability of lysine, methionine, cystine and arginine in the diets of chicks and rats. The cause of the improved performance of rats fed the heat-dried yeast (Vasconcellos et al., 1977) was not defined.

Yeast in Poultry Nutrition

Research on yeast in chick diets followed the work done with man and rats and occurred in the late 1960's. In a number of experiments

with broiler chicks, laying hens and turkeys, yeast has formed up to 20 per cent of practical type diets. Chepigo et al. (1967), Laine (1967), Shacklady and van der Wal (1968) and Shacklady (1969) all obtained acceptable results with no histological abnormalities.

In a series of experiments, Shacklady (1969) fed laying and breeding hens 12 to 14 per cent of yeast as the sole source of protein. Performance was normal. Chicks consumed 20 per cent yeast diets without detectable harm. Such a level is impractical from the standpoint of protein economy. In controlled feed intake trials, Waldroup and coworkers (1971) observed that broiler chicks performed as well on diets containing 30 per cent yeast as on any other practical type diet. Using a hydrocarbon-grown yeast preparation known as TOPRINA, Shannon and McNab (1972, 1973) obtained excellent chick performance when TOPRINA replaced all of the supplemental protein from fish meal or soybean meal.

Although most of the work done with yeast as a source of protein for poultry diets has been successful, occasionally poor responses have been obtained. Waldroup and Flynn (1975) found net protein utilization (NPU) values of yeast for chicks ranged from 40.8 to 73.5 as compared to 85.5 for soybean protein. Corresponding nitrogen efficiency ratios (NER) were 4.9 to 15.6 and 19.7. These authors concluded: "A large portion of the nitrogenous fraction of the yeasts is not in the form of amino acids which tend to downgrade the value of these products when compared on the basis of total nitrogen."

No relationship was established between yeast nucleic acid content and depressed chick performance. Waldroup and Hazen (1975) noted that 2.5, 5, 10 or 15 per cent hydrocarbon-grown yeast in isonitrogenous (16% Protein) and isocaloric (2970 ME kcal/kg) corn-soybean meal diets

had no adverse effects on laying hens. These results corroborate the results of other laboratories summarized by Shacklady (1974).

Yeast is a rich source of B vitamins and of protein. Mineral content and availability has received less attention. Thayer and Jackson (1975) fed 2.5 per cent yeast culture in corn-soybean meal diets and found that live yeast culture improved the efficiency of phosphorus utilization in growing chicks. The exact role of yeast in this connection was not established. One possible problem associated with the use of high levels of yeast in poultry diets is the lysine to arginine ratio. Yeast is high in lysine (Table I) so caution must be exercised when including yeast in broiler diets so as to maintain the critical lysine to arginine ratio for broiler diets (Shacklady, 1975).

Yeast in Livestock Feeding

Research on yeast as a source of protein for livestock has been reviewed by Braude (1942), Carter and Phillips (1944), Shacklady (1974, 1975) and Braude (1976). The pig has received some attention in the past 10 years. Little work has been done with cattle.

Shacklady and van der Wal (1968) and Shacklady (1969) fed yeast in several trials to pigs and obtained acceptable results with no histological abnormalities. Consistently larger litter size was obtained with sows fed fish meal or soybean meal diets containing 10 per cent yeast than when either fish meal or soybean meal was fed without yeast (Shacklady, 1969). A series of experiments by Shacklady (1970) with third generation pigs raised on hydrocarbon-grown yeast have proven yeast to be toxicologically safe. In their experiments, yeast replaced all the protein from fish meal and most of the protein from soybean

TABLE I

AMINO ACID COMPOSITIONS OF SOME SELECTED FEED PROTEINS AS COMPARED TO YEAST PROTEINS

	Feed						
	Soybean meal[1]	Fish meal[1]	Blood meal[1]	Brewers' yeast[1]	BP yeast[2]	Yeast SCP[3]	*Candida utilis*[4]
International reference no.	5-04-604	5-02-15	5-00-380	7-05-527	------	-----	7-05-534
Crude protein %	51.5	70.4	87.8	48.0	64.0	55.1	51.9
Amino acid (g/100 g CP)							
Arginine	7.0	4.1	4.4	4.9	4.9	3.3	4.4
Cystine	1.5	1.2	1.7	1.1	0.9	.5	---
Histidine	2.4	2.8	5.3	2.5	2.3	2.5	2.4
Isoleucine	5.4	5.0	1.3	4.7	4.9	5.1	4.7
Leucine	7.4	7.2	12.9	7.2	7.3	6.9	7.0
Lysine*	6.3	9.0	8.6	6.7	7.3	6.5	8.0
Methionine	1.3	3.1	1.1	1.6	1.8	1.1	.7
Phenylalnine	4.8	4.0	7.6	4.1	4.5	4.2	3.6
Threonine	3.7	4.0	4.6	4.7	4.7	3.4	5.0
Tryptophan	1.3	.8	1.4	1.1	1.3	1.5	---
Tryosine	3.0	4.6	2.3	3.4	3.7	3.8	3.0
Valine	5.2	6.3	8.1	5.1	5.5	5.6	5.2

[1] N.R.C. 1973. Figures recalculated to give amino acid as % of protein.

[2] Braude, 1976.

[3] Tegbe and Zimmerman. 1977. Figures recalculated to give amino acid as % of protein.

[4] Goulet et al., 1976.

*Values as high as 10.0, 11.4 and 11.6 g lysine nitrogen per 100 g of total nitrogen have been reported by Carter and Phillips, 1944.

meal without adversely affecting rate of gain, feed efficiency and carcass quality. In one instance a pig consumed 65 per cent yeast in its diet for 11 weeks with no ill effect.

Braude and Esnaola (1973) replaced all of the fish meal in cereal-based diets of young pigs with yeast and found that the addition of DL-methionine produced no added advantage. Although fish meal contains about twice as much methionine as yeast (Table I), the level of methionine in yeast can probably meet the young pig's low requirement for this amino acid. Yeast was found limiting in methionine for pigs in one report (Bodart, 1976). The experimental evidence in support of this finding was not provided. He compared purified paraffin-grown yeast with meat meal in the diets of 30 Belgian Landrace and 30 Pietrains pigs. With the Belgian Landrace pigs the inclusion of yeast in the diet lowered the live grade and the market value of the pigs. The opposite was found with the Pietrains. The cause of the breed differences was not explained and no other research supports this conclusion.

Work by Veum and Schmidt (1975) revealed that 2.5 per cent yeast culture in corn-soybean meal diets gave satisfactory performance in growing pigs and that carcass qualities were not adversely affected by including yeast culture in the diets. Similar results that favor yeast protein at higher levels of dietary yeast were reported by Tegbe and Zimmerman (1975). These workers replaced 0, 25 and 50 per cent of soybean meal with yeast, on weight basis, in diets of baby pigs and growing pigs. No appreciable differences were reported for pig performance.

In another experiment with baby pigs and growing pigs (Tegbe and Zimmerman, 1977), replacing soybean meal with increasing levels of yeast in 18.0 per cent CP dehulled soybean meal diets linearly decreased

plasma urea nitrogen, linearly increased plasma alpha-amino nitrogen and improved feed efficiency, nitrogen digestibility and nitrogen retention. Such results indicate that SCP may be a desirable protein source for swine.

Least studied in the area of SCP nutrition are ruminant animals. Lack of interest is probably due to high cost of SCP and protein degradation in the rumen. With two 10-week old milk-fed calves, Gaillard and van Weerden (1976) found that the polysaccharides of the yeast cell wall were poorly digested by intestinal enzymes. Good digestibility of yeast protein by swine should imply a possibility of equally good digestibility of yeast in the preruminant calf, but there is no research evidence in support of or against that idea.

The Chemical Composition of Yeast

Chemical differences in the composition of the yeast products explain some of the discrepancies among results of nutrition trials from different laboratories. Since yeasts grow on a wide variety of substrates (hydrocarbons, cereals, sugars, molasses, waste sulfite liquor, cheese whey, sewage; Braude, 1976), the substrate might influence the chemical composition of the final product. This might happen when undegraded substrate forms part of the final yeast product. Strains of yeast also might differ in chemical composition.

At the end of the nineteenth century, yeast was known to contain a high level of protein (Thaysen, 1943) but the chemical nature of the yeast protein was not known. The first comprehensive chemical analyses of the yeast protein were done by Pringsheim in 1913 and by Meisenheimer in 1915 (cited by Still and Koch, 1928). Hawk and associates (1919)

determined bakers yeast contained 52.4 per cent protein, 1.7 per cent fat, 37.1 per cent carbohydrate and 8.7 per cent ash. They did not examine the amino acid properties of their yeast, but after they fed this yeast to human subjects, they concluded that compressed bakers' yeast was a satisfactory food for man. This would imply that the bakers' yeast used in their experiments was complete in indispensable amino acids. Subsequent chemical analysis of the yeast has elucidated the amino acid profile of the yeast protein but nucleic acid content of the yeast cell has received little attention. Various reports concerning the chemical composition of yeast as determined by proximate analysis are shown in Table II. Although variations due to strain of yeast, substrate residues and handling methods might cause differences in the protein content of yeast as determined by different workers, the average protein level of the yeast is still 54.1%.

The level of yeast protein (N x 6.25) in Table II has limited usefulness since non-protein nitrogen portion is included in the nitrogen value used for calculating protein. To a protein nutritionist, the amino acid composition of the yeast protein is of utmost importance. Many strains of yeast have been evaluated and compared to more common protein sources such as soybean meal, fish meal and blood meal. Table I illustrates the amino acid contents of some strains of yeast and selected conventional protein feeds.

Although differences in protein content and amino acid composition of the protein occur between strains of yeast (Table I), yeast protein is generally comparable to conventional sources of food protein in relation to amino acid content. Most critics of SCP base their argument on the high nucleic acid content of single cell organisms. Nucleic

TABLE II

CHEMICAL COMPOSITION OF YEAST AS DETERMINED BY PROXIMATE ANALYSIS

Type of Yeast	Component (% on Dry Matter Basis)			
	Protein*	Fat	Carbohydrate	Ash
Compressed bakers' yeast[1]	52.4	1.7	37.1	8.7
Dried brewers' yeast[2]	47.6	1.0	33.4	8.4
BP yeast[3]	62.0	1.6	----	5.7
Red star yeast[4]				
fresh	57.3	0.3	28.7	5.7
freeze-dried	52.3	0.4	36.8	4.8
heat-dried	49.0	0.2	28.2	5.8
Hydrocarbon-grown yeast[5]	57.8	3.6	30.8	7.9

[1] Hawk et al., 1919.
[2] Carter and Phillips, 1944.
[3] Braude, 1976.
[4] Vasconcellos et al., 1977.
[5] Tegbe and Zimmerman, 1977.
* Protein defined as N X 6.25.

acid totals 8 to 25 grams of nucleic acid per 100 grams of protein in single cell organisms (Young and Scrimshaw, 1975). This is high as compared to the 4 grams of nucleic acid per 100 grams of wheat flour protein (Kihlberg, 1972). With the exception of man and non-human primates, mammals can degrade nucleic acid into allantoin which they excrete readily in the urine (Keilin, 1959; Christen et al., 1970; Young and Scrimshaw, 1975; Braude, 1976). Consequently the relatively high nucleic acid content of yeast is of more concern in human than in animal nutrition.

The maximum limit for nucleic acid intake for man is 2 grams per day above that in the normal diet; this is equivalent to about 15 grams of SCP (Waslien et al., 1968; Edozien et al., 1970; Scrimshaw, 1975; Oser, 1975) which is still an appreciable amount of protein supplement. Several methods are available for removing nucleic acids from SCP (Sinskey and Tannenbaum, 1975) which attempt to reduce nucleic acid to 4 g per 100 g SCP (Oser, 1975).

Sources of Nitrogen for Ruminants

The anaerobic bacteria and protozoa resident in the ruminoreticulum of ruminant animals convert most sources of dietary nitrogen into ammonia. Ammonia can be used for synthesis of microbial nitrogen (Blackburn, 1965; Smith, 1969; Hume et al., 1970; Hatfield, 1970; Burroughs et al., 1975; Chalupa, 1975; Hogan, 1975; Satter and Roffler, 1975). This microbial activity in the ruminoreticulum is advantageous to both the animal and the livestockman if ammonia nitrogen was well utilized and converted into microbial protein, and if all the microbial protein that reaches the abomasum and the small intestine was readily

digested. Such are not always the case (McDonald, 1968; Waldo, 1968; Hume et al., 1970; Chalupa, 1975; Hogan, 1975).

One advantage of the ruminant over the nonruminant is the ability of the former to utilize NPN (Chalupa, 1975). The quantity of microbial protein synthesized depends on many factors, including the numbers and type of microbes, availability of energy, rate of ammonia release and rate of passage of ingesta through the ruminoreticulum (Church, 1971). Often much ammonia remains unmetabolized and is lost via ammonia absorption and excretion as urea. Excess ammonia can precipitate toxicity (Visek, 1968; Chalupa, 1972; Bartley et al., 1976). Besides degrading NPN, microorganisms also degrade protein of low or high quality, thereby increasing the biological value of low quality proteins and reducing the value of high quality protein. Consequently, ruminants are well suited to utilize low quality protein sources and NPN. On the other hand, with high rates of production, the quantity of microbial protein may limit performance (Reis and Tunks, 1969; Braman et al., 1973). Even at the highest rate of microbial protein synthesis in the rumen, the animal may still be protein deficient unless substantial amounts of dietary protein bypass the rumen (Reis and Schinckel, 1963; Little and Mitchell, 1967; Schelling and Hatfield, 1968; Peter et al., 1971).

To increase passage of dietary protein into the abomasum, various methods have been employed. Most of the methods aimed at protecting dietary protein from microbial degradation in the rumen (Chalupa, 1975). Methods include heat treatment of feed protein (Sherrod and Tillman, 1964; Goering and Waldo, 1974) and chemical treatments to reduce protein solubility at the ruminal pH (MacRae et al., 1972; Hatfield, 1973; Hemsley et al., 1973). Such methods are often expensive and may lower

the postruminal protein digestibility as well (Reis and Tunks, 1969; Faichney and Weston, 1971; Barry, 1973; Dinius et al., 1975). Since some yeast protein is a washed precipitation product (Smith et al., 1977), it may resist ruminal degradation and partially escape ruminal degradation.

High postruminal digestibility of yeast cell protein would be expected since the ruminant intestines normally digest microbial cells and digestibility of yeast protein by nonruminants is high. Further, nucleic acids should not be of concern since the ruminant animal can metabolize nucleic acids with ease (Condon, 1971).

CHAPTER III

WHEY-GROWN YEAST AS A SOURCE OF PROTEIN FOR EARLY-WEANED PIGS

Summary

Three experiments were conducted to examine whey-grown Kluyveromyces fragilis yeast as a protein source for early-weaned pigs. In experiment 1, 25 Yorkshire male pigs averaging 3.5 weeks of age and 6.05 kg were randomly assigned to four diets containing 0.9% total lysine with 0, 25, 50 and 75% lysine furnished by whey-yeast and the rest from soybean meal, and one ration at 0.8% lysine with all supplemental lysine from soybean meal. In experiment 2, 25 pigs were allotted on a basis of litter to four diets containing 0.9% lysine with 0, 50, 75 and 83% lysine furnished by whey-yeast and one ration at 0.8% lysine with all supplemental lysine from soybean meal. The data from experiment 2 were adjusted for litter effects and pooled with the data from experiment 1 for a combined analysis. In experiment 3, 24 pigs were assigned to four diets containing 0.9 or 1.0% lysine from soybean meal or whey-yeast.

At 0.9% dietary lysine, increasing the level of whey-yeast lysine linearly improved rate of gain ($P < .05$), feed efficiency ($P < .01$) and protein efficiency ratio ($P < .05$) but linearly decreased protein consumption ($P < 0.01$). Rate of gain, feed efficiency and protein efficiency ratios were improved by 21, 22 and 47%, respectively, at the highest level of yeast fed. In experiment 3, at both 0.9 and 1.0%

levels of dietary lysine, whey-yeast produced 35 and 25% higher ($P < .05$) rates of gain, 40 and 35% better ($P < .05$) feed utilization and 65 and 59% more efficient ($P < .01$) protein utilization, respectively, than soybean meal.

It was concluded that whey grown K. fragilis yeast, at levels up to 11% of the diet has no adverse effect on early-weaned pigs and that whey-yeast protein was superior to soybean meal protein for growth rate, feed efficiency and protein efficiency of early-weaned pigs.

Introduction

A comprehensive description of the conventional protein sources for swine has been summarized (Pond and Maner, 1974). Most of the common protein sources used in swine rations are also protein sources for man. Therefore, man and swine often compete for the same products. This competition has frequently resulted in high costs of feed for swine. Crop failures due to adverse weather conditions and other factors (pests, diseases) have often worsened the problem (Ganzin, 1974). Despite this competition, the conventional protein sources have produced the most economical gains in swine to date. Recent increases in protein cost, however, have stimulated the study of alternate protein sources.

It seems logical to investigate alternate protein sources for which there would be little or no competition from man whose production would not be limited by severe weather or space availability. This is particularly relevant if waste products can be utilized to produce the protein. Previous reports (Braude, 1942; Mateles and Tannenbaum, 1968;

Davis, 1974; Tannenbaum and Wang, 1975) have favored single-cell protein (SCP) sources. Yeast cell protein has received more research attention than algae, fungal, or bacterial protein during the last decade.

Inclusion of yeast in swine rations have produced no histological abnormalities (Shacklady and van der Wal, 1968; Shacklady, 1969). Consistently larger litter sizes have been obtained when yeast formed 10% of corn-based fish meal or soybean meal diets than when all fish meal or soybean meal supplemented diets were fed to sows (Shacklady, 1969). From sow through the third generation, pigs raised on hydrocarbon-grown yeast proved yeast to be toxicologically safe (Shacklady et al., 1970). Satisfactory performance of baby pigs and growing pigs fed yeast have been obtained (Veum and Schmidt, 1975; Tegbe and Zimmerman, 1975; 1977).

Variations in results due to different strains of yeast, the nature of substrate used in producing yeast and differences in preparation and handling procedures indicate that further research on the nutritional properties of yeast is warranted. In this study, feed consumption, growth and efficiency of feed and protein utilization by swine fed whey-grown Kluyveromyces fragilis (formerly Saccharomyces fragilis) yeast were investigated. The whey-yeast product (Table III) evaluated in this study was prepared using the method described by Smith and associates (1977).

Materials and Methods

Three feeding experiments were conducted with a total of 74 Yorkshire male pigs to evaluate whey-grown Kluyveromyces fragilis yeast as a dietary protein source for early-weaned pigs. The three experiments differed in design and treatment combinations but were similar in that

TABLE III

ANALYSIS OF WHEY-GROWN KLUYVEROMYCES FRAGILIS YEAST AND SOYBEAN MEAL

Item	% of As-fed Product	
	K. fragilis[1]	Soybean meal[2]
Moisture	8.59	11.0
Calcium	.27	.32
Phosphorus	.40	.67
Protein (N x 6.25)	63.07-67.04	45.8
Amino acid (g/100 g C.P.)		
Alanine	3.99	-----
Arginine	1.89	3.20
Aspartic acid	8.18	-----
Cystine	3.33	.67
Glutamic acid	11.21	-----
Glycine	1.90	-----
Histidine	1.23	1.10
Isoleucine	3.36	2.50
Leucine	8.24	3.40
Lysine	6.92	2.90
Methionine	1.49	.60
Phenyalanine	2.89	2.20
Proline	3.62	-----
Serine	3.63	-----
Threonine	3.88	1.70
Tryptophan	-----	.60
Tyrosine	2.53	1.40
Valine	3.53	2.40

[1]Analysis done by Analytical Biochemistry Laboratories, Inc., P.O. Box 1097, Columbia, MO, 65201.

[2]Figures obtained from N.R.C. (1973).

- Value not determined.

the same diet preparation and animal management practices were used. Each experiment was five weeks in duration. Corn and the whey-yeast used in all the three experiments were ground through 3.2 mm and 1.6 mm screens, respectively.

Animal Management and Data Collection

Individual feeding crates equipped with self-feeders and automatic waterers were used to house pigs. Feed and water were supplied *ad libitum*. Quantity of feed offered was recorded at each feeding time. Feed wastage and feed refusal were collected separately, dried to as-fed moisture content and weighed weekly. The crates used were adjusted to minimize contamination of waste feed with feces or urine. The sum of the feed wastage and feed refusal was subtracted from the total feed offered during the week to obtain the amount of feed consumed by the pig during that week. Pigs were weighed at the beginning of the experiment and weekly thereafter. All measurements were done on an individual animal basis. Throughout each experiment, the animals were closely observed for any abnormal behavior and illness.

Design of Experiments

In experiment 1, 25 pigs averaging 3.5 weeks of age and 6.05 kg were assigned to five treatments in a completely randomized design (Steel and Torrie, 1960). Treatments comprised five corn-soybean meal diets formulated on the basis of total dietary lysine. Whey-yeast furnished 0, 0, 25, 50, and 75% of the total dietary lysine in diets 1, 2, 3, 4, and 5, respectively. Composition of the diets is given in Table IV.

TABLE IV

COMPOSITION OF DIETS USED IN EXPERIMENT 1

Ingredients	International Reference No.	% Composition (As-fed) Diet No. 1	2	3	4	5
Corn	4-02-931	73.10	69.59	74.26	79.15	83.93
Soybean meal	5-04-604	23.20	26.79	18.82	10.63	2.54
Whey-grown yeast	---	-----	-----	3.25	6.50	9.76
DL-Methionine (98% pure)	---	0.22	0.20	0.15	0.10	0.05
Dicalcium phosphate	6-01-080	1.22	1.14	1.28	1.43	1.57
Calcium carbonate	6-01-069	1.16	1.18	1.14	1.09	1.05
Vitamin T.M. Premix[a]	---	0.75	0.75	0.75	0.75	0.75
Salt, iodized	6-04-151	0.30	0.30	0.30	0.30	0.30
Aureomycin 50	---	0.05	0.05	0.05	0.05	0.05
Calculated analysis						
Crude protein (N x 6.25)[b]		16.65	17.91	17.08	16.23	15.41
Calcium		0.80	0.80	0.80	0.80	0.80
Phosphorus		0.60	0.60	0.60	0.60	0.60
Lysine, total		0.80	0.90	0.90	0.90	0.90
Methionine + cystine		0.74	0.74	0.76	0.76	0.76
Tryptophan*		0.20	0.22	0.18*	0.13*	0.09*
Arginine:lysine ratio		1.33	1.30	1.10	0.91	0.64
Yeast lysine (% of total)		-----	-----	25	50	75

[a]Vitamin T.M. premix supplied 661,380 I.U. Vitamin A; 49,604 I.U. Vitamin D; 661 mg Riboflavin; 3307 mg. Pantothenic acid; 4960 mg Niacin; 132,276 mg Choline; 2.48 mg Vitamin B_{12}; 1653 I.U. Vitamin E; 331 mg Menadione sodium bisulfate; 33.1 mg iodine; 14.88 gm iron; 3.31 gm manganese; 1.65 gm copper; 14.88 gm zinc and 16.53 mg selenium per 100 kg of feed.

[b]Values determined by Kjeldahl procedure.

*Additional tryptophan was expected to come from whey-yeast.

In the second experiment, 25 pigs (five from each of five litters) averaging 3.5 weeks of age and 6.13 kg were allotted to five diets in a randomized complete-block design. Blocking was done by litter with five pigs per treatment and each litter represented in a treatment (Snedecor and Cochran, 1967). The treatments (Table V) consisted of diets 1 and 2 from experiment 1 plus diets 3, 4 and 5 in which whey-yeast lysine formed 50, 75 and 83% of total dietary lysine. All the soybean meal was replaced by whey-yeast in diet 5.

In experiment 3, 24 pigs (8 sired by each of three boars) averaging 3.5 weeks of age and 5.90 kg were allotted to four dietary treatments on a basis of sire and initial weight. A randomized block design with a 2 x 2 factorial arrangement of treatments (Cochran and Cox, 1957) was used. Treatments consisted of a low (0.90%) or a high (1.00%) total dietary lysine, based on corn-soybean meal or corn-whey-yeast. Composition of the diets is given in Table VI.

Statistical Analysis

Separate analysis of experiments 1 and 2 were performed following the procedures of Steel and Torrie (1960) for a completely randomized experiment and Snedecor and Cochran (1967) for a randomized block experiment (Appendix Tables XIII and XIV). Since the two experiments differed in two out of six dietary treatments (Tables IV and V), the data from experiment 2 were adjusted for litter (block) effects and pooled with the data from experiment 1 for combined analyses. Treatments 1 and 2 containing 0.80 and 0.90% total dietary lysine, respectively, from corn-soybean meal, were compared on the basis of the analysis of variance shown in Appendix Table XV. Diets 2 through 6 containing 0, 25,

TABLE V

COMPOSITION OF DIETS USED IN EXPERIMENT 2

Ingredients	International Reference No.	% Composition (As-fed) Diet No. 1	2	3	4	5
Corn	4-02-931	73.10	69.59	79.15	83.93	85.45
Soybean meal	5-04-604	23.20	26.79	10.63	2.54	-----
Whey-grown yeast	---	-----	-----	6.50	9.76	10.80
DL-methionine (98% pure)	---	0.22	0.20	0.10	0.05	-----
Dicalcium phosphate	6-01-080	1.22	1.14	1.43	1.57	1.62
Calcium carbonate	6-01-069	1.16	1.18	1.09	1.05	1.03
Vitamin T.M. Premix[a]	---	0.75	0.75	0.75	0.75	0.75
Salt, iodized	6-04-151	0.30	0.30	0.30	0.30	0.30
Aureomycin 50	---	0.05	0.05	0.05	0.05	0.05
Calculated analysis						
Crude protein (N x 6.25)[b]		16.65	17.91	16.23	15.41	15.13
Calcium		0.80	0.80	0.80	0.80	0.80
Phosphorus		0.60	0.60	0.60	0.60	0.60
Lysine, total		0.80	0.90	0.90	0.90	0.90
Methionine + cystine		0.74	0.74	0.76	0.76	0.76
Tryptophan*		0.20	0.22	0.13*	0.09*	0.08*
Arginine:lysine ratio		1.33	1.30	0.91	0.64	0.65
Yeast lysine (% of total)		-----	-----	50	75	83

[a]Vitamin T.M. premix supplied 661,380 I.U. Vitamin A; 49,604 I.U. Vitamin D; 661 mg Riboflavin; 3307 mg. Pantothenic acid; 4960 mg Niacin; 132,276 mg Choline; 2.48 mg Vitamin B_{12}; 1653 I.U. Vitamin E; 331 mg Menadione sodium bisulfate; 33.1 mg iodine; 14.88 gm iron; 3.31 gm manganese; 1.65 gm copper; 14.88 gm zinc and 16.53 mg selenium per 100 kg of feed.

[b]Values determined by Kjeldahl procedure.

*Additional tryptophan was expected to come from whey-yeast.

TABLE VI

COMPOSITION OF DIETS USED IN EXPERIMENT 3

Ingredients	International Reference No.	% Composition (As-fed) Diet No. 1	2	3	4
Corn	4-02-931	69.61	85.17	69.45	85.02
Soybean meal	5-04-604	26.71	-----	26.74	-----
Whey-grown yeast	---	-----	10.79	-----	10.81
DL-Methionine (98% pure)	---	0.27	0.10	0.27	0.10
Lysine hydroxychloride (78.5% lysine)	---	-----	-----	0.13	0.13
Dicalcium phosphate	6-01-080	1.12	1.63	1.12	0.63
Calcium carbonate	6-01-069	1.19	1.21	1.19	1.21
Vitamin T.M. Premix[a]	---	0.75	0.75	0.75	0.75
Salt, iodized	6-04-151	0.30	0.30	0.30	0.30
Aureomycin 50	---	0.05	0.05	0.05	0.05
Calculated analysis					
Crude protein (N x 6.25)[b]		17.91	14.39	17.90	14.39
Calcium		0.80	0.80	0.80	0.80
Phosphorus		0.60	0.60	0.60	0.60
Lysine, total		0.90	0.90	1.00	1.00
Methionine + cystine		0.73	0.73	0.73	0.73
Tryptophan*		0.22	0.08*	0.22	0.08*
Arginine:lysine ratio		1.30	0.65	1.17	0.59

[a]Vitamin T.M. premix supplied 661,380 I.U. Vitamin A; 49,604 I.U. Vitamin D; 661 mg Riboflavin; 3307 mg. Pantothenic acid; 4960 mg Niacin; 132,276 mg Choline; 2.48 mg Vitamin B_{12}; 1653 I.U. Vitamin E; 331 mg Menadione sodium bisulfate; 33.1 mg iodine; 14.88 gm iron; 3.31 gm manganese; 1.65 gm copper; 14.88 gm zinc and 16.53 mg selenium per 100 kg of feed.

[b]Values determined by Kjeldahl procedure. *Additional tryptophan was expected to come from whey-yeast.

50, 75 and 83% yeast lysine of the 0.90% total dietary lysine (Table VII), were analyzed separately by the least squares procedure (Appendix Table XVI). The data from experiment 3 were analyzed according to the procedure of Cochran and Cox (1957) for a 2 x 2 factorial, randomized block experiment.

In all the three experiments, treatment effects on feed consumption, rate of gain, feed efficiency, protein intake and protein efficiency ratio were estimated by analysis of variance. Differences between means were tested by the least significant difference test if the F-test was significant ($P < .05$). Unless stated otherwise, differences were declared statistically significant if the probability of their chance occurrence was less than 5% ($P < .05$).

Results and Discussion

Problems encountered with early weaning of pigs include digestive disorders associated with diarrhea and/or depressed appetite and slow adaptation to dry feed. These problems are more common with some diets than others (Manners, 1970; Pond and Maner, 1974). The data obtained during the first week of experiments 1 and 2 were analyzed to determine the effect of whey-yeast on diet palatability, appetite and length of period required to adapt to dry feed in baby pigs, as measured by intake.

During the first week of experiments 1 and 2, no significantly different responses of pigs to the treatments were observed with respect to average daily gain, average daily feed consumption and average gain: feed ratio. Although the level of protein in the diet decreased as graded levels of whey-yeast lysine were substituted for 25, 50, 75 and 83% of total lysine (Table VII), average daily protein intake values

TABLE VII

AVERAGE PERFORMANCE OF PIGS IN EXPERIMENTS 1 AND 2-COMBINED ANALYSIS*

Item	Diet Number					
	1	2	3	4	5	6
Total lysine, %	0.80	0.90	0.90	0.90	0.90	0.90
Whey-yeast lysine, % of total	----	----	25	50	75	83
			During the First Week			
Number of pigs	10	10	5	10	10	5
Initial weight, kg	5.67	6.22	5.68	5.84	6.29	6.75
Daily feed, kg/day**	0.196	0.253	0.241	0.283	0.276	0.262
Daily gain, kg/day	0.034	0.051	0.086	0.061	0.077	0.077
Gain:feed ratio	-0.013	0.055	0.321	0.126	0.219	0.174
Daily protein, kg/day	0.037	0.057	0.048	0.056	0.052	0.044
Gain:protein ratio	-0.071	0.269	1.603	0.647	1.159	0.957
			Over the Entire 5-Week Period			
Number of pigs	10	10	5	10	9	5
Daily feed, kg/day**	0.656	0.684	0.721	0.676	0.684	0.694
Daily gain, kg/day[a]	0.227	0.287	0.357	0.317	0.348	0.348
Gain:feed ratio[b,c]	0.329	0.414	0.496	0.470	0.507	0.506
Daily protein, kg/day[b,c]	0.125	0.147	0.144	0.133	0.131	0.126
Gain:protein ratio[b]	1.720	1.909	2.479	2.389	2.651	2.807

*See Appendix Tables XV and XVI for analyses of variance.

**Average daily feed dry matter intake.

[a]Linear effect of increasing whey-yeast lysine level, diets 2 through 6, $P < .05$.

[b]Linear effect of increasing whey-yeast lysine level, diets 2 through 6, $P < .01$.

[c]Diet 1 vs. diet 2, $P < .05$.

were not significantly affected. Since carryover effects of previous nutritional management and change of housing environment were common to all pigs, these results indicate that whey-yeast has no adverse or positive effects on diet palatability, appetite or duration of adaptation to the diet in early-weaned pigs.

Over the whole 5-week period of experiments 1 and 2 (Table VII), the inclusion of whey-yeast in the diet did not significantly affect feed consumption. This suggests that whey-grown yeast and soybean meal, when diluted this much, are equally acceptable to early-weaned pigs. Rate of gain was improved linearly ($P < .05$) when 25, 50, 75, and 83% of the .90% dietary lysine was furnished by whey-yeast. Average daily gain was improved by 24, 10, 21 and 21% when 25, 50, 75 and 83% of the dietary lysine was supplied by whey-yeast, respectively. Feed efficiency (gain:feed) and protein efficiency (gain:protein) ratios were significantly higher when whey-yeast was added to the diet. Improvements of 22 and 47% in feed efficiency ratio and protein efficiency ratio, respectively, were obtained at the highest level of whey-yeast fed. Tegbe and Zimmerman (1977) reported quadratic (increasing at a decreasing rate) average daily gain and average daily feed and linear (positive) feed efficiency ratio responses of pigs fed graded levels of yeast in corn-soybean meal diets. Our results indicate linear (positive) responses to average daily gain ($P < .05$), feed efficiency ($P < .01$) and protein efficiency ratio ($P < .01$). Advantage of whey-grown yeast over soybean meal as a source of protein for early-weaned pigs, when fed with corn as an energy source, was implicated by these experiments. Incidents and severity of pig scours during the first week of each experiment decreased with decreasing dietary protein level which accompanied increasing levels of dietary

whey-yeast (visual observations). The exact reasons for this previously observed (Kornegay et al., 1974) relationship were not apparent.

When contrasting protein level, lysine level and source interaction in young pigs, Lunchick and associates (1978) observed that at 1.08% dietary lysine, increasing protein level (14, 16, 18 and 20%) produced increased rates of gain. Our study indicates that gain increased at decreasing protein levels when lysine was maintained at .90% while the percentage of whey-yeast in the diet was increasing. Improved overall pig performance was obtained with the decreasing level of dietary protein (Table VII).

The results of experiment three are presented in Table VIII. Since source by level of lysine interactions were not statistically significant (Table XVII), the results of this experiment are discussed in light of main effects. At both .90 and 1.00% dietary lysine, the source of protein did not affect feed consumption. Corn-whey-yeast diets produced faster growth rates ($P < .01$) at both levels of dietary lysine than did corn-soybean meal diets. Within a given level of dietary lysine (.90 or 1.00%) corn-whey-yeast diets produced better feed efficiency ($P < .01$) and protein efficiency ($P < .001$) ratios than corn-soybean meal diets. The level of dietary lysine had no effect on pig performance ($P > .10$) when pigs were fed corn-soybean meal diets but when fed corn-whey-yeast diets, pigs showed significantly better feed efficiency ratio and protein efficiency ratio at 1.00% than at .90% dietary lysine.

If lysine and protein were the limiting factors of performance, then the pigs fed 1.00% lysine in the corn-soybean meal diet at 17.90% protein should have performed better than those fed .90% lysine in the corn-whey-yeast diet at 14.39% protein. Since pigs fed the higher

TABLE VIII

PERFORMANCE OF PIGS OVER THE 5-WEEK PERIOD OF EXPERIMENT 3*

Item	Diet Number				Main Effects	
					Source	Level
	1	2	3	4	CWY-CSBM	1.00% - 0.90%
Diet description**	CSBM	CWY	CSBM	CWY		
Total lysine, %	0.90	0.90	1.00	1.00		
Number of pigs	6	5	5	5		
Avg. daily feed, kg/day[d]	0.639	0.616	0.589	0.647	-0.035	-0.061
Avg. daily gain, kg/day[b]	0.240	0.323	0.281	0.351	0.078	0.039
Avg. gain:feed ratio[a,c]	0.373	0.524	0.473	0.638	0.163	0.114
Avg. daily protein, kg/day[a]	0.125	0.102	0.119	0.094	-0.024	-0.008
Avg. gain:protein ratio[a,d]	1.916	3.152	2.345	3.720	1.325	0.555

*See Appendix Table XVII for analysis of variance.

**CSBM - corn/soybean meal diet; CWY - corn/whey-yeast diet.

[a]Main effects - Source, $P < .01$.

[b]Main effects - Source, $P < .05$.

[c]Main effects - Level, whey-yeast only, $P < .025$.

[d]Main effects - Level, $P < .05$.

level of lysine and protein did not perform as well, this suggests that protein was not a limiting factor and that lysine and/or other dietary indispensable amino acids were more readily available from whey-yeast than from soybean meal. Chavez and Bayley (1976) reported maximum rates of gain in 5 - 7 kg pigs when fed 1.3 to 1.5% lysine in diets containing 18.0% protein. The report of Lunchick and associates (1978) shows maximum rates of gain at .92% lysine in a 16% protein diet. Our results suggest that as long as the baby pig's requirement for dietary lysine is met, the level of protein in the diet is a function of the source of protein and can be as low as 14% for whey-yeast based cereal diets without depressing rate of gain. In this experiment, pigs fed corn-whey-yeast at 14.39% protein and .90% dietary lysine had 35, 40 and 65% greater ($P < .05$) rate of gain, gain:fed ratio and gain:protein ratio, respectively, than those pigs fed the corn-soybean meal diet at 0.90% lysine and 17.91% crude protein. At 1.00% lysine (14.3% protein), the corn-whey-yeast diet produced 25, 35 and 59% greater ($P < .01$) average daily gain, feed efficiency ratio and protein efficiency ratio, respectively, over the 17.90% protein corn-soybean meal diet. No adverse effects of whey-yeast on early-weaned pigs were observed in this study. This corroborates the reports of Shacklady and associates (1970).

The results of this study have confirmed whey-yeast protein to be of a consistently superior quality to soybean meal protein for early-weaned pigs. The reasons for this are unclear; however, whey-yeast protein (Table III) contains higher levels of most of the pig's dietary indispensable amino acids than soybean meal protein (Table III). The possibility of whey-yeast lysine being more readily available to the pig than soybean meal lysine is implied in this study. On the basis of

these experiments, it is suggested that further work be done to determine the first, second and the third limiting amino acids and the exact level of dietary lysine that will produce maximum rates of gain in baby pigs fed corn-whey-yeast diets.

CHAPTER IV

WHEY-GROWN YEAST AS A PROTEIN SOURCE FOR LAMBS

Summary

The potential of whey-grown Kluyveromyces fragilis yeast as a protein supplement for lambs was studied. Four treatments comprising 11.0% protein based on soybean meal (LS) or whey-yeast (LY) and 14.0% protein based on soybean meal (HS) or whey-yeast (HY) were fed to 48 crossbred lambs. In experiment 4, the four diets were fed to 40 ewe lambs averaging 34.15 kg in a 2 x 2 factorial growth experiment. Average daily feed intakes ($P > .05$) were 1.29, 1.26, 1.32 and 1.20 kg/day for diets LS, LY, HS and HY, respectively. Corresponding values for daily gain were 284, 296, 278 and 269 g/day ($P > .05$); for gain/feed .22, .24, .21 and .22 ($P > .05$) and for gain/protein 1.59, 2.16, 1.50 and 1.59 ($P < .01$). In experiment 5, the same diets were fed to 8 ram lambs in a replicated 4 x 4 latin square. For diets LS, LY, HS and HY, digestibility coefficients averaged 65.6, 70.0, 68.0 and 69.2% for dry matter ($P > .05$) and 78.0, 80.9, 84.3 and 83.6% for nitrogen ($P < .05$), respectively. Higher urinary nitrogen ($P < .01$) and higher urine volume ($P < .05$) values were observed for sheep fed the high protein diets than those fed the low protein rations. Rumen ammonia-N ($P > .05$) and plasma urea-N ($P < .01$) averaged 20.7, 19.4, 22.8 and 21.0 mg/100 ml and 10.2, 11.1, 13.5 and 15.1 mg/100 ml, respectively, for diets LS, LY, HS and HY. The corresponding values for average daily nitrogen retention of 6.0, 6.5,

8.9 and 5.1 g/day were not different statistically. Lower rumen pH ($P < .10$) values were observed with yeast diets at both protein levels. Results suggest that K. fragilis has a promise as a single-cell protein supplement for lambs.

Introduction

Anaerobic microbes in the ruminoreticulum degrade much of dietary nitrogen into ammonia. The ammonia produced can be used for synthesis of microbial protein which may be digested and absorbed post-ruminally (Blackburn, 1965; Burroughs et al., 1975; Chalupa, 1975). Faced with rising costs of high quality protein sources, animal nutritionists were prompted to seek non-protein nitrogen (NPN) sources for use in ruminant feeds. Although well managed NPN-based diets have been successfully used in ruminant nutrition, at high protein supplementation levels natural protein generally supports a faster growth rate for domestic ruminants than NPN (Reis and Tunks, 1969; Braman et al., 1973). At the highest rate of microbial protein synthesis in the rumen, the animal may still receive suboptimal protein for maximum production unless substantial amounts of dietary protein bypass the rumen (Little and Mitchell, 1967; Schelling and Hatfield, 1968; Peter et al., 1971).

Various methods to protect dietary protein against rumen microbial degradation have been used in attempts to increase passage of dietary protein to the abomasum (Driedger and Hatfield, 1972; Atwal et al., 1974; Glenn et al., 1977). Such methods often have proved costly and have lowered post-ruminal digestibility (Barry, 1973; Dinius et al., 1975). The search continues for sources of preformed protein which could bypass the rumen without further protection and be highly digesti-

ble post-ruminally.

The objectives of this study were to determine the influence of supplemental whey-grown Kluyveromyces fragilis yeast or soybean meal on growth performance, digestibility and nitrogen balance in lambs.

Materials and Methods

Preliminary Determinations

The test product, "whey-yeast", was prepared by growing Kluyveromyces fragilis yeast on cottage cheese whey (Smith et al., 1977). Soluble nitrogen of the whey-yeast was determined at pH 6.5 and at pH 5.5, stimulating normal rumen conditions in buffer solutions by the method of Prigge et al. (1976). In vitro 24-hr day matter digestibility (IVDMD) and in vitro nitrogen digestibility (IVND) of the same material were determined using rumen fluid obtained from a fistulated steer.

Experiment 4

Forty crossbred ewe lambs ranging from 24.6 to 40.0 kg in body weight were grouped according to weight and assigned to four dietary treatments in a randomized block design with a factorial arrangement of treatments (Cochran and Cox, 1957). Diets were based on soybean meal or on whey-yeast and provided (11%) or (14%) protein (Table IX). All diets were supplemented with .86% urea as a source of readily available nitrogen to aid microbial protein synthesis.

Lambs were housed in individual elevated wooden feeding pens with slatted floors which facilitated cleaning. Small quantities of feed were fed during the 5-day adjustment period. Thereafter, feed and

TABLE IX

COMPOSITION OF THE DIETS USED IN EXPERIMENTS 4 AND 5

Ingredients*	International Reference No.	% Composition (As-fed)** Diet No. 1	2	3	4
Corn	4-02-931	62.48	64.13	54.18	58.87
Soybean meal	5-04-604	4.51	-----	12.81	-----
Whey-grown yeast	---	-----	2.86	-----	8.12
Cotton seed hulls	1-01-599	25.00	25.00	25.00	25.00
Urea	---	0.86	0.86	0.86	0.86
Molasses, cane	4-04-696	5.00	5.00	5.00	5.00
Calcium carbonate	6-01-080	0.85	0.85	0.85	0.85
Trace-Minearlized salt	---	0.50	0.50	0.50	0.50
Sodium sulfate	6-04-292	0.50	0.50	0.50	0.50
Potassium chloride	---	0.30	0.30	0.30	0.30
Crude protein, N x 6.25***					
Calculated		11.09	11.09	14.00	14.00
Determined		11.07	10.97	14.00	13.98
Calcium***		0.42	0.42	0.45	0.44
Phosphorus***		0.25	0.24	0.28	0.24

*1,000 I.U. of Vitamin A palimtate and 140 I.U. of Vitamin D were added per kg of each diet.
**Diets 1, 2, 3 and 4 contained 87.6, 88.0, 87.5 and 87.5% dry matter, respectively.
***Values based on 100% dry matter.

clean water were available ad libitum. Feed intakes were recorded.

After the adjustment period, lambs were weighed full to obtain initial weights, and subsequently every two weeks for the six-week trial.

Experiment 5

A nitrogen balance trial was conducted to determine the effects of diets on protein metabolism, rumen ammonia release and plasma urea levels. Eight crossbred ram lambs, closely related to the ewe lambs used in the growth trial, were used in two 4 x 4 latin squares (Table X).

Lambs were housed in individual metabolism cages. Feed and water were available ad libitum. Each period lasted 14 days with urine and feces collected the final 5 days. Urine was collected daily in plastic buckets containing 30 ml of 6N HCl to acidify the urine. Ten percent aliquots by weight of urine per day were saved and composited for each animal within each collection period and stored frozen for later analysis. Feces were collected and ten percent composited and stored frozen for later analysis. At the end of each collection period, and before animals were switched to the next diet in the square, rumen fluid and blood samples were obtained 1 hr post-feeding. About 40 ml of rumen fluid were obtained by suction through a stomach tube and strained through 4 layers of cheesecloth. After the pH was determined, the rumen fluid was acidified with .5 ml of concentrated sulfuric acid and frozen for later analysis. Via jugular venipuncture, 20 ml of blood were collected with preheparinized disposable syringe and a number 18 needle and transferred to a preheparinized plastic centrifuge tube.

TABLE X

ARRANGEMENT OF THE DOUBLE 4 X 4 LATIN SQUARE DESIGN USED IN EXPERIMENT 5*

Square No.	Sheep No.	Period No.** 1	2	3	4
1	344	HY	LS	LY	HS
	347	HS	HY	LS	LY
	103	LY	HS	HY	LS
	642	LS	LY	HS	HY
2	644	HY	LS	LY	HS
	526	HS	HY	LS	LY
	643	LY	HS	HY	LS
	213	LS	LY	HS	HY

LS - Low protein soybean meal based diet 1.

LY - Low protein whey-yeast based diet 2.

HS - High protein soybean meal based diet 3.

HY - High protein whey-yeast based diet 4.

*Composition of diets is shown in Table IX.

**Each period started with 9 days of adjustment in order to minimize carry-over effects.

Blood samples were centrifuged at 3,000 g for 15 min and the plasma saved frozen for later analysis.

All frozen samples were defrosted immediately prior to analysis. Urine was analyzed for total nitrogen, and feces were analyzed for nitrogen and dry matter (A.O.A.C., 1970). Rumen fluid samples were analyzed for ammonia nitrogen by the method of Chaney and Marbach (1962). Blood plasma samples were analyzed for plasma urea nitrogen using the spectrophotometric technique summarized by Preston and Osmond (1971) from the works of Fawcet and Scott (1960) and Searcy et al. (1961).

Statistical Analysis

The growth trial (Experiment 4) was analyzed as a 2 x 2 factorial experiment with a randomized complete-block design using the procedure outlined by Steel and Torrie (1960). Treatment effects on feed consumption, rate of gain, feed utilization efficiency, protein intake and protein efficiency ratio were estimated by the analysis of variance method. The nitrogen balance trial (Experiment 5) was analyzed as a 2 x 2 factorial experiment with a replicated 4 x 4 latin square design (Cochran and Cox, 1957). Analysis of variance was performed to estimate treatment effects on the variables listed in Table XII.

For both experiments 4 and 5, differences between means were tested by the least significant difference test if the F-test was significant at $P < .10$. Unless stated otherwise, differences were declared statistically significant if the probability of their chance occurrence was less than 5% ($P < .05$).

Results and Discussion

Preliminary Determinations

Solubility of the protein from the whey-yeast product was 2.75 ± .25% at pH 6.5 and 2.25 ± .15% at pH 5.5. Under similar conditions, solubility values of 41.4, 13.0, and 7.2% have been reported for protein from dried whey, soybean meal and cottonseed meal, respectively (Wohlt et al., 1973). Degradation of protein nitrogen in the rumen tends to increase with protein solubility (Chalmers et al., 1954; Tagari et al., 1962; Nishimuta et al., 1973). Consequently, the whey-yeast protein may escape destruction in the rumen. In vitro dry matter digestibility (IVDMD) and in vitro nitrogen digestibility (IVND) values for the whey-yeast averaged 28.9 ± 1.6 and 20.0 ± 2.2%, respectively, for 12 replicates. Values of IVDMD and IVND for soybean meal average 67 and 65%, respectively (F. N. Owens, unpublished data). With the low nitrogen solubility and low IVND values obtained in the preliminary work, it was anticipated that whey-yeast protein should resist degradation in the rumen.

Experiment 4

The performance trial results are summarized in Table XI. Lambs had an initial mean weight of 34.2 kg with those fed the yeast diets slightly lower in body weight than those fed the soybean meal diets at the beginning of the experiment. However, palatability problems were not observed with any ration. Throughout the experiment, neither source nor level of dietary protein affected rate of gain or efficiency of feed utilization, although the whey-yeast fed lambs averaged 0.5 percent

TABLE XI

AVERAGE PERFORMANCE OF LAMBS IN EXPERIMENT 4*

Item	Diet Number				Main Effects	
					Source	Level
	1	2	3	4	Yeast - Soy	High - Low
Diet description**	LS	LY	HS	HY		
Protein level (N x 6.25), %	11.07	10.97	14.00	13.98		
Number of lambs	10	10	10	10		
Initial weight, kg	34.90	33.29	35.06	33.35		
Daily feed, kg/day	1.287	1.256	1.321	1.200	-0.090	-0.007
Daily gain, kg/day	0.284	0.296	0.278	0.269	0.001	-0.016
Gain:feed ratio	0.219	0.237	0.210	0.223	0.015	-0.012
Daily protein[a,c]	0.142	0.138	0.185	0.168	-0.010	0.036
Gain:protein ratio[b,c]	1.592	2.164	1.497	1.592	0.133	-0.476

*See Appendix Table XVIII for the analysis of variance.

**See footnotes to Table X for diet definitions.

[a]Main effects - Source, $P < .025$.

[b]Main effects - Source, $P < .05$.

[c]Main effects - Level, $P < .01$.

faster gains and 7.2 percent greater efficiency of feed utilization than the soybean meal fed lambs. The feed efficiency ratios obtained in this study are superior to those reported by Sherrod and Tillman (1962) for heat treated soybean meal and cotton seed meal, and by Reynolds et al., (1978) for formaldehyde treated alfalfa meal. At this point the reasons for the differences are unclear.

Average daily protein intake was lower for the low than the high protein diets, mainly due to the differences in diet protein levels (Table XI). Superior protein utilization was achieved with the low protein whey-yeast diet ($P < .01$). For all the variables measured, protein source and level interactions were not significant statistically (Appendix Table XVIII).

Experiment 5

Results of the nitrogen balance trial are presented in Table XII and the analysis of variance table is given in Table XIX. At 11.0 and 14.0% crude protein, the source and level of protein had no significant statistical effect on feed consumption or fecal dry matter output. Consequently, diets did not differ in dry matter digestibility. However, yeast diets averaged 2.8 percent higher in digestibility. Feed intake values agreed closely with those obtained for the same diets in experiment 4 (Table XI), suggesting that animal sex has little effect on feed consumption. The higher level of dietary nitrogen increased daily nitrogen intake as in experiment 4. Source and level of protein fed were without effect on total fecal nitrogen. The higher ($P < .05$) nitrogen digestibility coefficients at 14.0 percent protein can be attributed to more supplemental protein and less corn protein in those

TABLE XII

NITROGEN METABOLISM OF LAMBS IN EXPERIMENT 5*

Item	Diet**				
	LS	LY	HS	HY	SE[1]
Crude protein, % as-fed	11.07	10.97	14.00	13.98	
Number of lambs	8	8	8	8	
Dry matter intake, kg/day	1.202	1.184	1.169	1.127	.112
Fecal dry matter, kg/day	.380	.353	.375	.346	.033
Dry matter digest, %	65.56	69.95	67.96	69.15	2.54
Nitrogen intake, g/day[b]	21.3	20.8	26.2	25.2	2.0
Fecal nitrogen, g/day	4.02	3.89	4.10	4.07	.31
Nitrogen digestibility, %[b]	78.03	80.88	84.28	83.58	2.56
Urinary nitrogen, g/day[a,b,c]	10.6	10.0	13.2	15.8	1.1
Nitrogen retained, g/day	6.02	6.51	8.86	5.10	.22
As % of intake	28.26	31.34	33.81	20.24	11.07
Urine volume, l/day[b]	1.100	1.157	1.370	1.464	.135
Rumen ammonia-N, mg/100 ml	20.56	19.39	22.75	20.99	2.45
Plasma urea-N, mg/100 ml[a]	10.17	11.09	13.52	15.10	1.48
Rumen fluid pH[d]	6.39	6.18	6.34	6.11	.17

*See Appendix Table XIX for the analysis of variance.

**See footnotes to Table X for diet definitions.

[1]Standard error of difference between 2 treatment combination means.

[a]Level within source, $P < .01$.

[b]Level within source, $P < .05$.

[c]Source within level, $P < .01$.

[d]Source within level, $P < .10$.

rations. In the former experiment, the highest rate of gain and protein efficiency ratio were produced by the low nitrogen whey-yeast diet. In this experiment, nitrogen balance was high with this diet, but at the high protein level, soybean meal produced a slightly higher nitrogen balance than yeast protein.

Irrespective of protein source, higher ($P < .05$) volumes of urine were excreted by animals fed the high nitrogen diets. The same relationship existed for the plasma urea-N values (Table XII). Since urine is the normal route of urea excretion in ureolytic vertebrates such as sheep, this relation was normal. There were no dietary effects on rumen ammonia-N. This questions whether increased protein bypass with whey-yeast was achieved. There appeared to be no relationship between rumen ammonia-N and plasma urea-N. However, rumen fluid pH values were lower ($P < .10$) when whey-yeast protein diets were fed. Level of protein had no effect on rumen fluid pH. No protein source and level significant interactions were statistically (Appendix Table XVIX).

Little literature information is available regarding whey-yeast as a protein supplement for ruminants. The performance and nitrogen balance data obtained in this study suggest that as a supplemental protein source, its value is at least equal to soybean meal. Microbial protein is added to or present in certain feedstuffs like fermented whey and liquid supplements for cows on range. Whether microbial protein in liquid supplements is of this high value is not known.

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A P P E N D I X

TABLE XIII

ANALYSIS OF VARIANCE FOR AVERAGE PERFORMANCE OF PIGS IN EXPERIMENT 1

Source	df	Mean Squares				
		ADF[a]	ADG[b]	ADP[c]	FER[d]	PER[e]
		During the First Week				
Treatment	4	.00778	.00424	.00040	.16692	4.41069
Error	20	.00910	.01302	.00036	.43025	11.61005
		Over the Entire 5-Week Period				
Treatment	4	.00381	.01936*	.00064	.04052***	1.17514***
Error	19[f]	.00785	.00622	.00029	.00745	.18835

[a]Average daily feed intake.

[b]Average daily gain.

[c]Average daily protein intake.

[d]Average feed efficiency ratio (gain:feed).

[e]Average protein efficiency ratio (gain:protein).

[f]One pig died after the first week.

*P < .05.

**P < .01.

***P < .005.

TABLE XIV

ANALYSIS OF VARIANCE FOR AVERAGE PERFORMANCE OF PIGS IN EXPERIMENT 2

Source	df	Mean Squares				
		ADF[a]	ADG[b]	ADP[c]	FER[d]	PER[e]
			During the First Week			
Treatment	4	.00582	.00226	.00030	.02115	.59121
Litter	4	.01041	.01236***	.00041	.07278***	1.98136***
Error	16	.00542	.00227	.00022	.01378	.36147
			Over the Entire 5-Week Period			
Treatment	4	.00250	.00973**	.00039	.01900**	.86627***
Litter	4	.00929	.00414	.00035	.00343	.08552
Error	16	.00557	.00216	.00022	.00447	.11539

[a]Average daily feed dry matter intake.
[b]Average daily gain.
[c]Average daily protein intake.
[d]Average feed efficiency ratio (gain:feed).
[e]Average protein efficiency ratio (gain:protein).
*P < .05.
**P < .025.
***P < .01.

TABLE XV

ANALYSIS OF VARIANCE FOR COMPARING TREATMENTS 1 AND 2 - COMBINED ANALYSIS OF EXPERIMENTS ONE AND TWO

Source	df	Mean Squares				
		ADF[a]	ADG[b]	ADP[c]	FER[d]	PER[e]
		During the First Week				
Experiment	1	0.001044	0.001020	0.000001	0.079026	1.907678
Treatment	1	0.016138	0.001469	0.001898	0.022886	0.578810
Error	17	0.009357	0.010644	0.000382	0.414001	11.192951
		Over the Entire 5-Week Period				
Experiment	1	0.002268	0.000402	0.000009	0.002262	0.012324
Treatment	1	0.004053	0.018518	0.002392*	0.035340*	0.179372
Error	17	0.010480	0.005802	0.000407	0.007429	0.178806

[a]Average daily feed intake.

[b]Average daily gain.

[c]Average daily protein intake.

[d]Average feed efficiency ratio (gain:feed).

[e]Average protein efficiency ratio (gain:protein).

*$P < .05$.

TABLE XVI

ANALYSIS OF VARIANCE FOR COMPARING TREATMENTS 2, 3, 4, 5 AND 6 - COMBINED ANALYSIS OF EXPERIMENTS ONE AND TWO

Source	df	Mean Squares				
		ADF[a]	ADG[b]	ADP[c]	FER[d]	PER[e]
		During the First Week				
Experiment	1	0.000137	0.000184	0.000007	0.002276	0.062108
Treatment	4					
Linear	1	0.003867	0.002319	0.000213	0.074140	2.460886
Quadratic	1	0.000496	0.000147	0.000043	0.021024	0.383870
Cubic	1	0.004561	0.002277	0.000466	0.100680	2.525643
Quartic	1	0.000766	0.001625	0.0000004	0.095762	2.290534
Experiment x Treatment[f]	2	0.002656	0.004837	0.0000048	0.102617	2.706715
Error	32	0.006222	0.007077	0.000256	0.100554	2.479111
		Over the Entire 5-Week-Period				
Experiment	1	0.000195	0.000529	0.0000003	0.001387	0.020371
Treatment	4					
Linear	1	0.000226	0.018047*	0.002363***	0.048911***	3.783789***
Quadratic	1	0.000137	0.000105	0.000007	0.000259	0.001483
Cubic	1	0.006707	0.006463	0.000045	0.004837	0.299816
Quartic	1	0.001661	0.002746	0.000228	0.001668	0.000564
Experiment x Treatment[f]	2	0.003602	0.004298	0.000200	0.007607	0.20567
Error	31	0.003652	0.003570	0.000148	0.005458	0.135728

[a]Average daily feed intake. [b]Average daily gain. [c]Average daily protein intake.
[d]Average feed efficiency ratio (gain:feed). [e]Average protein efficiency ratio (gain:protein).
[f]2 df for interaction are lost because 2 of the treatments were not present in both experiments, i.e., missing cells.
*P < .05. ***P < .01.

TABLE XVII

ANALYSIS OF VARIANCE FOR AVERAGE PERFORMANCE OF PIGS IN EXPERIMENT 3

Source[1]	df	Mean Squares				
		ADF[2]	ADG[3]	ADP[4]	FER[5]	PER[6]
Source	1	.0134	.0169*	.0036***	.1061***	7.5069***
Level	1	.0185*	.0058	.0003	.0591**	1.3095*
Source x Level	1	.0041	.0014	.0001	.0003	.0277
Error[a]	9	.0035	.0037	.0001	.0068	.1848

[1]Only the sources of variation of major importance in this experiment are considered in this table.

[2]Average daily feed dry matter intake.

[3]Average daily gain.

[4]Average daily protein intake.

[5]Average feed efficiency ratio (gain:feed).

[6]Average protein efficiency ratio (gain:protein).

*P < .05.

**P < .025.

***P < .01.

[a]Unequal subclass numbers.

TABLE XVIII

ANALYSIS OF VARIANCE FOR AVERAGE PERFORMANCE OF LAMBS IN EXPERIMENT 4 - OVER THE ENTIRE SIX-WEEK PERIOD

		Mean Squares				
	df	ADF[1]	ADG[2]	ADP[3]	FER[4]	PER[5]
Block	9	.02023	.00391	.00035	.00142	.10131*
Source	1	.08122	.00001	.00182**	.00196	.17639*
Level	1	.00050	.00262	.01808***	.00133	2.26101***
Source x Level	1	.02264	.00105	.00039	.00007	.02397
Error	27	.02027	.00236	.00030	.00058	.04273

[1]Average daily feed dry matter intake.

[2]Average daily gain.

[3]Average daily protein intake.

[4]Average feed efficiency ratio (gain:feed).

[5]Average protein efficiency ratio (gain:protein).

*P < .05.

**P < .025.

***P < .01.

TABLE XIX

ANALYSIS OF VARIANCE FOR THE DIGESTIBILITY AND NITROGEN BALANCE TRIAL - EXPERIMENT 5

Source	df	Mean Square				
		ADM[1]	FDM[2]	DMD[3]	ANI[4]	FCN[5]
Square	1	.0728	.0033	8.5604	.00004	.0000011
Sheep (square)	6	.18075*	.0349***	24.7041	.00006**	.0000018***
Period	3	.1705	.0149	511.0088***	.00006	.0000003
Square x Period	3	.0526	.0037	24.1549	.00002	.0000003
Source	1	.0070	.0063	62.3380	.000004	.00000005
Level	1	.0161	.0003	5.1567	.00017***	.00000014
Source x Level	1	.0011	.0000002	20.5055	.0000004	.00000002
Square x Source	1	.0086	.0007	56.7918	.0000032	.0000002
Squre x Level	1	.0837	.0081	9.9365	.000041	.000000003
Square x Source x Level	1	.0007	.0009	11.5342	.00000009	.0000003
Error	12	.0503	.00431	25.7866	.000016	.00000034

Source	df	Mean Square				
		ND[6]	URN[7]	ANR[8]	PNR[9]	URV[10]
Square	1	17.1540	.000023*	.00000006	393.8538	.4838**
Sheep (Squre)	6	19.7004	.000013	.000026	680.8462	1.0360***
Period	3	135.4805**	.000003	.000079***	2054.4402*	.0395
Square x Period	3	22.4072	.000006	.000007	331.6108	.3630**
Source	1	9.2989	.000008	.000022	.5202	.0451

TABLE XIX (Continued)

Source	df	Mean Square				
		ND[6]	URN[7]	ANR[8]	PNR[9]	URV[10]
Level	1	160.0669*	.000137***	.000001	59.0366	.6648**
Source x Level	1	25.1295	.000019	.000026	1528.3673	.0029
Square x Source	1	36.7382	.000003	.000016	912.5931	.3126
Square x Level	1	51.1936	.0000000004	.000042	1088.2376	.3906*
Square x Source x Level	1	3.5769	.000011	.000010	655.2969	.0159
Error	12	26.2916	.000005	.000013	490.2453	.0735

Source	df	Mean Square		
		RAN[11]	PUN[12]	RPH[13]
Square	1	7.5175	71.2520**	1.0915***
Sheep (square)	6	31.7229	43.9922***	.1231
Period	3	146.0110***	2.0280	.9726***
Square x Period	3	11.3777	8.9809	.0782
Source	1	17.2431	12.5626	.3894
Level	1	28.5957	108.2288***	.0248
Source x Level	1	.6991	.8613	.0009
Square x Source	1	9.0206	.7051	.0270
Square x Level	1	12.1895	35.1751	.0176
Square x Source x Level	1	24.9395	.0413	.0872
Error	12	24.0550	8.7907	.1160

TABLE XIX (Continued)

[1]Average daily feed dry matter intake.

[2]Average daily fecal dry matter voided.

[3]Average dry matter digestibility.

[4]Average daily nitrogen intake.

[5]Average daily fecal nitrogen voided.

[6]Average nitrogen digestibility.

[7]Average daily urinary nitrogen excreted.

[8]Average daily nitrogen retained.

[9]Average nitrogen retained as percentage of intake.

[10]Average daily urine volume.

[11]Rumen ammonia nitrogen.

[12]Plasma urea nitrogen.

[13]Rumen pH.

*$P < .05$.

**$P < .025$.

***$P < .01$.

VITA[2]

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