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In part one, two experiments were conducted. In experiment one, levels of dehydrated alfalfa meal greater than 60% of the diet significantly ($P < 0.01$) reduced the growth of rats fed isocaloric diets. Purified cellulose fed to provide the same amount of acid detergent fiber as contributed by the alfalfa did not affect growth. Feed intake was reduced at high alfalfa levels, indicating that palatability, rather than fiber level, was the factor responsible for reduced performance. In experiment two, the avoidance of heat during drying of alfalfa meal, through the use of freeze drying, resulted in improved growth and feed conversion. The results are consistent with the hypothesis that during conventional drying methods, bitter compounds are formed. No difference in crude protein, dry matter, or acid detergent fiber digestibility was found with oven dried (95°) compared to freeze dried alfalfa meal.

In part two, five experiments with rats and one with Japanese Quail were conducted to evaluate a commercially prepared alfalfa protein concentrate (APC) as a protein supplement. It was found to contain 36% crude protein with 5.5 g lysine and 2.5 g methionine plus cystine per 100 g amino acids.

Levels of up to 20% APC did not decrease performance of rats; APC as the sole protein source gave poor results. The Protein Efficiency Ratio (PER) of APC was significantly lower ($P < 0.01$) than those for herring meal, casein, or soybean meal (1.23, 2.82, 2.67, and 2.54 respectively). Upon supplementation with lysine and methionine the PER was significantly increased ($P < 0.01$) to 2.54. Lysine was the first-limiting amino acid; methionine was also deficient.

With rats the crude protein digestibility was 65% for APC, which was significantly lower ($P < 0.01$) than for soybean meal (88%). The low digestibility of APC protein contrasts with other reported values of about 80%.

The availability of lysine in APC was determined by using a bioassay with both rats and Japanese Quail. Low protein basal diets were used. To the basal diets the lysine references (casein for rats, lysine HCl for the quail) and APC were added as the lysine sources. The results were extrapolated from a graph of reference values using g gain vs. g lysine consumed. The availability of lysine was found to be about 80% for rats when compared to casein and about 86% for quail when compared to lysine HCl.

In part three, three swine studies were conducted. APC gave excellent results when used in grower-finisher rations. Carcass evaluation showed no significant differences; however, there was a trend towards lower market grades with higher dietary APC levels. With young growing swine (18 kg to 47 kg), gains with 20% APC were significantly lower ($P < 0.05$) than for the controls.

In creep-starter rations, APC fed at levels of 6.5 and 13% gave good results. Levels of 14, 28, and 34% which provided 29, 58, and 70% of the total dietary crude protein, significantly reduced growth ($P < 0.05$) in one trial but not another. In all tests with starter rations the feed conversion increased for the APC rations.

The experiments with swine indicate that the APC may be better utilized by growing-finishing swine than by younger animals. The growth performance indicates the APC can be a useful protein supplement for all classes of growing swine.

Utilization of Processed Alfalfa Meals
by Monogastric Animals

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INTRODUCTION

Alfalfa produces more crude protein per acre than most other crops grown in the United States. Peo (1972) has stated that under ideal conditions, alfalfa could produce 2700-3400 pounds of protein per acre compared to 1800-2000 pounds for soybeans, while under poor conditions, alfalfa would yield about 850 pounds compared to 350-450 pounds per acre from soybeans. Stahman (1968) obtained data on production of three forage crops, including alfalfa, and four seed crops based on recent yields obtained at USDA Experimental Stations in the midwest. He showed that alfalfa and other green forage crops produced three to ten times more crude protein per acre than major seed crops. Alfalfa produced 2400 pounds, sorghum-sudan forage 2000 pounds, corn seed 780 pounds, soybean seed 700 pounds and wheat 400 pounds of crude protein per acre.

Besides its high protein yield, other advantages of alfalfa noted by Kohler *et al.* (1973) include:

1) Alfalfa is a major forage crop, hence many varieties are available to fit differing conditions and agronomic practices.
2) It can be harvested frequently.
3) It maintains relatively high nutritional quality throughout hot

and cool seasons, so long as adequate moisture is available.

4) It is a perennial crop, hence time and costs of replanting are minimal.

5) It is a legume that, with the aid of symbiotic nodule bacteria, fixes nitrogen and fits well in crop rotation systems.

6) There is already a substantial processing industry that has solved many problems relating to farmers, i.e., harvesting and hauling, etc.

In spite of these apparent advantages, alfalfa has not been used as a major protein supplement for swine. Because of its high fiber content, low protein digestibility, and low energy content, and the fact that it is unpalatable to swine at high levels, direct use of alfalfa in swine rations as a protein source has not proven feasible. Even with air separation of alfalfa meals into leaf meals with crude protein contents of 25-28%, a high fiber content still exists and under many conditions the process is too costly (Kohler et al., 1973). Separation of the protein from the fiber of alfalfa and other green plants is required before it can be utilized efficiently by monogastric animals.

During World War II, work was started in England to develop mechanical processes for concentrating protein from green plants. When green plant material is crushed to break the cell walls and pressed, the juice contains much of the protein free from fibrous material. A practical economically feasible method of isolating protein from alfalfa and preparing a leaf protein concentrate or alfalfa protein concentrate (APC) has recently been developed by USDA scientists at the Western Regional Research Laboratory, Albany, California. The details of the techniques used have been described by Kohler et al. (1973). Essentially, the procedure involves pressing the juice from green alfalfa and coagulation with steam and then drying the protein curd. The USDA workers called this material Pro Xan (for Protein-Xanthophyll concentrate). The processing procedure has been adopted to commercial by Batley-Janss Enterprises, Brawley, California. This plant produces about 4000 tons per year of the protein concentrate, which they have given the trade name of "X-Pro" (from Pro Xan). This is a true protein concentrate containing about 35-38% crude protein with only about 2-3% fiber. The objective of this study was to evaluate this APC product as a protein supplement for swine.

LITERATURE REVIEW

The Utilization of Alfalfa by Monogastric Animals

Alfalfa has been used for several decades as a vitamin supplement and a source of unidentified growth and reproduction factors in swine rations. In addition it has been used quite extensively to restrict energy intake in swine gestation rations.

Attempts to use alfalfa at high levels in growing-finishing swine rations as a protein source or even as en energy source have been relatively unsuccessful. Bohman et al. (1953) observed fair gains with growing-finishing swine receiving rations up to 50% alfalfa; they noted little difference in gains for pigs fed 10-30% alfalfa rations when compared to no alfalfa in the ration. Becker et al. (1954) noted depressed gains and feed efficiency for growing-finishing swine receiving more than 20% alfalfa. Hanson et al. (1956) showed that rations containing up to 20% alfalfa did not lead to significant growth depression. Stevenson et al. (1960) reported significant reduction in gain when alfalfa was raised from 4 to 16% in the diet while Danielson et al. (1969) did not get significant depression in growth at the 16% level but did at the 32% dietary level for growing-finishing swine. One study did show a decrease in gain and feed efficiency with the inclusion of 20% alfalfa (Seerley and Wahlstrom,

1968) while in another study good results were obtained with a high quality (29% crude protein) dehydrated meal even when fed at the 35% dietary level (Charlet-Lery et al., 1955).

Part of the reason that alfalfa is utilized poorly at high levels is that its high fiber content reduces the total digestible nutrient content (TDN). Additions of corn oil to bring up the TDN to that of corn have been shown to be generally successful (Becker et al., 1954; Hanson et al., 1956) as has the addition of animal fat (De Costa, 1958). However, Hanson et al. (1956) showed with ad libitum feeding that with swine fed diets containing alfalfa plus corn oil at the same TDN level as a corn-soy control, decreased performance occurred, whereas when feed intake of the two rations was equalized, no differences in performance were noted. In another study it was observed that even with added fat, with dietary levels of alfalfa greater than 12.5%, decreases in performance were still noted with growing-finishing swine (De Costa, 1958). Therefore some factor(s) other than its low TDN content interfere with the utilization of alfalfa.

Forbes and Hamilton (1952), Whiting and Bezeau (1957), Cunningham et al. (1962) and Keys et al. (1972) noted that with increasing fiber levels in the diet, metabolic fecal nitrogen excretion by swine increases, and thereby increases the dietary protein requirement. Higher fiber levels in the diet have been shown to also decrease dry matter, energy, and crude fiber digestibility of the total rations (Keys et al., 1972). With growing-finishing swine, increasing the dietary fiber level above the 10% level resulted in decreased dry matter, energy and crude protein digestibility (Dinusson et al., 1969), while maximum growth and feed efficiency were shown to occur at between 6.5 and 7.5% crude fiber in growing-finishing swine rations (Axelsson and Ericksson, 1953). With increasing levels of alfalfa in the diet, decreased digestibility of dry matter, energy, crude fiber, and crude protein has been noted (Charlet-Lery et al., 1955; Danielson et al., 1969). The high fiber levels of alfalfa may account for some of the reported reduced performance of swine even when TDN has been corrected. In the study by Dinusson et al. (1969) in feeding high fiber rations to swine an increase in feed intake was noted with increasing fiber level so that the daily energy intake was the same; in some of the above studies with alfalfa a decreased intake has been shown at high levels (Becker et al., 1954; Stevenson et al., 1960). With alfalfa added to a typical corn-soy ration, about 25% alfalfa can be added before the crude fiber content will exceed 10%; however, many studies show growth inhibition at lower alfalfa levels (Hanson et al., 1956; Stevenson et al., 1960; Seerley et al., 1968). With equal fiber levels for a corn-soy basal with purified fiber, and a 20% alfalfa test ration, Peo et al. (1972) noted a significant decrease in performance when baby pigs were fed the alfalfa ration. Therefore some other factors must be involved.

Growth inhibition caused by alfalfa meal in chick diets can be largely overcome by dietary supplementation with 1% cholesterol (Peterson, 1950). The growth inhibition is caused by saponins (Heywang and Bird, 1954); cholesterol combines with saponins and prevents their absorption. Growth inhibition in chicks and rats fed alfalfa saponin was reported by Coulson (1957), who noted that high levels (2-3%) of alfalfa saponin were required to reduce growth of rats. Lower levels (1%) did not suppress growth in another study (Lindahl et al., 1957). Hanson et al. (1956) observed a slight response to cholesterol supplementation of swine fed high levels of alfalfa. Pederson et al. (1972) with rats fed low and high saponin alfalfa varieties found rats in the early stages of the experiment doing better on low saponin alfalfa diets; however, towards the end of the study no difference was noted. With swine no difference was noted when high and low saponin alfalfas were fed (Peo et al., 1972). Thus the saponin content of alfalfa does not seem to be the factor contributing to the reduced performance of swine fed high alfalfa diets.

Low palatability of alfalfa may be a significant factor. Peo et al. (1972) suggested that during dehydration with heat, bitter compounds could be formed from carmelization reactions. They observed better growth of baby pigs fed freeze-dried alfalfa over dehydrated alfalfa. In their work the alfalfa supplied one-half of the supplementary protein in a corn-soy ration. Heating protein supplements at high temperatures decreases crude protein digestibility (Donoso *et al.*, 1962; Ford and Salter, 1966; Heshiem and Carpenter, 1969). This has also been shown with forages (Hathout, 1962; Goering and Van Soest, 1967; Vielemeyer and Rubach, 1969). The availability of lysine is reduced because of browning reactions which involve reaction of the free ϵ-amino group with carbonyl group. This heat-stimulated reaction has been shown to occur in alfalfa (Hathout, 1962; Goering and Van Soest, 1967; Vielemeyer and Rubach, 1969). This in part explains the decrease in performance observed by Peo *et al.* (1972). However, Charlet-Lery and Zelter (1969) observed high biological values even for alfalfa meal dried at high temperatures.

Even though high levels of alfalfa may be unpalatable in swine rations, its high fiber content is enough to keep it from being used very extensively in high energy rations. Any means of separation of the fiber and protein portions and thereby making a leaf protein concentrate would be advantageous.

Leaf Protein Concentrates

Methods for the preparation of leaf protein concentrates have been studied for more than 20 years (Pirie, 1942; Morrison and Pirie, 1961; Chayen *et al.*, 1961). The processes involve basically crushing or pulping the fresh plant material, expressing the juice, precipitating out the protein with steam injection or by acid, then drying the protein curd. The result is a high protein, low fiber feedstuff. Byers (1961) and Byers and Sturrock (1965) have reported that leaf protein can be extracted from a large number of plant species.

Most leaf protein concentrates produced by the above methods contain about 30-60% crude protein depending on the process and plant species used. Leaf protein concentrates are reported to have adequate amounts of all the essential amino acids with marginal levels of methionine (Gerloff et al., 1965; Wilson and Tilley, 1965; Oelshleqel et al., 1969; Byers, 1971a). When compared to alfalfa meal, APC has a higher proportion of essential amino acids on a nitrogen equivalency basis (Wilson and Tilley, 1965). Alfalfa contains a higher proportion of soluble non-protein nitrogen which dilutes the essential amino acids. Byers (1971b) found that leaf protein coagulated by heat contained 10-15% less lysine than that precipitated by acid from the same extract upon amino acid analysis.

Leaf protein concentrates can be processed still further into chloroplastic and cytoplasmic fractions. This can be done by controlled heating (Subba Rau et al., 1969) or centrifuging (Chayen et al., 1969; Wilson and Tilley, 1965). Generally the amino acid composition of the fractionated leaf protein is very similar to the unfractionated except for lysine and histidine which are usually higher in the cytoplasmic fraction (Wilson and Tilley, 1965; Byers, 1971a).

From the amino acid analysis, leaf protein concentrates would be expected to be of high quality. However, early feeding trials suggested a low protein nutritive value (Davies _et al._, 1952; Carpenter _et al._, 1952; Cowlishaw _et al._, 1956). A review of early studies of leaf protein concentrates by Duckworth and Woodham (1961) showed that of the 27 samples produced from a variety of processes from clover, alfalfa, and grass (mostly ryegrass) only three gave results approaching what would be expected by their amino acid compositions. They included one alfalfa sample that was roller dried and two ryegrass samples that were solvent extracted after drying. These early failures may be the result of improper processing.

Studies by Duckworth and Woodham (1961) showed leaf protein concentrates to be as good as soybean meal for growing chicks and rats, if high drying temperatures were not used. They found that excessive damage to protein occurs with drying temperatures higher than 82°C. Further studies by Duckworth _et al._ (1961) showed that wheat leaf protein concentrate dried commercially was comparable to white fish meal when fed to young pigs. This agrees with the earlier work of Barber _et al._ (1959). Larson and Halverson (1962) obtained poor results with APC for rats; they obtained responses to methionine and/or lysine supplementation. They suggested that processing may have led to the lowered nutritive value. This supports the observation by Duckworth and Woodham (1961) that oven-drying at

$100^{\circ}C$ reduces the biological value and true digestibility. Similar conclusions have been reported by Subba Rau and Singh (1970) using the rat Protein Efficiency Ratio (PER) method and Saunders et al. (1973) using the rat true protein digestibility method for the evaluation of heated samples.

Buchanan (1969) compared the products of various methods of drying in biological value, net protein utilization, and PER with rats. Neither moist heat alone, moist heat followed by chloroform extraction, nor extraction with acidified solvent affected biological value significantly, but all decreased true digestibility, net protein utilization, and PER. He noted that the loss was reversed by mild solvent extraction. These studies are further supported by the finding of high biological value of freeze-dried leaf protein concentrates (Akeson and Stahmann, 1965) and the high PER of freeze-dried APC for rats (Booth et al., 1972). In addition, spray drying has been shown to give a high quality product (Hartman et al., 1967; Arkcoll, 1969).

It is often the availability of the essential amino acids, rather than their absolute amounts, which determines the nutritive value of a protein. Lysine, because of the many reactions that it can undergo rendering it unavailable, and methionine plus cystine, which are often limiting, are especially important. Addition of methionine to unfractionated leaf protein concentrates has been shown to improve rat diets (Shurpalekar et al., 1969). Addition of lysine (Larson and

Halverson, 1962; Henry and Ford, 1965; Shurpalekar *et al.*, 1969) or leucine (Reddy, 1971) to diets containing leaf protein concentrate had no effect except in one case with lysine (Larson and Halverson, 1962). Care should be taken in extrapolating these results to other species, as rats have a greater methionine requirement than most species (Eggum, 1970). The value of the cytoplasmic fraction even without supplemental methionine approaches that of casein (Henry and Ford, 1965). Subba Rau *et al.* (1969) also showed that the cytoplasmic fraction is nutritionally superior for rats. The poor *in vivo* results with chloroplastic protein may be a result of low amino acid availability. The availability of methionine in some barley leaf proteins as determined by microbiological tests (Ford, 1964) ranged from 100% for some cytoplasmic fractions to 40% in chloroplastic fractions. About 90% was available in unfractionated leaf protein concentrate (Ford, 1964, 1970). However, some of the methionine requirement can be met by cystine since it has a sparing effect (Miller and Samuel, 1968). Thus it is more accurate to take the total S-amino acids into account. Subba Rau *et al.* (1969) suggested that the compounds associated with chloroplasts (i.e., quinones, pigments, isoprene complexes) can nutritionally tie up S-amino acids.

The lack of response when lysine was added to diets containing unfractionated leaf protein concentrate (Subba Rau *et al.*, 1969; Shurpalekar *et al.*, 1969) indicates that, at least in their work, enough was nutritionally available to meet the rat's requirement. Using Carpenter's method (1960) for measuring "available" lysine, the available lysine in unfractionated leaf protein concentrate was shown to be between 70 to 80% of the total content. However, Larson and Halverson (1962) did get a response to lysine supplementation. This could be attributed to the processing method in which higher drying temperatures were used than in the other above mentioned studies.

Heat stimulates browning (Maillard reaction) which involves the reaction of the ϵ-amino acid group of lysine with a carbonyl group such as that found in reducing sugars (Carpenter, 1973). Many leaf protein preparations produced without the use of solvents have relatively high levels of unsaturated fat; also leaf protein preparations, especially chloroplastic fractions, contain many quinones and other phenols which can provide the reactive carbonyl groups (Pierpoint, 1970; Carpenter, 1973). This could also explain the poor performances of rats fed chloroplastic leaf protein fractions (Subba Rau et al., 1969).

Alfalfa Protein Concentrate

Several field and laboratory studies have been done with products of the Pro Xan process and its commercial product, X-Pro.

Booth et al. (1972) obtained a low PER (1.78) with rats fed diets containing APC. Upon supplementation with methionine better results were obtained. The best results were observed when the coagulum was freeze-dried. When methionine was added to the freeze-dried meal, PER's as high as that for casein were obtained. Similar results were obtained for APC by Hove et al. (1974). When the cytoplasmic fraction from the Pro-Xan process was isolated and freeze-dried, a PER greater than that for casein was obtained (2.63 vs. 2.50) (Saunders et al., 1973).

Halloran et al. (1972) observed no changes in growth or feed efficiency when levels of APC up to 8.8% in broiler rations were used. Kuzmicky et al. (1972) obtained good results with APC levels as high as 54% of the diet for broilers. Supplementation with cholesterol had no effects, indicating that saponins were not a problem. All of their rations contained supplemental methionine. A significant response in feed conversion was obtained upon supplementation of lysine to a 20% APC diet.

In recent work by Saunders et al. (1973) on various preparations made from the Pro-Xan process, crude protein digestibility values of 80.5% for commercial APC to 99.99% for freeze-dried water washed cytoplasmic protein were obtained.

PART ONE: ALFALFA STUDIES

Objective

The objectives of this experimental work include the following:

1) To determine the influence of alfalfa fiber on the growth-depressing effects to rats of high dietary alfalfa levels.

2) To determine if processing methods affect the nutritive value of alfalfa meal for rats.

Experimental Procedure

Two experiments with rats were conducted. The rats were young Long Evans rats obtained from Simonson Laboratory, Gilroy, California. They were housed in individual wire bottomed cages.

Experiment 1-1

Alfalfa, ryegrass straw and alphacellulose were compared at the same fiber levels corresponding to levels of 40, 50, 60, 70 and 80% alfalfa. The alfalfa used was a commercial dehydrated meal (20% crude protein). The ryegrass straw was obtained locally and ground to pass through a 2 mm screen. The alphacellulose was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Acid detergent fiber (ADF) was determined for both the ryegrass straw and alfalfa using the Van Soest method (1963) as modified by Waldern (1971); values of 27.2 and 47.3% respectively were obtained and used in formulating the rations.

Eighty male Long Evans rats of 80-100 g body weight were randomly distributed into 16 treatments. The treatments included: 40, 50, 60, 70, and 80% alfalfa; 23, 28.5, 34.5, 40, and 46% ryegrass straw; and 10.9, 13.6, 16.5, 19.0, and 21.7% alphacellulose. Lard was used to make all the diets approximately isocaloric (3380-3450 kcal/kg). The diets were also formulated to contain about 20% crude protein, except for the 80% alfalfa diet which contained about 16.5% crude protein. A control with no fiber was used and since this diet contained no fat, several drops of corn oil were given orally to each rat once weekly to provide essential fatty acids. The composition of the diets is given in Table 1. The rats were on test for 28 days and were given food and water ad libitum. Gain, feed intake, and dry volume intake were compared. The dry volume intake was computed from feed density, which was measured by filling a 50 ml beaker until overflowing, scraping of the excess with a spatula, and weighing the contents; no packing was done. The average of triplicate determinations was used.

Experiment 1-2

A comparison of freeze-dried (FD), air dried (AD) and

Table 1. Composition (g/kg) of Experimental Diets. Experiment 1-1.

Ingredient	Ration no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dehydrated Alfalfa	-	400	500	600	700	800	-	-	-	-	-	-	-	-	-	-
Ryegrass Straw	-	-	-	-	-	-	230	285	345	400	460	-	-	-	-	-
Alphacellulose	-	-	-	-	-	-	-	-	-	-	-	109	136	165	190	217
Casein	250	200	180	150	115	-	250	250	250	250	250	250	250	250	250	250
Lard	-	75	105	140	160	180	100	120	145	165	195	60	77.5	95	112.5	127.5
Corn Starch	700	310	190	75	-	-	370	295	210	135	45	529	486.5	440	397.5	355.5
Vitamin mixture[1]	10	10	10	10	10	5	10	10	10	10	10	10	10	10	10	10
Mineral mixture[2]	-	-	-	-	-	-	40	40	40	40	40	40	40	40	40	40
Na_2HPO_4	-	7.5	7.5	7.5	7.5	7.5	-	-	-	-	-	-	-	-	-	-
KH_2PO_4	-	7.5	7.5	7.5	7.5	7.5	-	-	-	-	-	-	-	-	-	-
% Acid Detergent Fiber[3]	-	10.9	13.6	16.5	19.0	21.7	10.9	13.6	16.5	19.0	21.7	10.9	13.6	16.5	19.0	21.7
Digestible Energy[4] (kcal/kg)	3420	3385	3380	3390	3390	3400	3450	3430	3440	3430	3460	3420	3410	3380	3400	3380

[1] Cheeke and Stangel (1972).

[2] Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

[3] For alfalfa = 21.16%, ryegrass straw = 47.33%, alphacellulose = 100%, other ingredients = none.

[4] Estimate using NRC data (1968). (Alfalfa = 2200 kcal/kg, Straw = 1200 kcal/kg, and alphacellulose = nil)

oven-dried (OD) alfalfa in rat rations was made. The alfalfa was cut in the pre-bloom stage, divided into three batches, and dried by the above methods. The AD material was dried in the field where it was cut, the OD was dried in a forced-air oven at 95°C and the FD was frozen immediately and then freeze-dried. All of the alfalfa types were ground to 2 mm or less before incorporation into experimental rations. Analysis for ADF (Waldern, 1971), lignin (Van Soest and Wine, 1968), and dry matter was done on the alfalfa meals.

Sixty male Long Evans rats of 60-80 g body weight were randomly distributed into 10 groups. Treatments consisted of substitutions of each of the three types of alfalfa meals in a corn-soy basal diet at levels of 20, 40, and 60%. In addition a control with no alfalfa was used. The rations were formulated to be isonitrogenous. There was a difference in the crude protein content of the meals as determined by the macrokjeldahl method, with the air-dried being 21% and the freeze-dried and oven-dried being 25%. The composition of the rations is given in Table 2.

The rats were on test for 16 days, except for those on the 60% alfalfa diets, which were terminated at 14 days (because the supply of FD alfalfa was exhausted). Fecal collections were made from day 4 to day 9, pooled for each treatment, and the digestibility of dry matter and ADF determined.

Table 2. Composition (g/kg) of Experimental Diets. Experiment 1-2.

Ingredient	0% Alfalfa	20% Alfalfa			40% Alfalfa			60% Alfalfa		
		FD	OD	AD	FD	OD	AD	FD	OD	AD
Alfalfa	-	200	200	200	400	400	400	600	600	600
Soybean meal	190	120	120	140	60	60	90	-	-	40
Ground corn	726	623	623	583	443	443	383	203	203	243
Lard	30	40	40	60	80	80	110	120	120	120
K_2HPO_4	-	8	8	8	8	8	8	8	8	8
NaCl	-	5	5	5	5	5	5	5	5	5
Vitamin mixture[a]	10	-	-	-	-	-	-	-	-	-
Mineral mixture[b]	40	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	60	60	-
DL-methionine	4	4	4	4	4	4	4	4	4	4

[a] Cheeke and Stangel (1972)

[b] Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio

FD = Freeze-dried; OD = Oven-dried; AD = Air-dried

In addition to the above experiment, a metabolism trial was carried out to determine the digestibility of the crude protein, dry matter, and ADF. In this trial only FD and OD alfalfa meals were compared. The FD and OD meals were prepared as previously described except from a different batch of alfalfa. The composition of the diets was 40% alfalfa (FD and OD), 4% mineral mix (Jones and Foster, 1942), 5% lard, 1% vitamin mix (Cheeke and Stangel, 1972) and 50% sucrose.

Each diet was fed to eight Long Evans female rats of 60-80 g for 10 days. The feces were collected on screens below the cages. The rats were fasted for 24 hrs before and at the end of the feeding period. No attempt was made to measure endogenous protein excretion. The feces were dried, and the dry matter, ADF and crude protein digestibility determined.

Results and Discussion

Experiment 1-1

With increasing levels of both alfalfa and ryegrass straw, decreased growth was observed (Table 3) while no effect was noted for increasing levels of alphacellulose. The daily feed intake on a dry weight basis of the alfalfa and ryegrass diets increased with increasing ADF levels (Table 4). For dry volume feed intake per day, both

Table 3. Effect of Fiber Level and Type on Rat Growth. Experiment 1-1.

% ADF	Total Gain (g)					
	Dehydrated Alfalfa		Ryegrass Straw		Alphacellulose	
	Mean	SD	Mean	SD	Mean	SD
0	119.8	8.8	119.8	8.8	119.8	8.8
10.9	118.8	19.0	125.6	20.2	129.2	9.2
13.6	105.8	11.7	97.0	14.1	117.0	11.0
16.5	88.0[b]	8.2	82.0[b]	4.4	115.0[a]	15.0
19.0	86.4[b]	14.6	55.0[b]	7.6	129.6[a]	8.2
21.7	46.6[b]	13.3	63.2[b]	4.7	114.6[a]	8.9

a significantly different from b ($P < 0.01$).

Table 4. Effect of Fiber Level and Type on Feed Intake of Rats. Experiment 1-1.

% ADF	Daily Feed Intake (g)					
	Dehydrated Alfalfa		Ryegrass Straw		Alphacellulose	
	Mean	SD	Mean	SD	Mean	SD
0	11.4	0.6	11.4	0.6	11.4	0.6
10.9	13.3	0.7	12.9	0.6	12.6	0.3
13.6	13.0	0.6	12.8	0.4	12.1	0.9
16.5	12.6	0.4	12.1	1.2	12.4	0.5
19.0	11.5[c]	1.0	11.6[b]	0.5	13.1[a]	0.8
21.7	9.6[b]	0.5	10.3[b]	0.7	13.0[a]	0.4

a significantly different from b ($P < 0.01$).

a significantly different from c ($P < 0.05$).

alphacellulose and ryegrass straw showed increases for increasing fiber levels while alfalfa decreased (Table 5).

Both the alphacellulose and the alfalfa diets were of similar bulkiness while the ryegrass straw diets were much bulkier at similar ADF levels (Table 6). Bell (1960) observed with mice that in addition to palatability, the bulk, caloric density, and gut capacity may also be important when comparing various fiber sources in isocaloric diets. Since both the alphacellulose and alfalfa diets were about the same bulk, any problem with palatability should elicit a decrease in feed intake (both volume and weight) and subsequently lower gains with increasing alfalfa levels in the diet, which was observed.

Thus the lowered performance observed in swine in previous studies (Becker et al., 1954; Danielson, 1969) could be in part due to the unpalatability of alfalfa as well as its high fiber and lowered caloric density. Hanson et al. (1956) did show that when isocaloric diets were fed ad libitum to swine, those on the alfalfa diet had depressed gains, but upon pair feeding no difference was noted. With swine Peo et al. (1972) observed depressed performance on alfalfa rations over a corn-soy ration with added fiber. With rats fed isocaloric diets, at levels of alfalfa above 40% performance drops off drastically (Cheeke, 1972).

The results of this experiment suggest that at high dietary alfalfa levels, it is palatability rather than fiber per se that limits performance.

Table 5. Effect of Fiber Level and Type on Dry Volume Feed Intake of Rats. Experiment 1-1.

% ADF	Daily Volume of Feed Intake (cc)		
	Dehydrated Alfalfa	Ryegrass Straw	Alphacellulose
0	16.0	16.0	16.0
10.9	22.7	30.7	21.4
13.6	21.9	32.0	22.0
16.5	22.7	32.9	23.6
19.0	21.5	34.5	25.9
21.7	18.1	34.1	28.6

Table 6. Feed Density of Experimental Diets. Experiment 1-1.

% ADF	Feed Density (g/cc)					
	Dehydrated Alfalfa		Ryegrass Straw		Alphacellulose	
	Unit	% of Control	Unit	% of Control	Unit	% of Control
0	.712	100	.712	100	.712	100
10.9	.586	82	.420	59	.590	83
13.6	.594	83	.400	56	.550	77
16.5	.556	78	.368	52	.526	74
19.0	.536	75	.336	47	.506	71
21.7	.530	74	.302	42	.454	64

Experiment 1-2

The OD alfalfa had consistently higher ADF levels, and more lignin than the other preparations (Table 7). Similar results were shown by DeGaillard (1962), who noted a decrease in xylans and an increase in lignin when forages were heated. In addition the AD meal had a lower crude protein content which could be attributed to its loss of leaves and higher proportion of stems over that of the other meals.

Table 7. Proximate Analysis of Alfalfa Samples (%). Experiment 1-2.

	Freeze-dried	Oven-dried	Air-dried
Dry matter	94.5	96.0	90.0
Crude protein	23.5	23.4	19.5
Acid detergent fiber	29.5	36.6	32.8
Lignin	6.3	10.0	9.2

The rats fed the FD alfalfa grew significantly faster at the 20% alfalfa level ($P < 0.01$) and at the 40% alfalfa level ($P < 0.05$) than the rats fed equivalent levels of OD alfalfa. The growth of the rats fed OD alfalfa was significantly lower than those fed AD alfalfa at the 40% level of dietary alfalfa (Table 8). No other growth differences were statistically significant. No apparent differences in growth and feed efficiency were apparent at the 60% alfalfa level. With increasing

Table 8. Effect of Processing Method on Growth Response of Rats to Alfalfa Meals. Experiment 1-2A.

Treatment	Ave. Daily Gain (g/day) Mean	SD	Ave. Daily Feed Intake (g)	% Dry Matter Digestibility	% Digestibility of Acid-Detergent Fiber	Gain per Rat 0-5 Days (g)
20% FD	5.7[a]	0.5	14.4	82.6	32.0	23.3[a]
20% OD	4.5[b]	0.6	13.0	80.9	20.6	15.8[b]
20% AD	5.1	0.6	13.4	79.4	30.7	17.7
40% FD	4.6[a]	0.5	12.4	73.6	21.2	22.1[a]
40% OD	3.8[c]	0.6	12.5	72.9	22.6	12.5[b]
40% AD	4.6[a]	0.3	11.8	73.6	21.5	14.0[b]
60% FD	2.9	0.4	9.8	69.1	21.3	12.9[a]
60% OD	3.0	0.3	10.0	66.1	23.2	- 1.6[b]
60% AD	2.6	0.3	9.4	68.0	24.2	- 0.5[b]
0 Alfalfa (Control)	5.8	0.8	15.1	84.8	29.5	23.2

a significantly different from b ($P < 0.01$)
a significantly different from c ($P < 0.05$)

FD = Freeze-dried alfalfa meal; OD = Oven-dried alfalfa meal; AD = Air-dried alfalfa meal alfalfa levels decreasing feed intake was noted for all three types of alfalfa. In general the results support those of Peo et al. (1972) indicating that with the avoidance of heat in the drying process, the feeding value of alfalfa is improved.

There was no apparent effect of processing method on the digestibility of dry matter or ADF (Table 8). The metabolism trial further supported this and in addition no difference was noted in crude protein digestibility (Table 9).

It has been shown that heat lowers the crude protein digestibility of protein supplements (Ford and Salter, 1966; Neshiem and Carpenter, 1969) and of forages for ruminants (Hathout, 1962; Goering and Van Soest, 1967). Hot air drying at 150°C can decrease protein digestibility by 17% in alfalfa (Vielemeyer and Rubach, 1969). The reason why no apparent differences were noted is crude protein digestibility in this study can be attributed to the lower drying temperature for the oven-dried alfalfa (95°C).

The susceptibility of alfalfa to browning (Maillard reaction) has been shown (Hathout, 1961; Goering and Van Soest, 1967) and could result in a decrease of available lysine. It has been observed that hot air drying (150°C) can decrease lysine by 45% in alfalfa (Vielemeyer and Rubach, 1969). The lowered performance of rats fed the oven-dried meals at the 20 and 40% levels (especially the 40% level) may be partially attributable to this since the diets were made isonitrogenous.

Table 9. Comparative Digestibilities of Freeze-dried and Oven-dried Alfalfa with Rats. Experiment 1-2B.

Group	% Apparent Digestibility					
	Dry Matter		Crude Protein		Acid Detergent Fiber	
	Mean	SD	Mean	SD	Mean	SD
Freeze-dried alfalfa	77.1	1.5	59.6	4.5	16.4	7.9
Oven-dried alfalfa	76.4	1.0	64.2	3.9	17.6	4.6

For the FD and OD alfalfa samples, the % dry matter, % crude protein and % ADF were 97.6 and 98.0, 23.0 and 23.0, 10.5 and 11.3, respectively.

During wilting of forages, such as in air drying, a decrease in protein quality can occur by enzymatic degradation of the proteins with an increase in non-protein nitrogen (Vielemeyer and Rubach, 1969). This could explain the somewhat lowered performance with the AD alfalfa over that of the FD alfalfa in the same groups.

Peo *et al.* (1972b) indicated that carmelization during heat treatment of alfalfa could lead to the formation of bitter compounds. In this experiment the differences in gain occurred within the first 5 days (Table 8). The greater acceptance of the freeze-dried alfalfa in the first few days at the 20% and 40% levels supports the idea that this method of preparation avoids the formation of bitter compounds.

Summary

Two experiments using rats were conducted. One experiment involved examination of the inhibitory effect of high levels of alfalfa. The second involved the effect of processing method on the utilization of alfalfa.

In the first experiment, feeding alfalfa at dietary levels above 40% in isocaloric diets depressed growth. When compared to alphacellulose diets fed at similar ADF levels, both growth and dry weight feed intake were depressed significantly ($P < 0.01$) with increasing levels of ryegrass straw and alfalfa. Dry volume intake increased with increasing levels of ryegrass straw and alphacellulose, while dry volume intake of alfalfa decreased with increasing alfalfa levels. The alphacellulose and alfalfa diets were similar in density while those containing dyegrass straw were much bulkier.

In experiment two, FD, OD and AD alfalfa meals were compared in rat diets at levels of 20, 40, and 60%. The gains for the rats fed the FD alfalfa were significantly greater than for those fed the OD alfalfa at 20% ($P < 0.01$) and 40% ($P < 0.05$). The response appeared to be due to greater palatability. No differences in dry matter and ADF digestibilities were noted. For OD and FD alfalfa only, no differences were found in ADF, dry matter or crude protein digestibilities. It is concluded that the primary factor causing reduced performance of rats fed high alfalfa levels is its low palatability.

PART TWO: NUTRITIONAL STUDIES WITH ALFALFA PROTEIN CONCENTRATE (APC)

Objective

The objectives of this experimental work in part two include:

1) To evaluate APC as a protein source in both practical and purified type diets for the rat.
2) To determine its limiting amino acids.
3) To determine the availability of lysine in APC by using bioassays with both the rat and Japanese quail (*Corturnix coturnix japonica*).
4) To determine crude protein and dry matter digestibility of APC in both purified and practical type diets for the rat.

Experimental Procedures

The APC used is a commercial preparation (X-Pro) which was obtained from Batley-Janss Enterprises of Brawley, California. Dry matter, crude protein, ether extract and ash values were obtained. In addition phosphorus, sodium, potassium levels were obtained by colorimetry or atomic absorption. An amino acid analysis was conducted by the Department of Biochemistry, Oregon State University, which involved a 200 mg sample hydrolyzed in 6N HCl for 20 hrs before analysis. Amino acid analysis of both APC and soybean meal was obtained.

Experiment 2-1

This experiment involved a preliminary evaluation of APC. Forty-two male Long Evans rats weighing 60-80 g were allotted to the treatments and were on test for 16 days. The treatments were: 10, 20, 29, and 56% APC, compared with diets containing all the supplementary protein from either soybean meal, cottonseed meal, or herring meal in corn based diets. The latter three treatments were directly comparable to the 29% APC level, which also provided all the supplementary protein. In the diet with 56% APC, the APC provided all the dietary protein. Composition of the diets is given in Table 10. All rats were given the diets as well as water *ad libitum* and were in individual wire bottomed cages. Daily gain, feed efficiency, feed intake, and PER were determined. The crude protein level of each diet was calculated using reference[1] values and the analytical values for APC and soybean meal.

Experiment 2-2

The PER was determined for APC, soybean meal, herring

[1]National Research Council (NRC, 1973).

Table 10. Composition of Experimental Diets (g/kg). Experiment 2-1.

Ingredient	Treatment no.						
	1	2	3	4	5	6	7
Soybean meal	140	70	-	-	220	-	-
Cottonseed meal	-	-	-	-	-	245	-
Herring meal	-	-	-	-	-	-	130
APC	100	200	290	560	-	-	-
Ground corn	703	665	666	-	700	675	790
Lard	40	50	30	80	30	30	30
Salt	5	3	2	1	-	-	-
KH_2PO_4	12	12	12	14	-	-	-
Mineral mixture[1]	-	-	-	-	40	40	40
Vitamin mixture[2]	-	-	-	-	10	10	10
Sucrose	-	-	-	345	-	-	-

[1]Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

[2]Cheeke and Stangel (1972).

meal, cottonseed meal and casein, using the method of Osborne *et al.* (1919). Additional treatments were mixtures of APC and herring meal, and APC and soybean meal, so that each provided 5% crude protein, and a group with APC supplemented with 0.3% lysine and 0.3% methionine. All diets were calculated to contain 10% crude protein. Composition of the diets is given in Table 11. These diets were fed to male Long Evans rats with initial weights of 80-100 g. Five rats were randomly allotted to each group. Each rat was individually caged and given feed and water *ad libitum*. The rats were on test for 28 days.

Experiment 2-3

The use of APC as a protein supplement for a corn-based ration was studied. Twenty-five male, Long Evans rats weighing 60-80 g were allotted to five treatments. The treatments were a corn soy control, a 20% APC basal, basal + 0.3% lysine, basal + 0.3% methionine and basal + 0.3% methionine + 0.3% lysine. Each rat was individually caged and given feed and water *ad libitum*. Composition of the diets is given in Table 12. The experiment was terminated after 19 days.

Experiment 2-4

In this experiment, two bioassays were conducted to determine

Table 11. Composition of Experimental Diets (g/kg). Experiment 2-2.

	Ration no.							
	1	2	3	4	5	6	7	8
Herring meal	145	-	-	-	-	72.5	-	-
Soybean meal	-	220	-	-	110	-	-	-
Cottonseed meal	-	-	245	-	-	-	-	-
APC	-	-	-	285	142.5	142.5	-	285
Casein	-	-	-	-	-	-	110	-
Corn starch	400	360	335	320	340	360	420	320
Sucrose	355	320	310	290	305	322.5	370	284
Lard	50	50	70	70	60	60	40	70
Vitamin mixture[1]	10	10	10	10	10	10	10	10
Mineral mixture[2]	40	40	40	20	30	30	40	20
Na_2HPO_4	-	-	-	5	2.5	2.5	-	5
L-Lysine HCl	-	-	-	-	-	-	-	3
DL-Methionine	-	-	-	-	-	-	-	3

[1]Cheeke and Stangel (1972).

[2]Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

Table 12. Composition of Experimental Diets (g/kg). Experiment 2-3.

	Ration no.				
	1	2	3	4	5
Soybean meal	220	70	70	70	70
APC	-	200	200	200	200
Corn	698	670	667	667	664
Lard	30	40	40	40	40
Vitamin Mixture[1]	10	-	-	-	-
Salt	-	4	4	4	4
Mineral Mixture[2]	40	-	-	-	-
KH_2PO_4	-	16	16	16	16
$CaCO_3$	-	5	5	5	5
DL-Methionine	2	-	3	-	3
L-Lysine HCl	-	-	-	3	3

[1] Cheeke and Stangel (1972)

[2] Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

the nutritionally available lysine in APC for rats and Japanese quail. In both trials a basal diet of a low level of soybean meal (10 and 21.5% for rats and quail respectively) was used to which varying amounts of reference and test proteins were added as the source of lysine. The requirements[2] of the other amino acids in the basal, reference and test diets were met with crystalline amino acids. The composition of the diets is given in Tables 13 and 14. In both studies APC was tested; in addition, soybean meal was tested with the quail. In the rat experiment, five reference and four test groups as well as a control were used. Each group was assigned five male rats of the Long-Evans strain weighing 60-80 g each. The reference protein in this study was casein in which 2, 4, 6, 8, and 10% levels were added to the basal. All rats were individually caged and given feed and water *ad libitum.* The rats were on test for 21 days.

With the quail, three reference groups, three test groups for APC, three test groups for soybean meal, and a basal group were set up. In this study three levels of lysine HCl (0.146, 0.293, and 0.439%) added to the basal were the reference diets. Levels of APC and soybean meal used are shown in Table 14. Duplicates of 10 baby quail (1 day old) were assigned to each treatment, and were fed the experimental rations for 16 days (on test from day 2 to day 16). Any

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[2] National Research Council (NRC, 1968a, 1971).

Table 13. Composition of Experimental Diets (g/kg). Experiment 2-4A (Rats).

Ingredient	Ration no.									
	1	2	3	4	5	6	7	8	9	10
Soybean meal	100	100	100	100	100	100	100	100	100	100
Corn starch	450	450	450	450	450	450	450	450	450	450
Alphacellulose	80	80	80	80	80	80	80	80	80	80
Mineral mixture[1]	40	40	40	40	40	40	40	40	40	40
Vitamin mixture[2]	10	10	10	10	10	10	10	10	10	10
Lard	50	50	50	50	50	50	50	50	50	50
Casein	-	20	40	60	80	100	-	-	-	-
Alfalfa protein concentrate	-	-	-	-	-	-	50	100	150	200
Amino acid mixture[3]	60	50	37.5	25	15	-	50	30	15	-
DL-Methionine	6	5	5	4	3	3	5	5	4	4
L Glutamic Acid	45	35	25	15	5	-	30	15	5	-
Sucrose	159	160	162.5	166	167	167	135	120	96	66

[1]Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

[2]Cheeke and Stangel (1972).

[3]Contains 3.87% DL-Tryptophan, 3.87% L-Histidine, 20.64% DL-Leucine, 12.90% DL-Isoleucine, 24.15% DL-Phenylalanine, 12.90% DL-Threonine, 18.06% DL-Valine and 3.22% DL-Tyrosine.

Table 14. Composition of Experimental Diets (g/kg) (Japanese quail). Experiment 2-4B.

	Ration no.									
	1	2	3	4	5	6	7	8	9	10
Soybean	215	257	300	343	215	215	215	215	215	215
Alfalfa Protein Concentrate	-	-	-	-	-	-	-	63	126	189.5
Amino Acid Mixture[1]	120	95	70	45	120	120	120	95	70	45
L-Lysine HCl	-	-	-	-	1.46	2.93	4.39	-	-	-
L-Glutamic Acid	80	60	40	20	80	80	80	60	40	20
DL-Methionine	8	7.5	7	6.5	8	8	8	7.5	7	6.5
Soy Oil	40	40	40	40	40	40	40	50	60	70
Cerelose	465	468.5	471	473.5	463.5	462.1	460.6	437.5	410	382

Each ration was supplemented with: 6 g Gordon's B Complex[a], 1000 IU Vitamin E, 3.9 g Choline Xanthate, 1500 ICU Vitamim D, 0.125 g BHT, 60 g Salts N[b], 1 g Salt N Modified (Se, Mo), and 30,000 IU Vitamin A. (Vitamin A was not included in the APC rations)

[a]Gordon and Sizer (1955)

[b]Spivey Fox and Briggs (1960)

[1]Contains 10.97% L Arginine HCl, 6.73% Glycine, 3.74% L-Histidine HCl, 11.47% DL Isoleucine, 20.70% DL Leucine, 20.70% DL Phenylalanine, 10.97% DL Threonine, 2.99% DL Tyrosine, and 11.72% DL Valine.

birds that died during the first 2 days of the test were replaced; beyond this any birds found dead were weighed and gains to that time were included in the final results.

In both studies the criterion used for determining lysine availability was the comparison of g of lysine consumed vs. g gain. The percent availability was extrapolated from a graph of the reference values. A similar procedure was used by Boctor and Harper (1968). The total lysine content for APC, and the soybean meal used in the rat experiment, was determined from amino acid analysis. For the casein, lysine HCl, and soybean meal used in the quail experiment, reference values[3] were used.

Experiment 2-5

Five metabolism trials were conducted to determine crude protein and dry matter digestibilities of APC and soybean meal using rats. Two trials were done involving purified diets with APC and soybean meal, and three trials involving practical type diets of APC plus corn, soybean meal plus corn, and one-half APC and one-half soybean meal with corn. The purified diets were formulated to contain 10% crude protein and the practical diets about 16%. The composition of the diets is given in Table 15.

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[3] National Research Council (NRC, 1968b).

Table 15. Composition of Experimental Diets (g/kg). Experiment 2-5.

Ingredient	Ration no.					
	Purified Diets			Practical Type Diets		
	1	2	B[1]	1	2	3
Alfalfa Protein Concentrate	285	-	-	300	-	150
Soybean meal	-	220	-	-	225	112.5
Sucrose	280	310	410	-	-	-
Corn starch	300	340	460	-	-	-
Alphacellulose	30	30	30	-	-	-
Lard	70	50	50	50	40	45
Corn	-	-	-	625	685	655
Na_2HPO_4	5	-	-	7.5	-	3.75
KH_2PO_4	-	-	-	7.5	-	3.75
Mineral mixture[2]	20	40	40	-	40	20
Vitamin mixture[3]	10	10	10	10	10	10

[1]Zero protein blank for determination of metabolic N excretion.

[2]Jones and Foster, Nutritional Biochemicals, Cleveland, Ohio.

[3]Cheeke and Stangel (1972).

Twelve Long-Evans female rats were used with an initial weight of about 60 g each. The rats were divided into two lots. One lot (lot 1) was fed the purified diets, one diet at a time for a feeding period of 10 days each. The other lot (lot 2) was fed the practical type diets, one diet at a time for a feeding period of 10 days each time. Therefore the rats in lot one were used twice and those in lot 2 were used three times. The rats were in individual wire bottom cages and were given feed and water ad libitum. They were fasted for 24 hours prior to the start and at the end of each feeding period. The feces were collected on copper screens under the cages.

After the conclusion of the trials with the purified diets, one group of lot 1 rats was fed a zero protein purified diet in order to measure metabolic nitrogen excretion. This was used in determination of the true crude protein digestibility of the above purified diets. The following formula was used:

$$\text{Protein digestibility \%} = \frac{\text{N in feed} - (\text{N in feces} - \text{metabolic N})}{\text{N in feed}} \times 100$$

Metabolic fecal nitrogen was not measured with the practical type diets.

Results and Discussion

The composition of APC is given in Table 16. Our analysis is similar to that reported by Kuzmicky et al. (1972). For comparison,

Table 16. Percentage Composition of APC.

	APC[2]	APC[3]	Soybean Meal[4]	Dehydrated Alfalfa Meal[4]
Crude Protein	36.0	38.8	45.8	20.6
Ether Extract	4.8	6.2	0.9	3.6
Fiber	ND	2.4	6.0	20.2
Ash	19.6	19.2	5.8	10.3
Calcium	ND	2.35	0.32	1.5
Phosphorus	1.04	0.44+	0.67	0.27
Sodium	0.35	0.30	0.34	0.86
Potassium	1.15	1.07	1.97	2.52
Magnesium	ND	0.35	0.27	0.35
Chloride	ND	1.00	0.27	0.58
Lysine	1.98	2.15	2.90	0.90
Methionine	0.68	0.79	0.60	0.30
Cystine	0.35[5]	0.43[5]	0.65	0.42
Dig. Energy (kcal/kg)	ND	(2800)[6]	3300	2200

[1] All values are on a wet weight basis.

[2] Oregon State University.

[3] As reported by Kuzmicky *et al.* (1972).

[4] NRC values (1973).

[5] Some may be lost due to oxidation.

[6] Estimate

ND = Not determined; + = Available

reported values[4] for soybean meal and dehydrated alfalfa are also given. Table 17 shows the amino acid composition of APC. The lysine content is lower than that of soybean and of that previously reported by Byers (1971b) for similarly processed unfractionated APC or by Hove et al. (1974) for APC. However, for many of the other essential amino acids, APC compares very favorably. It does show that APC has a higher methionine and cystine content than soybean meal. The first two limiting amino acids in APC would appear to be methionine plus cystine and lysine for both the rat and the pig. With soybean meal the order of limitation would be methionine plus cystine first for the pig and the rat followed by isoleucine for the pig and valine for the rat.

Experiment 2-1

When fed diets containing 10 or 20% APC rats performed favorably as compared to performance with other protein supplements. When all the supplemental protein was provided by APC, the growth, feed conversion, and protein efficiency were significantly reduced ($P < 0.01$), while performance was very poor when all the protein was supplied by APC (Table 18). These results suggest that while APC alone gives unsatisfactory performance with rats, it can

[4] National Research Council (NRC, 1973)

Table 17. Amino Acid Composition (g/100 g of Amino Acids) of APC.

	APC[1]	Soybean Meal[1]	Reported Values for APC[2]	Reported Values for APC[3]
Lysine	5.5	6.7	6.3-6.9	6.2
Histidine	2.6	2.8	2.0-2.5	2.3
Arginine	6.7	7.5	5.8-7.4	ND
Threonine	5.3	4.1	4.6-5.7	4.0
Valine	6.5	4.7	5.9-6.5	6.7
Cystine	0.6[4]	0.8[4]	0.6-1.5	0.5[4]
Methionine	1.9	1.5	1.6-2.2	1.7
Isoleucine	5.2	4.5	5.0-6.6	5.7
Leucine	9.7	8.1	9.2-9.8	10.3
Tyrosine	4.7	3.9	4.5-5.1	3.7
Phenylalanine	7.1	5.7	4.1-6.4	6.7

[1]Oregon State University.

[2]As reported by Byers (1971b) for unfractionated APC.

[3]As reported by Hove et al. (1974) for X-Pro.

[4]Some may be lost due to oxidation.

ND = Not determined

Table 18. Utilization of APC by Rats. Experiment 2-1.

Treatment	Average Daily Gain (g)		Average Daily Feed (g)	% Crude Protein in Diet[1]	Gain/g Protein Consumed (g)	
	Mean	SD			Mean	SD
10% APC	5.7[b]	0.3	15.5	16.9	2.11[b]	0.01
20% APC	5.4[b]	0.4	16.0	16.7	2.02[b]	0.12
29% APC[2]	3.9[a]	0.5	14.4	16.4	1.66[a]	0.13
56% APC[3]	1.9[a]	0.5	11.4	20.2	0.82[a]	0.18
22% Soybean Meal[2]	6.1[b]	0.5	17.2	17.4	2.03[b]	0.20
24.5% Cottonseed Meal[2]	4.3[a]	0.4	16.9	16.5	1.55[a]	0.17
13% Herring Meal[2]	5.7[b]	0.7	16.2	16.2	2.18[b]	0.21

[1]Proximate analysis data for soybean meal and APC; NRC data (1973) used for other ingredients.

[2]At this level, provided all the supplementary protein.

[3]Provided all the protein in the diet.

a significantly different from b ($P < 0.01$)

be used up to levels of at least 20% in combination with soybean meal, with no detrimental effects on growth.

Experiment 2-2

The PER of APC was significantly lower ($P < 0.01$) than that obtained for herring meal, soybean meal, or casein (Table 19). It was similar to that of cottonseed meal. When APC was mixed with herring meal or soybean meal on an equal protein basis increases in PER were noted; with the herring meal the increase was significant ($P < 0.05$). A great increase in PER ($P < 0.01$) was obtained upon supplementation with lysine and methionine.

These results compare quite well with reported PER values for similarly processed APC (Subba Rau et al., 1969; Shurpalekar et al., 1969). At the 15% dietary crude protein level, Booth et al. (1972) obtained a PER of 1.78 for APC while Hove et al. (1974) obtained a PER of 1.60 at the 10% crude protein level. Upon supplementation with methionine an increase in PER was noted by Shurpalekar et al. (1969) (from 1.39 to 2.77) but lysine failed to give similar results. The PER obtained by Booth et al. (1972) and Hove et al. (1974) showed a response to methionine supplementation; however, they did not test lysine. Larson and Halverson (1962) obtained a response to lysine supplementation.

Table 19. Protein Efficiency Ratio of APC Compared to Other Proteins. Experiment 2-2.

Treatment	Average Gain per Rat (g)	Average Feed Consumed/ Rat (g)	% Crude Protein in Diet	Gain per g Protein Consumed	
				Mean	SD
Herring Meal	152.4	491.2	10.94	2.82[a]	0.14
Soybean Meal	100.6	476.4	9.01	2.34[a]	0.08
Cottonseed Meal	74.6	478.0	10.66	1.46[b]	0.07
APC	45.0	360.4	10.08	1.23[bc]	0.25
1/2 Soybean Meal, 1/2 APC	85.2	476.2	9.66	1.84	0.17
1/2 Herring Meal, 1/2 APC	93.6	470.0	10.98	1.81[d]	0.07
Casein	126.4	466.2	10.12	2.67[a]	0.08
APC + Lysine and Methionine	150.0	512.4	11.12	2.54[a]	0.13

a significantly different from b ($P < 0.01$).

c significantly different from d ($P < 0.05$).

The results of this experiment show that for rats this APC is of poor quality when compared to other protein sources. The poor quality could be almost entirely attributed to the lysine and/or methionine content.

Experiment 2-3

The gain per g protein consumed was significantly less ($P < 0.01$) with the APC diet than with the corn-soy control (Table 20). Supplementation with methionine alone had little effect, while lysine supplementation significantly ($P < 0.05$) increased gain per g protein consumed. The best response was observed with supplementation with both lysine and methionine (Table 20). These results indicate that for rats, fed a corn-based diet such as would be typical for swine under commercial conditions, lysine is the first-limiting amino acid in APC, but both lysine and methionine are deficient. Larson and Halverson (1962) also observed a response to lysine, while Shurpalekar, Singh and Sundaravalli (1969) reported a response to methionine but not to lysine. Hove et al. (1974) with APC from the same source as ours, reported a response to methionine, but did not test lysine.

This experiment shows that APC is lacking in methionine and lysine for maximum growth of rats, and that when these deficiencies

Table 20. Evaluation of the Protein Quality of APC with Rats. Experiment 2-3.

	Average Daily Gain (g)		Daily Feed Intake (g)	% Crude Protein in Diet[3]	Gain/g Protein Consumed	
	Mean	SD			Mean	SD
Corn-Soy Control[1]	5.53	0.74	16.08	17.6	1.95[a]	0.12
20% APC Basal[2]	4.34	0.34	15.98	16.7	1.62[be]	0.05
Basal + 0.3% Methionine	4.97	0.59	17.72	16.9	1.66[b]	0.06
Basal + 0.3% Lysine	4.96	0.69	16.62	16.9	1.75[c]	0.07
Basal + 0.3% Lysine + 0.3% Methionine	6.40	0.22	19.17	17.2	2.09[d]	0.09

[1] Contains 0.2% DL Methionine

[2] Contains 7% Soybean meal in a corn based diet.

[3] Calculated using proximate analysis data and NRC (1973) data for other ingredients for soybean meal and APC.

a significantly different from b ($P < 0.01$)

e significantly different from c ($P < 0.05$)

d significantly different from b ($P < 0.01$)

d significantly different from c ($P < 0.01$)

are corrected, excellent growth is obtained. Thus the value of APC is limited by protein quality, rather than by other factors such as saponins.

Experiment 2-4

For rats, the lysine in APC was about 80% as available as that in casein; the values ranged from 76-85%. In the Japanese quail the lysine was found to be slightly higher with a value of about 86% when compared to lysine HCl. In the same study with quail the lysine in soybean meal was found to be essentially 100% available. These values were obtained by extrapolation from a graph of the reference values. The final results are reported in Tables 21-24.

These values for APC compare well with reported values of 70-80% available lysine for other leaf protein concentrates, using Carpenter's (1960) chemical method (Woodham, 1965; Glencross, 1969). The relatively low lysine availability could be attributed to the heat used in the processing of APC. It contains many polyphenolic compounds, reducing sugars, and is high in unsaturated fat that in the presence of heat can react with the ε-amino group of lysine and render it nutritionally unavailable (Pierpoint, 1969; Carpenter, 1973).

Table 21. Lysine Availability Studies of APC with Rats. Experiment 2-4A.

Treatment	% Lysine[1] in Diet	Average Gain per Rat (g)		Lysine Consumed per Rat (g)	Gain per g Lysine Consumed	
		Mean	SD		Mean	SD
Basal	0.32	22.8	4.7	0.619	36.7	6.8
Basal + 2% Casein	0.46	59.2	17.6	1.243	47.8	3.5
Basal + 4% Casein	0.60	75.8	9.9	1.616	46.8	4.2
Basal + 6% Casein	0.74	95.8	7.0	2.174	44.1	1.0
Basal + 8% Casein	0.88	92.6	13.5	2.561	36.1	2.2
Basal + 10% Casein	1.02	100.0	9.8	3.122	32.0	2.6
Basal + 5% APC	0.42	40.8	6.6	1.006	40.6	6.3
Basal + 10% APC	0.52	54.0	5.7	1.395	38.8	3.2
Basal + 15% APC	0.62	61.6	5.2	1.727	35.6	2.6
Basal + 20% APC	0.72	72.4	5.9	2.124	34.6	4.6

[1]Calculated using amino acid analysis for alfalfa protein concentrate and soybean meal, standard values used for casein (from NRC, 1968b).

Table 22. Percent Availability of Lysine in APC for Rats Compared to a Casein Reference Diet. Experiment 2-4A.

Treatment	Gain/g Lysine Consumed		% Availability
	Expected[1]	Actual	
Basal + 5% APC	47.9	40.6	85
Basal + 10% APC	47.2	38.8	82
Basal + 15% APC	46.2	35.6	77
Basal + 20% APC	44.3	34.6	78

[1]Extrapolated from a graph of the reference values of the casein groups (lysine in casein was assumed to be completely available).

Table 23. Lysine Availability Studies of APC with Quail. Experiment 2-4B.

Treatment	No. of Birds	Lysine Level in Diet (%)	Ave. Gain/ Bird (g)	Ave. Feed Intake/ Bird (g)	Ave. g Lysine Consumed/ Bird	Gain/g Lysine Consumed/ Bird
21.5% Soybean Meal Basal	15	0.624	10.7	47.2	0.295	37.4
25.7% Soybean Meal	20	0.745	15.0	69.9	0.521	28.7
30.0% Soybean Meal	20	0.870	16.7	77.6	0.674	24.8
34.3% Soybean Meal	20	0.995	24.3	81.5	0.811	30.0
Basal + 1.46 Lysine HCl	19	0.744	13.7	65.0	0.484	28.4
Basal + 2.93% Lysine HCl	20	0.864	15.8	71.4	0.616	25.7
Basal + 4.39% Lysine HCl	20	0.984	18.6	70.6	0.695	26.8
Basal + 6.3% APC	16	0.750	13.9	81.1	0.609	22.8
Basal + 12.6% APC	20	0.876	14.8	70.4	0.617	23.9
Basal + 18.95% APC	20	1.003	17.6	76.5	0.768	23.0

Table 24. Percent Availability of Lysine in APC in Quail Diets. Experiment 2-4B.

Basal + Lysine HCl	Basal + Soybean Meal	Lysine Available[2] (%)	Basal + APC	Lysine Available[2] (%)
28.4[1]	28.7[1]	101	22.8[1]	80
25.7	24.8	96	23.9	93
26.8	30.0	112	23.0	86

[1]Gain per g lysine consumed.

[2]As compared to lysine HCl at the same lysine level.

Experiment 2-5

The 65.1% true digestibility of the crude protein of APC was significantly lower ($P < 0.01$) than the 86.8% obtained for soybean meal (Table 25). In addition the dry matter digestibility of the APC diet was also significantly lower ($P < 0.01$) than the soybean diet.

With the APC in corn diets the apparent crude protein digestibility and dry matter digestibility were significantly lower ($P < 0.01$) than for the corn-soy diet. With the mixture of APC and soybean meal on a protein equivalency basis, the crude protein and dry matter digestibilities were improved but still are significantly lower ($P<0.01$) than for soybean meal alone.

The results of the experiment compare very poorly to results of about 80% crude protein digestibility reported by Saunders et al. (1973) and Hove et al. (1974) for APC. The explanation for this difference in results with the same product is not apparent. The poor digestibility of this APC could partially explain the poor performance of rats fed unsupplemented APC.

Summary Part Two

Five experiments were conducted using rats and Japanese quail to evaluate the potential of APC as a protein source in monogastric animal rations.

Table 25. Crude Protein Digestibility Studies of APC with Rats. Experiment 2-5.

Treatment	% Crude Protein in Diet	% Crude Protein Digestibility		% Dry Matter Digestibility	
		Mean	SD	Mean	SD
		With Purified Diets[1]			
APC	10.08	65.1[a]	0.8	81.0[a]	0.6
Soybean meal	9.01	88.8[b]	1.8	88.5[b]	0.7
		With Practical Diets[2]			
APC with Corn	16.24	67.7[b]	3.9	76.1[b]	2.1
Soybean Meal with Corn	15.45	76.7[a]	2.4	88.1[a]	1.0
1/2 APC, 1/2 Soybean Meal with Corn	15.40	70.4[b]	3.6	78.6[bd]	1.2

[1]Metabolic fecal nitrogen excretion determined, true digestibility.

[2]Metabolic fecal nitrogen excretion not determined, apparent digestibility.

a significantly different from b ($P < 0.01$).

c significantly different from d ($P < 0.05$).

In experiment 2-1, APC was fed at levels up to 20% with no detrimental effects on performance of rats. In experiment 2-2, the PER of APC was significantly lower ($P < 0.01$) than that of herring meal, casein, or soybean meal at the 10% dietary crude protein level. Upon supplementation with lysine and methionine the difference was overcome. Lysine was first limiting in APC-corn based diets, with methionine also limiting for the rat. The availability of lysine was 80% for the rat when compared to casein and 86% for the quail when compared to lysine HCl in experiment 2-4. Finally, in experiment 2-5 the true crude protein digestibility and dry matter digestibility of APC were significantly lower ($P < 0.01$) than for soybean meal.

The results of these experiments indicate that the APC used in this study is a relatively poor protein source, due to its low lysine and methionine content, and low protein digestibility.

PART THREE: SWINE EXPERIMENTS WITH ALFALFA PROTEIN CONCENTRATE

Objective

This work was designed to examine the use of APC in swine starter, grower, and finisher rations. Varying levels of APC were used to determine its potential as a protein supplement.

Experimental Procedures

Experiment 3-1

This experiment consisted of two parts to evaluate APC as a protein supplement for growing-finishing swine.

In the first part a preliminary examination of APC as a protein supplement for swine was made. Thirty-six Yorkshire x Berkshire barrows, with average initial weights of 36-40 kg, were randomly allotted to six groups. The treatments were 0, 5, 10, 15, 20, and 24% APC. The 24% level provided all the supplementary protein in a barley-based ration. All diets were formulated to contain at least 15% crude protein. Composition of the rations is given in Table 26. Each pig was penned individually and given feed and water *ad libitum*. Because of limited quantities of APC, only the control and the 24% APC fed hogs were fed to market weight of about 91 kg. The others

Table 26. Composition of Rations (%). Experiment 3-1A.

Ingredient	Treatment 1	2	3	4	5	6
APC	-	5	10	15	20	24
Soybean meal	10	8	5	-	-	-
Meat and bone meal	7.5	5.5	5	5.5	2.5	-
Barley	80.8	79.8	78.3	77.9	75.9	74.4
Tricalcium phosphate	0.2	0.2	0.2	0.2	0.3	0.3
Trace mineralized salt	0.5	0.5	0.5	0.5	0.3	0.3
Bentonite	1	1	1	1	1	1
Calculated Composition[1]						
% Crude Protein	16.2	16.0	16.0	15.7	15.8	15.9
% Lysine	0.79	0.76	0.75	0.72	0.72	0.70
% Methionine + Cystine	0.52	0.52	0.53	0.55	0.56	0.58
% of Protein provided by APC	0	11.2	22.5	34.4	45.6	54.3

Each ration was supplemented with: 230 g $ZnSO_4$, 120,000 IU Vitamin D, 1,200,000 IU Vitamin A, 40 g Chlortetracycline, 40 g Sulfamethazine, and 20 g Penicillin per 910 kg.

[1]From using NRC data (1968b, 1973) and our own analysis for APC or that reported by Kuzmicky et al. (1972).

were terminated when 1 ton (910 kg) had been consumed. All rations were pelleted. Carcass data were obtained for the control and 24% APC groups after a 24 hour fast.

The second part consisted of a further evaluation of APC with groups of swine. Fifty Yorkshire x Berkshire swine (25 barrows and 25 gilts) were randomly assigned into 5 pens of 5 barrows and 5 gilts each. The pens were located in a semi-sheltered barn. This experiment was done in two phases, a grower phase and a finisher phase. In both the grower and finisher phases, two controls were used, one with suncured alfalfa and another with dehydrated alfalfa to represent the two common types of alfalfa in swine rations. For the growers the level of suncured and dehydrated alfalfa was 7% and for finishers it was 5%. These rations were compared to test grower rations of 7, 14, and 20% APC, and finisher rations of 5, 11, and 16% APC. The grower rations were formulated to contain at least 15.5% crude protein and 13% for the finishers. Composition of the rations is given in Tables 27 and 28. All diets were pelleted. The pigs received feed and water *ad libitum.*

The pigs started the grower phase at about 17-30 kg each and were taken off as a group when the pigs averaged 50-60 kg and were put on the finisher phase. The pigs were taken off test upon reaching at least 91 kg, fasted for 24 hours and carcass information obtained.

Table 27. Composition of Grower Rations (%). Experiment 3-1B.

Ingredient	Ration no.				
	1	2	3	4	5
Soybean meal (44%)	9.0	7.5	5.0	-	-
Meat meal (50%)	7.0	7.0	7.0	7.0	3.5
Suncured alfalfa	7.0	-	-	-	-
Dehydrated alfalfa	-	7.0	-	-	-
APC	-	-	7.0	14.0	20.0
Yellow corn	73.4	75.4	78.1	76.4	71.25
Tricalcium phosphate	0.5	0.5	0.3	-	-
Monosodium phosphate	-	-	-	0.2	0.45
Trace mineralized salt	0.5	0.5	0.5	0.3	0.2
Antibiotic premix[1]	0.1	0.1	0.1	0.1	0.1
Dried whey	2.0	2.0	2.0	2.0	2.0
Calculated Composition[2]					
% Crude protein	15.8	15.6	15.8	15.9	15.7
% Lysine	0.73	0.70	0.70	0.71	0.68
% Methionine + cystine	0.49	0.49	0.51	0.53	0.55
% Calcium	0.86	0.87	0.86	0.91	0.77
% Phosphorus	0.70	0.69	0.69	0.74	0.71
Digestible energy (kcal/kg)[3]	3320	3400	3455	3425	3300
% Protein provided by alfalfa or APC	7.4	9.2	16.0	31.8	45.9

Each ration was supplemented with: 240,000 IU Vitamin D, 2 g Riboflavin, 15 g DL Calcium Pantothenate, 10 g Niacin, 12 mg Vitamin B_{12}, and 335 g $ZnSO_4$ per 910 kg.

[1] Contains 20 g Chlortetracycline, 20 g Sulfamethazine and 10 g Penicillin per 454 g.

[2] Using NRC data (1968b, 1973) and our own analysis of APC or that reported by Kuzmicky et al. (1972).

[3] Estimate of 2800 kcal/kg for APC.

Table 28. Composition of Finisher Rations (%). Experiment 3-1B.

Ingredient	Ration no.				
	1	2	3	4	5
Soybean meal (44%)	7.2	6.5	4.0	-	-
Tankage (60%)	3.0	3.0	3.0	3.0	1.5
Suncured alfalfa meal	5.0	-	-	-	-
Dehydrated alfalfa meal (20%)	-	5.0	-	-	-
APC	-	-	5.0	11.0	16.0
Yellow corn	81.2	81.6	84.6	82.9	79.55
Dried whey	2.0	2.0	2.0	2.0	2.0
Ground limestone	0.2	0.2	-	-	-
Tricalcium phosphate	0.9	0.9	0.9	0.5	0.4
Monosodium phosphate	-	-	-	0.25	0.35
Trace mineralized salt	0.5	0.5	0.5	0.35	0.2
<u>Calculated Composition</u>[1]					
% Crude protein	13.4	13.3	13.2	13.4	14.0
% Lysine	0.53	0.52	0.51	0.51	0.54
% Methionine + cystine	0.43	0.43	0.44	0.46	0.49
% Calcium	0.65	0.67	0.62	0.62	0.62
% Phosphorus	0.59	0.58	0.62	0.64	0.65
Digestible energy (kcal/kg)[2]	3390	3425	3480	3455	3425
% Crude protein provided by alfalfa or APC	6.2	7.7	13.6	29.6	41.1

Each ration was supplemented with: 200,000 IU Vitamin D, 335 g $ZnSO_4$, 2 g Riboflavin, 12 mg Vitamin B_{12}, 15 g DL Calcium Panthothenate and 10 g Niacin per 910 kg.

[1] Using NRC values (1968b, 1973) and our own analysis of APC or that reported by Kuzmicky <u>et al</u>. (1972).

[2] Estimate of 2800 kcal/kg for APC.

Experiment 3-2

In the second experiment, APC was evaluated using young growing swine. Levels of 7.5, 12.5, and 20.0% APC were used in barley-based pelleted rations. Two controls were used; one with 7.5% suncured alfalfa and one without. Composition of the rations is given in Table 29. At the 20% APC level, a treatment without salt was included for the purpose of determining if the high ash content of APC had detrimental effects that could be reduced by lowering the salt level.

Nine Yorkshire x Berkshire barrows were assigned to each treatment, at an average weight of 16-18 kg. They were fed the experimental rations until their average weights were about 45 kg. The pigs were penned in groups of three and given feed and water *ad libitum.*

Experiment 3-3

In this experiment APC in creep-starter rations was investigated. This study was done in two parts. The first part was with wheat based rations in which both pre-weaning and post-weaning trials were done. The second part involved only post-weaned pigs with corn based starter rations.

Table 29. Composition of Grower Rations (%). Experiment 3-2.

Ingredient	Treatment 1	2	3	4	5	6
APC	-	-	7.5	12.5	20	20
Soybean meal	10	9	5	2	-	-
Herring meal	2.5	2.5	2.5	2.5	2.5	2.5
Meat and bone meal	5	5	5	5	1	1
Barley	79	72.5	76.5	74.8	73.0	73.3
Ground alfalfa	-	7.5	-	-	-	-
Molasses	1	1	1	1	1	1
Trace mineralized salt	0.5	0.5	0.5	0.4	0.3	-
Dicalcium phosphate	1	1	1	0.8	1.2	1.2
Bentonite	1	1	1	1	1	1
Calculated Composition[1]						
% Crude Protein	16.5	16.6	16.7	17.0	16.6	16.6
% Lysine	0.88	0.88	0.88	0.88	0.82	0.82
% Methionine + Cystine	0.56	0.55	0.59	0.61	0.63	0.63
% Protein provided by APC	0	7.5	16.2	24.4	43.4	43.4

Each diet was supplemented with: 200,000 IU Vitamin D, 1,500,000 IU Vitamin A (Ration 1 only), 230 g $ZnSO_4$, 60 g Chlortetracycline, 60 g Sulfamethazine, and 30 g Penicillin per 910 kg.

[1]From using NRC data (1968b, 1973) and our own analysis of APC or that reported by Kuzmicky et al. (1972).

In the first part the OSU creep ration was used as the control. The APC was tested at the 6.5 and 13.0% levels (Table 30). It was substituted for soybean meal in the control ration. Five litters of pigs received each treatment. In the pre-weaning stage the feed was given *ad libitum* to the pigs in creep feeders until weaning (about 9-11 kg average). They were then grouped together as litters as much as possible and given the same feed as a starter. The pigs were on test until the pigs averaged about 27 kg. All feed was pelleted.

In the second part, an attempt was made to feed higher levels of APC than in the first part of this experiment. A control and three test treatments of 14, 28, and 34% APC were set up. The APC replaced one-half of the soybean meal, all of the soybean meal, and all the fish meal plus soybean meal respectively for the three APC levels. The composition of the rations is given in Table 31. All feed was pelleted. Each treatment was assigned two pens of randomly chosen pigs weighing between 9 and 14 kg. Of the two pens, one pen had 15 pigs (9 gilts, 5 barrows) and the other 12 pigs (8 gilts, 4 barrows). The pigs were given feed and water *ad libitum* and were on test until the pigs in the pens averaged 20-27 kg.

Table 30. Composition of Creep-Starter Rations (%). Experiment 3-3A.

Ingredient	Treatments 1	2	3
Wheat	66	64.5	63.25
Alfalfa Meal (sundried)	5	5	5
Herring Meal	5	5	5
Soybean Meal	10	5	-
Dried Buttermilk	6	6	6
Dried Whey	2	2	2
Ground Limestone	0.5	0.5	0.5
Trace Mineralized Salt	0.5	0.5	0.5
Molasses	5	5	5
APC	-	6.5	13.0
Calculated Composition[1]			
% Crude Protein	17.6	17.5	17.4
% Lysine	0.96	0.94	0.92
% Methionine + Cystine	0.74	0.76	0.78
% Protein provided by APC	0	13.4	26.9

[1]Using NRC data (1968b, 1973) and our own analysis for APC or that reported by Kuzmicky et al. (1972).

Table 31. Composition of Starter Rations (%). Experiment 3-3B.

Ingredient	Ration no.			
	1	2	3	4
APC	-	14	28	34
Yellow corn				
Ground oats	20	20	20	20
Soybean meal (44%)	18.5	9	-	-
Fish meal (65%)	3	3	3	-
Dried whey	5	5	5	5
Animal fat	2	4	5	5.5
Dehydrated alfalfa meal (20%)	5	-	-	-
Dried brewer's yeast	3	3	3	3
Tricalcium phosphate	1.4	1.2	0.25	-
Ground limestone	0.5	-	-	-
Monosodium phosphate	-	0.25	1.2	1.2
Antibiotic Premix[1]	0.25	0.25	0.25	0.25
Trace mineralized salt	0.2	0.25	0.2	0.2
Calculated Composition[2]				
% Crude protein	18.9	18.5	18.9	18.8
% Lysine	1.02	0.97	0.98	0.93
% Methionine + cystine	0.62	0.64	0.67	0.66
% Calcium	0.98	0.94	0.94	0.85
% Phosphorus	0.74	0.84	0.97	0.89
Digestible energy (kcal/kg)[3]	3225	3310	3260	3315
% Protein provided by APC	0	27.2	53.4	65.2

Each ration was supplemented with: 340 g $ZnSO_4$; 240,000 IU Vitamin D and 20 mg Vitamin B_{12} per 910 kg.

[1] Contains 20 g Chlortetracycline, 20 g Sulfamethazine and 10 g Penicillin per 454 g.

[2] Using NRC data (1968b, 1973) and our own analysis of APC or that reported by Kuzmicky et al. (1972).

[3] Estimate of 2800 kcal/kg for APC.

Results and Discussion

Experiment 3-1

In the first part, the performance of swine fed APC was excellent at all levels (Table 36). In contrast to the results with rats reported in experiment 2-1, the growth of the pigs receiving APC as the sole protein supplement was not depressed. Because the treatments were not all terminated at the same time, the gains are presented for three time intervals. The mean for two treatments was influenced by a mild respiratory infection that affected several animals (Table 32); the affected animals were off feed for several days. They were treated with antibiotic; the infection was not identified. For the control and 24% APC groups respectively, carcass data were obtained and are presented in Table 33. No real differences are noted; however, only a small number of pigs were evaluated.

In trial two, the performance of the pigs, this time in groups fed under practical type conditions, was excellent at all levels and in both the grower and finisher phases (Table 34). In the grower phase the pigs on the highest APC level (20%) grew significantly faster than the two controls or the low APC level (7%) ($P < 0.05$). Two pigs in the control with dehydrated alfalfa meal died during the grower phase; the apparent cause was rectal constricture. One pig on the highest APC level died several days prior to scheduled slaughter of

Table 32. Performance of Market Hogs Fed APC. Experiment 3-1A.

Group	Treatment	Average Daily Gain (g) 0-36 Days Mean	0-36 Days SD	0-42 Days Mean	0-42 Days SD	0-49 Days Mean	0-49 Days SD	Feed Conversion (kg/kg gain) Mean	Feed Conversion SD	Daily Feed Intake (kg)
1	Control	1008	136 (6)	1003	145 (6)	976	159 (4)	3.41	0.47	3.42
2	5% APC	953	82 (6)	903	64 (4)	-	-	2.96	0.21	2.76
3	10% APC	1022	77 (6)	1067	73 (3)	-	-	3.16	0.20	3.26
4	15% APC	913[b]	145 (5)	908	123 (5)	-	-	3.42	0.47	3.19
5	20% APC	872[a]	132 (6)	881	136 (6)	-	-	3.29	0.36	2.95
6	24% APC	972[b]	82 (5)	985	95 (5)	999	136 (3)	3.47	0.27	3.45

[a] Three pigs in this group contracted an unidentified infection during the experiment (see Results and Discussion).

[b] Two pigs in each of these groups contracted an unidentified infection during the experiment in which one pig in each group died.

Numbers in parentheses refer to number of pigs.

Table 33. Carcass Information of Market Hogs Fed APC. Experiment 3-1A.

Group	Treatment	Dressing %		Ham Loin %		Ave. Backfat (cm)	
		Mean	SD	Mean	SD	Mean	SD
1	Control	74.34	0.69 (5)	34.10	0.77 (5)	3.86	0.23 (5)
6	24% APC	72.97	1.42 (3)	34.01	1.56 (4)	3.50	0.28 (4)

[1]After a 24-hour fast.

Numbers in parentheses refer to number of pigs.

Table 34. Performance of Swine Fed APC. Experiment 3-1B.

Group	Treatment	Ave. Starting Weight (kg)	Ave. Finish Weight (kg)	Ave. Daily Gain (g) Mean	SD	Feed Conversion (kg/kg gain)	Daily Feed Intake (kg)	Ave. no. Days on Test
				Grower Phase				
1	7% Suncured alfalfa	28.9	51.8	623[a]	91	3.65	2.3	37
2	7% Dehydrated alfalfa	30.1	52.2	595[a]	95	3.60[1]	2.1	37
3	7% APC	27.5	48.7	572[a]	118	3.33	1.9	37
4	14% APC	26.6	52.2	690	159	3.32	2.3	37
5	20% APC	27.4	55.8	767[b]	150	3.04	2.3	37
				Finisher Phase				
1	5% Suncured alfalfa	56.8	94.0	804	118	3.38	2.7	46.9
2	5% Dehydrated alfalfa[2]	56.3	95.7	799	123	3.24	2.6	49.9
3	5% APC	53.0	87.4	726	195	3.47	2.5	49.0
4	11% APC	57.0	92.0	808	141	3.13	2.5	44.1
5	16% APC	61.7	96.9	831	91	3.10	2.6	42.5

[1] Estimate

[2] Two pigs died in the early part of the grower phase.

a significantly different from b ($P < 0.05$)

internal bleeding from an apparent ulcer. The feed conversion in the grower phase is rather high in all treatments; this could be explained by the unusually cold weather at the time the test was done and the fact that the pigs were in a semi-sheltered barn.

While the test was conducted, problems arose and feed for the finisher phase was not available when the pigs were taken off the grower phase. Since the grower feed was used up, the standard OSU grower was fed until the finisher feed was made. This explains the different weights at the end of the grower phase and start of finisher phase (Table 34). The total time on the OSU grower was about 1 week.

From the carcass data presented in Table 35, no significant differences were noted. There was a trend of decreasing percent lean cuts and subsequent lower market grades with increasing APC levels. Pond (1966) and Clawson _et al._ (1963) noted decreasing percent lean cuts with lower lysine levels; Holme _et al._ (1965), Wallace (1965), and Davey and Morgan (1969) noted the same with lower protein levels. Perhaps the lower percentage lean cuts show the lower protein quality and low lysine availability of APC as found in the earlier experiments with rats.

In addition, the pigs on the highest levels of APC did appear visually to be a bit "pot bellied." Further study is needed with this to determine if this could be a problem when high levels of APC are used.

Table 35. Carcass Information of Swine Fed APC. Experiment 3-1B.

	Group No.				
	1	2	3	4	5
No. of Pigs	10	8	10	10	10[1]
No. Barrows & Gilts	5-5	4-4	5-5	5-5	5[1]-5
Avg. Live Weight (kg)	92.7	95.0	91.9	91.8	94.2
Dressing %					
Mean	77.6	77.3	76.9	77.8	77.8
SD	1.2	1.5	1.8	1.2	1.1
Carcass Length (cm)					
Mean	76.40	77.39	77.01	75.31	76.07
SD	1.60	2.82	2.54	2.44	2.54
Back Fat Thickness (avg.) (cm)					
Mean	3.73	3.84	3.66	4.09	3.91
SD	0.51	0.46	0.51	0.46	0.23
% Lean Cuts (4 main cuts)					
Mean	52.51	52.00	51.94	50.49	50.95
SD	2.42	1.73	2.45	3.31	2.30
Color Score (1 poor-5 excellent)					
Mean	3.00	3.00	3.00	3.10	3.20
SD	0.45	0	0	0.30	0.40
Marbling Score (1 poor-5 excellent)					
Mean	3.40	3.50	3.10	3.60	3.10
SD	0.49	0.50	0.54	0.49	0.30
U.S. Market Grades					
No. 1	6	3	5	3	3
No. 2	2	3	3	3	4
No. 3	2	2	2	2	1
No. 4	0	0	0	2	2

[1]One pig missing--died of internal bleeding several days prior to scheduled slaughter. The missing plot technique was used to calculate data for this animal.

Experiment 3-2

In experiment 3-2 there was a significant ($P < 0.01$) depression in gain with the 20% APC level (Table 36). The deletion of salt had no measurable effect. Feed consumption data were obtained and presented; however, variable wastage had occurred that went undetected with three pigs per pen. Two pigs in the first control died early in the experiment of an unknown cause.

These results indicate that with young pigs, APC at high levels may not be utilized as efficiently as soybean meal. Further work needs to be done with pigs to determine if this is a result of amino acid inadequacy or poor protein digestion. Growth at the 7.5% or 12.5% APC levels was excellent, indicating at least at this level that alfalfa protein is well utilized. The reduction of the level of meat and bone meal at the 20% APC level may also have contributed to the poorer performance.

Because of the high ash content of the APC, diarrhea problems might be expected. There was a tendency for loose feces at the higher APC levels, but not to the extent of being detrimental. Similar results were also noted in experiment 3-1 and 3-3. This problem was minimized by proper mineral supplementation to utilize the APC ash to its fullest extent. Kuzmicky et al. (1972) noted excessively wet feces from poultry fed high levels of APC but they noted that with proper mineral supplementation the problem could be minimized.

Table 36. Performance of Young Growing Swine Fed APC. Experiment 3-2.

Group	Treatment	Average Daily Gain (g): To 91 kg, Mean	To 91 kg, SD	To 104-109 kg, Mean	To 104-109 kg, SD	Feed Conversion (kg/kg gain), Mean[1]	Feed Conversion, SD	Daily Feed Intake (kg)
1	Control (no alfalfa)	695	86 (6)	-	-	2.80[2]	0.08	1.70[2]
2	Control (+ alfalfa)	658	123 (9)	799	54 (3)	2.62[3]	0.06	1.92[3]
3	7.5% APC	708[a]	86 (9)	853	41 (3)	2.62[3]	0.07	1.92[3]
4	12.5% APC	662	91 (9)	-	-	2.92	0.15	1.93
5	20% APC (+ salt)	608[b]	104 (9)	-	-	3.19	0.09	1.93
6	20% APC (- salt)	608[b]	100 (9)	-	-	2.83	0.20	1.73

[1] For 3 groups of 3 pigs each.

[2] Estimate due to sickness and injury to 3 pigs in the group.

[3] Includes data from 3 pigs up to 54-60 kg.

a different from b ($P < 0.01$).

Experiment 3-3

In the first part of this experiment, the results show no differences among the pigs fed the APC diets over the control. This was the case for both the pre and post weaning phases (Table 37). A slight increase in feed conversion was noted for the APC diets.

In the second part of this experiment two separate trials were done. The pigs in the first trial were on test for 23 days or from 9-14 kg to 20 kg average per pen. The pigs on the second trial were on test from 9-11 kg to 29 kg average per pen or for 35 days. The results (Table 38) show that in the first trial a significant reduction in growth occurred for the APC rations ($P < 0.05$ for the 14% level and $P < 0.01$) for the 28 and 34% levels). In the second trial no difference was noted. The difference between replicates might be explained by the fact that the APC is utilized poorly by the smaller pigs or that the pigs needed a period of adjustment to the APC diets. In either case the weight could have been made up later at heavier weights (compensatory growth). In both cases the feed conversion was increased with APC in the rations.

The increase in feed conversion of all the pens fed the APC diets supports the idea that APC may be poorly utilized by the young pig. Kuzmicky et al. (1972) reported similar results with broilers; they found a significant response in feed conversion with lysine

Table 37. Performance of Pigs Fed APC Creep-Starter Rations. Experiment 3-3A.

Group	Treatment	No. of Litters	Ave. Start Weight (kg)	Ave. Finish Weight (kg)	Ave. Daily Gain (g) Mean	Ave. Daily Gain (g) SD	Ave. no. Days on Test
				Pre-weaning Phase			
1	Control OSU	5 (44)	4.9	9.2	204	77	20.05
2	6.5% APC	5 (34)	5.3	10.2	213	77	22.03
3	13% APC	5 (43)	5.4	9.7	181	45	24.86

Group	Treatment	No. of Pigs	Ave. Start Weight (kg)	Ave. Finish Weight (kg)	Ave. Daily Gain (g) Mean	Ave. Daily Gain (g) SD	Feed Conversion (kg/kg gain)	Daily Feed Intake (kg)	Ave. Days on Test
					Post-weaning Phase				
1	Control OSU	31 (15-16)	9.7	28.8	459	141	2.03	0.94	43.1
2	6.5% APC	36 (14-22)	10.4	29.4	449	118	2.13	0.90	44.2
3	13% APC	43 (22-21)	9.7	28.4	422	83	2.17	0.91	44.9

[1] Numbers in parentheses refer to number of pigs in the pre-weaning phase and to the number of males and females in the post-weaning phase.

Table 38. Performance of Pigs Fed APC Starter Rations. Experiment 3-3B.

Group	Treatment	No. of pigs[1]	Ave. Starting Weight (kg)	Ave. Finish Weight (kg)	Ave. Daily Gain (g) Mean	Ave. Daily Gain (g) SD	Feed Conversion (kg/kg gain)	Daily Feed Intake (kg)
23 Day Test (Part A)								
1	14% APC	13 (4-9)	12.6	21.7	395[b]	95	2.67	1.04
2	28% APC	14 (6-8)	12.8	21.7	386[c]	86	2.67	1.02
3	34% APC	14 (6-8)	13.9	22.3	368[c]	77	2.88	1.05
C	Control	15 (6-9)	11.6	22.6	477[a]	100	2.12	1.01
35 Day Test (Part B)								
1	14% APC	12 (4-8)	10.6	27.0	463	54	2.29	1.04
2	28% APC	12 (4-8)	11.2	26.9	445	68	2.59	1.19
3	34% APC	12 (4-8)	11.9	27.5	445	118	2.21	0.95
C	Control	10 (3-7)	11.5	28.0	463	54	2.08	0.85

[1] Numbers in parentheses refer to number of males-females.

a significantly different from b ($P < 0.05$)

a significantly different from c ($P < 0.01$)

supplementation. Earlier studies presented in this thesis with rats support the idea that APC is of relatively poor protein quality. Another explanation for the poor feed conversion could be overestimation of the digestible energy. It was estimated to have 2800 kcal/kg.

Summary Part Three

Three experiments were designed to evaluate APC as a protein source in swine rations.

In experiment one, individual and group trials were done with barley and corn-based diets respectively. In the individual trial, APC was fed at levels of 0, 5, 10, 15, 20, and 24% in the diet. All levels supported excellent rates of growth. With the group trial, grower and finisher rations were fed. The growers contained 7, 14, and 20% APC while the finishers contained 5, 11, and 16% APC. All levels supported good growth when compared to controls. No significant differences in carcass data were observed; however, there was a trend toward lower market grades with increasing APC in the diet.

The second experiment involved the use of young growing swine. Levels of 7.5 and 12.5% APC supported excellent growth when compared to the controls in barley based rations. At the 20% level growth was significantly depressed ($P < 0.01$).

The third experiment involved the use of APC in creep-starter rations. In the first trial with wheat based diets, both 6.5 and 13% APC levels supported good growth in both pre and post weaned pigs. In the second trial one group was fed to 20 kg while another was fed to 27 kg with rations containing 0, 14, 28, and 34% APC in corn based diets. With those fed to 20 kg average weight, the growth was significantly depressed at all levels ($P < 0.05$ at the 14% level, $P < 0.01$ at the 28 and 34% levels). With those fed to 27 kg, no difference was noted. In all cases feed conversion increased somewhat with APC in the diet.

It is concluded that APC can be a useful protein supplement for swine.

SUGGESTIONS FOR FUTURE WORK

The use of alfalfa as a protein source in monogastric rations up to now has been impractical. It was found in this study that alfalfa is unpalatable when fed at high levels. The cause of this warrants further work, including isolation of the factors involved. Changes in or modification to processing methods used for preparation of alfalfa meal might produce a more useful product for swine.

The use of APC in animal diets up to now has been limited to laboratory animals. Further field studies with swine should be done. In addition work should be done to see if methionine and/or lysine supplementation can improve utilization and carcass grade for swine. The effect of APC on swine reproduction should also be studied. The potential problem of photosensitization with APC should be looked at in swine. Other possible swine studies include the feeding of APC with varying combinations of other protein supplements and grain sources to find optimal combinations. Crude protein digestibility and digestible energy values need to be determined.

With rats, studies should be centered on methods of improving the utilization and digestibility of APC. One method might include solvent extraction of the fat, since heat, which is used to dry the protein curd, has been found to lower protein digestibility in the presence of fat (Buchanan, 1969).

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