LCP COTTONSEED FLOUR AS A
PROTEIN SOURCE FOR SWINE

by

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CHAPTER I

INTRODUCTION

In recent years, a rapidly expanding population has stimulated the search for new sources of protein suitable for the human diet. One potential source, cottonseed, has been the subject of much research (Harden, 1975). The annual worldwide production of cottonseed has been about 24 million metric tons (Gardner et al., 1973; Ridlehuber, 1973). Assuming that this cottonseed contained approximately 20% protein (Gardner et al., 1973; Ridlehuber, 1973), 4.8 million metric tons of cottonseed protein have been produced annually. This production represented approximately 6% of the world's protein resources (Ridlehuber, 1973).

Although cottonseed protein was recognized as a potential source of edible protein during the early stages of its processing history (Harden, 1975), its use in diets of the monogastric species (in which man, swine and poultry are included) was severely handicapped by the presence of a toxic substance known as gossypol (Martinez et al., 1970; Vix et al., 1971; Gardner et al., 1973). Gossypol is a phenolic compound which is found primarily in cottonseed pigment glands (Gastrock, 1968; Martinez et al., 1970). During conventional oil extraction procedures, these glands are ruptured and the gossypol becomes mixed with the other constituents of the cottonseed. A chemical reaction then occurs between a portion of the gossypol and other seed constituents,

primarily the lysine residue of the protein (Gastrock, 1968). The relatively stable complex is termed "bound" gossypol and is essentially inactive physiologically (Smith et al., 1958). That portion of the gossypol which does not react is termed "free" gossypol and is the primary toxic substance (Withers and Carruth, 1915).

Until recently, attempts to produce a cottonseed product suitable for human consumption utilized either moist heat and pressure to convert free gossypol to the bound form (Harden, 1975) or special solvents to remove free gossypol (Bressani, 1965). In 1968, workers at the U.S. Southern Research Laboratory, New Orleans, LA, completed the development of a new process, termed the Liquid Cyclone Process (LCP), by which the pigment glands were removed intact from the cottonseed (Vix et al., 1971). This process, which permits production of a cottonseed flour containing approximately 65% protein and acceptably low levels of free gossypol, was recognized by industry as "the quickest and most reliable method available to introduce cottonseed flour to the nation's food supply" (Ridlehuber, 1973).

Recognizing the potential of the LCP, the Plains Cooperative Oil Mill, Lubbock, TX, began construction in 1972 of a new cottonseed processing facility incorporating the LCP principles (Ridlehuber, 1973). This facility, which is capable of producing 25 tons of flour per day (Ridlehuber and Gardner, 1974), became operational in 1974.

The production of LCP cottonseed flour on a commercial basis is related to swine nutrition through the production of a quantity of flour which fails to meet the standards established for edible

cottonseed protein. This "off-specification" flour represents a potential source of high quality protein for use in swine diets. However, due to the uniqueness of this product, little is known of its nutritional value for swine.

The objectives of the research reported in this thesis were:

1. The determination of the nutritional value of LCP cottonseed flour protein relative to soybean meal (SBM) protein.
2. The determination of a feasible manner of utilizing LCP cottonseed flour as a protein source in swine diets.

CHAPTER II

LITERATURE REVIEW

The research concerning the use of LCP cottonseed flour for swine diets is limited. However, as Bressani (1965) stated, the difference between cottonseed meal (CSM) and flour should be small when both are produced by modern solvent extraction processes. Therefore, the following literature review will present the available information concerning the use of cottonseed products as protein sources for swine.

The Gossypol Problem

The use of CSM in the diets of swine always has been limited by the presence of a polyphenolic aldehyde, known as gossypol, which is found only in the pigment glands of plants of the *Gossypium* species (cited by Withers and Carruth, 1915). The early research concerning the use of CSM for swine adequately demonstrates the toxic nature of gossypol (Curtis and Carson, 1892; Curtis, 1895; Georgeson et al., 1895; Dinwiddie, 1903; Edgerton and Morris, 1912).

The gossypol contained in the pigment glands of cottonseed is released when the integrity of the gland is disrupted during comminution required for oil extraction processes (Gardner et al., 1973; Ridlehuber and Gardner, 1974). Although the LCP was developed to minimize gossypol content of the flour, some glands are disrupted during comminution of the seed, and other glands are not removed

during processing. Comminution of the seed is one of the most critical parts of the LCP. Severe comminution will result in amounts of free gossypol which are greater than 450 ppm, the maximum level established by the Food and Drug Administration (Ridlehuber and Gardner, 1974; CFR, 1975). Therefore, off-specification LCP cottonseed flour may contain appreciable amounts of free gossypol.

The total gossypol content of processed cottonseed generally is divided into two categories. Free gossypol is that portion which is soluble in aqueous acetone, while the remainder is termed bound gossypol (Pond and Maner, 1974). The division serves a practical purpose in determining the properties of a cottonseed product. A determination of the free gossypol content of a particular cottonseed product is an accurate measure of the toxicity of that product (Withers and Carruth, 1915). Bound gossypol is a physiologically inactive complex which frequently involves the epsilon amino group of the amino acid, lysine (Baliga and Lyman, 1957; Smith et al., 1958). The complex is relatively resistant to digestion (Carruth, 1947), and this resistance affects the availability of amino acids in cottonseed protein. Thus, a measure of bound gossypol is an indication of protein quality (Lyman et al., 1953).

The symptoms of gossypol toxicity in swine are well documented (Dinwiddie, 1903; Hale et al., 1958; Smith et al., 1972), and are described as dyspnea with violently labored respiration (thumping), progressive weakness and emaciation. The symptoms are associated with the effects of gossypol on the oxygen transporting capacity of

the blood. Gossypol exhibits a chelating effect on tissue iron stores; it interferes with protein synthesis, thereby lowering serum hematocrit and hemoglobin content (Skutches et al., 1974); and it exhibits a hemolytic effect on red blood cells in vitro (Menaul, 1923).

Methods of Detoxifying Gossypol

In 1913, Withers and Brewster discovered that the toxic effects of gossypol could be prevented by the addition of iron salts to the diet. A number of methods for preventing toxicity have since been devised. These methods may be divided into two major groups according to their mode of action. One group is based on detoxifying gossypol through chemical reactions, while the second group involves the use of special processing techniques to remove the free gossypol.

The addition of iron salts to the diet is the most common method of detoxifying free gossypol in swine diets (Smith, 1970a). The formation of an insoluble iron salt of gossypol in the digestive system, which effectively prevents absorption, is the most widely accepted theory regarding the mode of action of iron salts in detoxifying gossypol (Withers and Carruth, 1917; Smith and Clawson, 1965; Pond and Maner, 1974). A variety of iron salts is effective in preventing gossypol toxicity. These salts are ferrous sulfate, ferrous fumarate, ferric citrate, ferric ammonium citrate, ferrous oxalate and ferric chloride (Withers and Carruth, 1917; Gallup, 1928; Stephenson et al., 1965). Ferrous sulphate, as the monohydrate, is the most effective form of iron for this purpose (Stephenson et al.,

1965; Tanksley, 1970). In order to prevent toxicity and promote growth, iron must be added to the diet in a 1:1 weight ratio with free gossypol (Clawson and Smith, 1966; Smith, 1970a; Smith and Clawson, 1970). Although swine are among the most susceptible animals to gossypol toxicity (Bressani et al., 1969), they are able to tolerate .01% free gossypol in the diet without supplementary iron (Hale and Lyman, 1957; Hale et al., 1958).

Moist heat is effective in converting free gossypol to the bound form (Edgerton and Morris, 1912; Gallup, 1928; Robison, 1934). Since the bound form is physiologically inactive, cooking detoxifies the cottonseed product. This method is used in the production of commercial cottonseed flours now available, and has been instrumental in the development of several vegetable protein mixtures containing cottonseed in South America (Bressani, 1965). Although effective in reducing the free gossypol content of the product, this method also reduces the quality of the cottonseed protein by reducing lysine availability.

Abnormally high protein content in the diet will effectively alleviate gossypol toxicity, presumably through the availability of sufficient amounts of amino acids to overcome the inhibiting effect of gossypol on protein synthesis (Hale and Lyman, 1957; Sharma et al., 1966). This method is impractical in commercial swine production, due to the expense of protein sources.

In the 1940's, the Southern Regional Research Center, New Orleans, LA, developed two processing methods which removed much of

the free gossypol from cottonseed (Gastrock, 1968). These were flotation (Vix et al., 1947) and differential settling (Spadaro et al., 1950). In 1968, the LCP was developed from these methods (Gastrock, 1968). The LCP is a hexane extraction procedure which utilizes a cyclone to separate a liquid slurry into an essentially pigment gland free high protein fraction and a high protein fraction with high pigment gland content (Gastrock, 1968; Vix et al., 1971). This method for the removal of gossypol and production of a high protein concentrate from cottonseed has been accepted as the most reliable method for the production of food grade cottonseed proteins (Ridlehuber, 1973).

Indications of Other Toxic Substances in Cottonseed

Although gossypol is considered to be the principal toxic substance of cottonseed, there are several reports of clinical syndromes which apparently are unrelated to gossypol toxicity. Wood and Larson (1961) described a hepatoma in rainbow trout which was later attributed to the feeding of CSM (Wolf and Jackson, 1963). The histological lesions of this syndrome did not resemble those of gossypol toxicity, as described by Hale et al. (1958) and Smith et al. (1972). Dunbar and Herman (1971) attributed visceral granuloma, a chronic inflammatory disease of brook trout, to the feeding of diets containing CSM. Their findings were based on a series of experiments in which fish were fed either a synthetic diet, the same diet with cottonseed meal (22.5% by weight), the synthetic diet with 333 ppm free gossypol, or a modified Cortland meat-meal diet with and without CSM. The incidence of

visceral granuloma in fish fed these diets was 0, 21, 2.6, 91.3 and 4.7%, respectively.

The Quality of Cottonseed Protein

The significance of bound gossypol in animal feeds has been reported (Carruth, 1947; Baliqa and Lyman, 1957; Smith et al., 1958). Hale et al. (1958) have stated that the quality of cottonseed protein varies more than that of any other major protein source. Although some of the variation is due to variation in amino acid composition, much variation is due to the binding of amino acids, particularly lysine, by gossypol during the application of the heat necessary in some processing techniques. Since lysine is the first-limiting amino acid in CSM when used for swine diets (Tanksley, 1970), this binding can have a significant effect on cottonseed protein quality.

Protein quality is determined by amino acid composition and availability. Several methods of determining protein quality have been used to evaluate cottonseed protein. These include a chemical index method, amino acid analysis, amino acid availability determination, nitrogen balance, biological value, protein efficiency ratio (PER), animal growth rate and efficiency of gain.

To predict cottonseed protein quality, Lyman et al. (1953) developed a chemical index. This method is based on the theory that binding of lysine alters the chemical properties of cottonseed protein and reduces their solubility in dilute alkali (Lyman et al., 1953). The chemical index is the ratio of nitrogen solubility in 0.2 N sodium

hydroxide to the total gossypol content. Lyman et al. (1953) found that this index was highly correlated with chick growth. Chang et al. (1955) determined that nitrogen solubility was highly correlated to growth rate of the chick ($r=.82$). Growth rate was negatively related to total gossypol content ($r=-.90$).

A reference protein of known value is used for comparison when protein quality is evaluated by amino acid content. The Foreign Agriculture Organization (FAO) has developed a reference pattern of amino acids to meet the needs of an adult human. Casein, egg and soybean proteins frequently are used as the reference protein for animals.

Smith (1970a) stated that cottonseed protein contained less lysine, isoleucine and leucine than soybean meal. Harden and Yang (1975) demonstrated that LCP cottonseed flour was lower in lysine, threonine, isoleucine, leucine and tyrosine than SBM or casein. LCP cottonseed flour was deficient only in methionine and isoleucine in comparison to the FAO reference pattern (Harden and Yang, 1975). Bressani (1965) stated that the pattern of essential amino acids in cottonseed flour compared favorably with the requirements for human adults. About 30% of the amino acids in cottonseed flour are in the essential group (Bressani, 1965). This may be compared to roast beef protein, in which 50% of the amino acids are in the essential group.

Amino acid availability is as important as amino acid composition in determining protein quality. Lysine is the most important amino acid in cottonseed protein in terms of availability, due to the variation produced by different processing methods. Kuiken and Lyman

(1948) determined that the availability of lysine in a commercial cottonseed flour was 65%. Kuiken (1952) observed marked variations in availability between processing methods, species of cottonseed and amino acids. Lysine availability was reported as 64% for a commercial cottonseed flour and 84% for a hexane extracted "degossypolized" meal. Arginine, lysine and methionine availabilities for the flour were reported as 93%, 64% and 82%, respectively. Lysine availability was 95% for a glandless cottonseed variety. Almquist and Halloran (1975) reported that a CSM containing 1.8% lysine had an effective lysine level of 1.6%, indicating an availability of 88.9%.

Graham et al. (1969) studied malnourished children and determined that nitrogen balance could be obtained using cottonseed as the sole source of protein. However, higher levels of dietary protein intake were necessary to establish nitrogen balance for cottonseed protein than for milk protein. Other nitrogen balance studies with babies indicated that cottonseed protein was inferior to milk protein (cited by Bressani, 1965). A nitrogen balance index determined for dogs produced values of .60 for cottonseed protein and .78 for casein (cited by Bressani, 1965). The addition of lysine and methionine increased the index for cottonseed flour to .85.

The biological value of cottonseed protein was reported as 62.1 and 57.0 for children and infants, respectively (cited by Bressani, 1965). Comparable values obtained for cow's milk were reported as 82.1 and 79.0 for children and infants, respectively.

PER is a measure of the relative efficiency of gains produced by

feeds being tested. In the rat, Bressani (1965) cited PER values of 2.14 and 2.38 for cottonseed protein and casein, respectively. Castro (1974) obtained values of 2.61, 2.46 and 1.76 for protein from casein, cottonseed and soybean isolates, respectively. Barnes et al. (1966) reported PER values of 2.7, 3.0 and 1.9 for casein, azeotropically extracted cottonseed flour, and commercially heat processed cottonseed flour, respectively. For five samples of LCP cottonseed flour, Olson (1973) determined PER values ranging from 2.51 to 2.67, with PER for casein established at 2.50. At low levels of dietary protein (approximately 8.5%) Bressani et al. (1966) obtained PER values of 1.92, 2.13 and 1.81 for casein, LCP cottonseed flour, and a commercially heat processed cottonseed flour, respectively. However, at approximately 16% protein, the values obtained for the same proteins were 2.33, 2.18 and 1.85, respectively.

Hale et al. (1958) stated that any comparison of growth rate and efficiency of gain concerning cottonseed products was meaningful only if the processing conditions or the chemical nature (i.e., nitrogen solubility) of the product were known. In view of the influence exerted by the method of processing on cottonseed protein quality, this stipulation would appear to be valid. Therefore, either processing method or nitrogen solubility of cottonseed products used in the research reviewed will be presented where available. Those reports which contain no such information should be regarded as incomplete.

In 1965, screw-press, direct solvent extraction, prepress solvent extraction and hydraulic press methods accounted for 51, 22, 25 and

2%, respectively, of the processed CSM in the United States. Screw-pressed CSM is high in residual lipids, lowest in free gossypol and lowest in protein quality. The CSM produced by solvent extraction methods are lowest in lipids and higher in free gossypol and protein quality (Smith, 1970b). Individual samples produced by any processing method may vary more than samples from different processing techniques (Hale et al., 1958).

Nitrogen solubility, as previously discussed, is a good indicator of protein quality for cottonseed products. Hale et al. (1958) established that a nitrogen solubility of greater than 75% indicated a cottonseed protein of acceptable quality for diets of monogastrics.

Hale et al. (1958) reported that a screw-pressed CSM supported gains and feed efficiencies which were equal to those from a SBM when each was used as the sole source of supplementary protein. CSM produced by hydraulic pressing was inferior to SBM or screw-pressed CSM in this experiment. Haines et al. (1957), in studies utilizing a solvent extracted CSM with a nitrogen solubility of 89%, obtained gains and feed efficiencies in growing-finishing swine equal to those of a corn-SBM diet when CSM was used as 25, 50 or 75% of the protein supplement. For weanling pigs, Haines et al. (1955) improved weight gains by substituting a solvent extracted CSM for one-half of the SBM in a corn-SBM diet. Sewell et al. (1957) obtained equal gain and feed efficiency from a protein supplement containing one-half SBM and one-half CSM when compared to an all SBM supplement. The CSM had a nitrogen solubility of 89.9%. Hale and Lyman (1957), using a CSM of

unknown characteristics, obtained equal performance in gain and feed efficiency from growing pigs when CSM was substituted for one-half the SBM in a sorghum-SBM diet. Dyer et al. (1952) found that a CSM of unspecified characteristics did not perform as well as SBM at any level tested for weanling pigs.

Palatability of Cottonseed Products

Swine diets must be palatable to sustain maximum feed intake. Reported research indicates that cottonseed products are not highly palatable for swine. Curtis and Carson (1892) observed that hogs would consume normal amounts of a diet containing cottonseed for a period of 2 to 8 days. After that time, consumption was markedly reduced. Georgeson et al. (1895) found that pigs would eat relatively large amounts of CSM only "under protest". Tribble (unpublished data), in a palatability trial comparing various levels of CSM, found that the sorghum-SBM diet was preferred by finishing hogs over any level of CSM tested. Tanksley and Calvez (1973), in feeding trials with lactating sows, found that sows fed a SBM-sorghum diet consumed more ($P < .05$) than those fed a SBM-CSM-sorghum diet.

Methods of Conducting Palatability Trials

Two methods of conducting palatability tests for swine have been developed. Aldinger et al. (1959) described a free choice method in which pigs were offered their choice of rations on a continuous basis. Results were most consistent when only two rations were compared. The data indicated that pigs were not able to detect small differences

between diets when fed by this method. Aldinger and Fitzgerald (1966) described a single stimulus technique in which each diet was offered on an alternate feeding schedule. A feeding interval of 2 to 4 hours was optimum for preference testing with this technique. In a comparison with the free choice technique, the single stimulus method was found to be more consistent and more sensitive to small differences in diets (Aldinger and Fitzgerald, 1966; Wahlstrom et al., 1974).

CHAPTER III

LCP COTTONSEED FLOUR AS A PROTEIN SOURCE FOR SWINE

Summary

Four experiments, involving a total of 352 pigs, were conducted to evaluate liquid cyclone process cottonseed flour as a protein source for swine. In experiment 1, two trials were conducted to determine the relative palatability of diets containing various levels of cottonseed flour and SBM. When fed in meal form in trial 1, a diet containing 10% cottonseed flour was preferred ($P < .05$) to a SBM-sorghum control diet. Diets containing 15 or 18% cottonseed flour were less palatable ($P < .05$) than the control. When similar diets were fed in pellet form in trial 2, no preferences were shown in the comparisons made.

In the second experiment, two trials were conducted to determine the growth-promoting properties of cottonseed flour for weanling pigs. Average daily gain (ADG), feed/gain, average daily feed intake (ADFI), and a lysine efficiency ratio (LER) were determined for each trial. In all criteria evaluated in trial 1, pigs fed an 8% cottonseed flour diet performed as well as those fed a SBM control diet. ADG was reduced ($P < .05$) and feed/gain increased ($P < .05$) for pigs fed 16 and 24% flour diets. ADFI was reduced ($P < .05$) for the pigs fed the 24% flour diet. In trial 2, ADG and ADFI were lower ($P < .05$) for

the pigs receiving 8 and 12% flour diets than for the control pigs. There were no differences among treatments in feed/gain or LER. In all criteria evaluated, pigs fed a diet containing 4% flour performed as well as the control pigs.

Experiment 3 was conducted to determine the growth-promoting properties of cottonseed flour for growing and finishing swine. Diets containing cottonseed flour were compared to a SBM-sorghum control and to a diet containing 10% CSM. ADG, feed/gain, ADFI and LER were determined for the growing and the finishing period. During the growing period, pigs fed a 5% cottonseed flour diet performed as well as the control pigs. The ADG for pigs fed 10 or 15% cottonseed flour diets were lower ($P < .05$) than those of the pigs fed the control diet. The LER of the pigs fed the 10% CSM diet was lower ($P < .05$) than that for any other group. There were no differences among treatments in feed/gain or ADFI. During the finishing period, the LER values of the pigs fed diets containing 4 and 12% cottonseed flour were higher ($P < .05$) than values of the control pigs. The LER values of the pigs fed the diets containing cottonseed flour were higher ($P < .05$) than those of the pigs fed cottonseed meal. No differences among treatments were found in ADG, feed/gain or ADFI.

Experiment 4 was conducted to investigate the toxic properties of CSM and cottonseed flour. A sample of 20 pigs from experiment 3 were slaughtered and examined for gross and microscopic lesions in the heart, kidney, spleen and liver. Lesions were found in 100% of the livers from pigs receiving the CSM or cottonseed flour. No

similar lesions were found in the control pigs. The lesions did not resemble those of gossypol toxicity.

Introduction

During the 1960's, workers at the Southern Regional Research Laboratory, New Orleans, LA, developed a process for the production of high quality, low gossypol cottonseed flour for use as a human food. Application of this process to commercial cottonseed flour production first began in 1974 with the operation of a new facility owned by the Plains Cooperative Oil Mill, Lubbock, TX. A portion of this product which fails to meet the specifications for food grade cottonseed flour may become available for use in livestock feeds. Due to the newness of this product, little is known of its characteristics as an animal feed.

The properties of cottonseed meal as a protein source for swine have been investigated by Hale (1930); Sewell et al., (1957); Hale et al., (1958); Kornegay et al., (1961); Stephenson et al., (1965); and others. Gossypol, which is a toxic substance found in cottonseed (Withers and Carruth, 1915), is a limiting factor in the use of cottonseed meal in swine diets. Cottonseed flour which fails to meet the specifications for use in human diets may contain toxic quantities of this substance (CFR, 1975). The addition of iron, as ferrous sulphate, has been shown to prevent toxicity (Withers and Brewster, 1913; Clawson and Smith, 1966; Smith and Clawson, 1970).

The experiments reported here were conducted to compare liquid

cyclone process cottonseed flour and SBM as protein sources for swine.

Experimental Procedure

The animals in these experiments were crossbred (Hampshire X Duroc X Yorkshire) or purebred Duroc pigs. In each experiment, pigs were allotted to treatment on the basis of body weight, litter and sex. All pigs were housed in enclosed buildings with partial environmental control. The buildings used in experiments 1 and 2 had totally slatted aluminum or plastic floors. The building used for experiment 3 had partially slatted aluminum and concrete floors. All diets were formulated to meet or exceed NRC recommendations. Diets were calculated to maintain a constant level of lysine. Iron, as ferrous sulphate, was added in a 1:1 ratio to free gossypol. Feed and water were supplied ad libitum unless otherwise specified.

Data for experiment 1 were analysed by the method of Steele and Torrie (1960) for comparison of sample means with paired observations. Data from experiments 2 and 3 were analyzed by least squares analysis of variance. If the F ratio was significant ($P < .05$), the least significant difference was used to determine treatment differences.

Experiment 1

Two trials were conducted to determine the palatability of diets containing various levels of cottonseed flour in relationship to a SBM-sorghum diet. Experimental design and composition of diets for experiment 1 are presented in tables 1 and 2, respectively. Average initial weight of the pigs in this experiment was 9.7 and 12.7 kg.

TABLE 1. EXPERIMENTAL DESIGN, EXPERIMENTS 1 AND 2

Experiment	Trial	Diet form	Treatment	Diet(s)	No. of replicates	No. of pigs/rep.
1	1	Meal	A	Control vs CSF10	4	4
		Meal	B	Control vs CSF15	4	4
		Meal	C	Control vs CSF18	4	4
1	2	Pellet	D	Control vs CSF10	4	4
		Pellet	E	Control vs CSF14	4	4
		Pellet	F	Control vs CSF18	4	4
2	1	Meal	1	Control	2	8
		Meal	2	CSF8	2	8
		Pellet	3	Control	2	8
		Pellet	4	CSF8	2	8
		Pellet	5	CSF16	2	8
		Pellet	6	CSF24	2	8
2	2	Meal	1	Control	4	5
		Meal	2	CSF4	4	5
		Meal	3	CSF8	4	5
		Meal	4	CSF12	4	5

TABLE 2. COMPOSITION OF DIETS, EXPERIMENTS 1 AND 2

Experiment	Trial	Diet	Ingredient ^a , %							Premix ^b
			44% SBM	60% CSF	Deflour. Phosphate	CaCO ₃	NaCl	ASP250	FeSO ₄	
1	1 & 2	Control	75.8	20.2		.72	1.03	.25	...	1.50
		CSF10	77.2	8.9	10.0	.28	1.37	.25	.01	1.50
		CSF14	77.8	4.4	14.0	.12	1.51	.25	.02	1.50
		CSF15	77.9	3.2	15.0	.06	1.55	.25	.02	1.50
2	1	CSF18	78.3		17.9	...	1.59	.25	.02	1.50
		Control	67.2	28.5		1.12	.96	.25	20g	1.50
		CSF8	68.3	19.5	8.0	.77	1.24	.25	20g	1.50
		CSF16	69.4	10.4	16.0	.42	1.51	.25	20g	1.50
2	2	CSF24	70.4	1.4	24.0	.07	1.79	.25	20g	1.50
		Control	64.0	31.8		1.10	.90	.25	20g	1.50
		CSF4	63.5	28.2	4.0	.90	1.10	.25	20g	1.50
		CSF8	63.3	24.7	8.0	.70	1.20	.25	20g	1.50
2	2	CSF12	62.8	21.1	12.0	.60	1.30	.25	20g	1.50

^aAs fed basis.

^bSupplied vitamins and trace minerals.

for trials 1 and 2, respectively. In each trial, two replicates were fed by each of the methods described below for a period of 7 days.

Free Choice Method. The pigs on each treatment were allowed constant access to the diets being compared. Feed consumption was determined at the end of each 24-hr period. The feeders were stationary, but the diets were rotated between feeders each day.

Modified Free Choice Method. The pigs on each treatment were allowed to consume the diet of their choice during 1-hr periods at 8 am, 12 noon and 5 pm. The feeders were removed at the end of each period and feed consumption was determined. The feeders were placed in the same position during each period, but feed was rotated among feeders between feedings.

Experiment 2

Two trials were conducted to determine growth-promoting properties of cottonseed flour for the weanling pig. Experimental design and composition of diets are shown in tables 1 and 2, respectively. Average initial weight of the pigs in trials 1 and 2 was 7.5 and 7.7 kg, respectively. Pigs were fed for 35 days in both trials. ADG, feed/gain and ADFI were determined. An LER was determined for each treatment:

$$\text{LER} = \frac{\text{Units of weight gain}}{\text{Units of lysine consumed.}}$$

Experiment 3

One trial was conducted to determine the growth-promoting properties of cottonseed flour for growing and finishing swine. The trial was divided into a 42-day growing period and a 42-day finishing period. One group of 80 pigs with an average initial weight of 28.8 kg was allotted to two replicates of the five treatments. The treatments were: 1) control diet in both periods, 2) CSF 5 in growing period, followed by CSF 4, 3) CSF 10 in growing period, followed by CSF 8, 4) CSF 15 in growing period, followed by CSF 12 and 5) CSM 10 in growing period, followed by CSM 8. Composition of diets for experiment 3 is shown in table 3. ADG, feed/gain, ADFI and LER were determined for each period.

Experiment 4

Four pigs were selected at random from each treatment of experiment 3. Each pig was continued on its respective dietary treatment until normal slaughter weight was reached. At this time, each pig was slaughtered and examined for gross abnormalities. Samples of liver, kidney, heart and spleen were collected and preserved in a 10% buffered formalin solution. Tissues were later imbedded in paraplast and sectioned at approximately 8 microns. Slides for histological examination were prepared using hematoxin and eosin and Periodic Acid-Schiff staining techniques.

TABLE 3. COMPOSITION OF DIETS, EXPERIMENT 3

Period	Diet	Ingredient ^a , %						FeSO ₄	Premix ^b
		Sorg	44% SBM	60% CSF	41% CSM	Deflour. Phosphate	CaCO ₃	Salt	
Growing	Control	78.0	18.8			.74	1.02	.50	20g 1.0
	CSF5	78.6	13.1	5.0		.52	1.20	.50	20g 1.0
	CSF10	79.3	7.5	10.0		.30	1.37	.50	20g 1.0
	CSF15	80.0	1.9	15.0		.08	1.54	.50	20g 1.0
	CSM10	73.5	13.2		10.0	.35	1.41	.50	20g 1.0
Finishing	Control	83.3	13.9			.27	1.02	.50	20g 1.0
	CSF4	83.8	9.4	4.0		.09	1.15	.50	20g 1.0
	CSF8	84.4	4.9	8.0		...	1.21	.50	20g 1.0
	CSF12	84.9	.4	12.0		...	1.18	.50	20g 1.0
	CSM8	79.7	9.5		8.0	...	1.29	.50	20g 1.0

^aAs fed basis.^bSupplied vitamins and trace minerals.

Results and Discussion

Experiment 1

The results of experiment 1 are shown in table 4. Feeding method did not influence the results of either palatability trial. Therefore, no attempt was made to distinguish between methods of feeding in the statistical analyses. When the diets were fed in meal form in trial 1, the pigs receiving treatment A consumed more ($P < .05$) of the CSF 10 diet than of the control. However, the pigs receiving treatments B and C consumed more ($P < .05$) of the control diet. In trial 2 the diets were pelleted and no preference was shown for any diet in the comparisons made.

The results of experiment 1 indicated that cottonseed flour was acceptable to weanling pigs when fed at low levels in meal diets or at higher levels in pelleted diets. These results agree with those of Harden and Yang (1975) whose data indicated that rats offered no choice would consume more of a cottonseed flour diet than of a SBM diet at 10% dietary protein. However, when the protein and cottonseed flour levels were increased, the rats consumed more of the SBM diet. The data presented here indicated that diets containing 10% cottonseed flour were more palatable than a SBM diet. Tribble (unpublished data) found that a diet containing 5% CSM was less palatable than a SBM diet. The palatability of cottonseed flour diets in this experiment was apparently greater than that reported for CSM diets.

TABLE 4. RELATIVE PALATABILITY OF COTTONSEED FLOUR DIETS IN EXPERIMENT 1

Trial	Treatment	Diet	Diet form	Consumption	
				Total, kg ^a	%
1	A	Control	Meal	6.1	30.2
		CSF10	Meal	14.1*	69.8
	B	Control	Meal	17.0	87.8
		CSF15	Meal	2.4*	12.2
	C	Control	Meal	18.2	89.5
		CSF18	Meal	2.1*	10.5
	D	Control	Pellet	7.5	38.4
		CSF10	Pellet	12.1	61.6
	E	Control	Pellet	13.5	69.2
		CSF14	Pellet	6.0	30.8
	F	Control	Pellet	10.0	48.2
		CSF18	Pellet	10.7	51.8

^aAverage of 4 replicates.

* Different from control ($P < .05$).

Experiment 2

Table 5 presents the ADG, feed/gain, ADFI and LER for the pigs receiving the treatments in trial 1. The pigs fed diets containing 8% cottonseed flour, in both meal and pellet form, were similar to their respective controls for all criteria evaluated. ADG was reduced ($P < .05$) and feed/gain increased ($P < .05$) for the pigs receiving CSF 16 and CSF 24 diets. ADFI was reduced ($P < .05$) for the pigs fed

CSF 24. Pelleting improved ($P<.05$) feed/gain and LER and reduced ($P<.05$) ADFI for the pigs fed the control and CSF 8 diets.

TABLE 5. PERFORMANCE OF WEANLING PIGS FED VARIOUS LEVELS OF COTTON-SEED FLOUR IN EXPERIMENT 2, TRIAL 1

Item	Diet			
	Control	CSF8	CSF16	CSF24
ADG, kg				
Meal	.35	.32		
Pellet	.36 ^a	.32 ^{ab}	.28 ^{bc}	.22 ^c
Feed/gain				
Meal	2.34	2.34		
Pellet	1.96 ^a	2.02 ^{ab}	2.24 ^{bc}	2.28 ^c
ADFI, kg				
Meal	.81	.77		
Pellet	.70 ^a	.66 ^a	.62 ^a	.52 ^b
LER				
Meal	48.0	50.8		
Pellet	57.5	58.8	56.6	59.0

^{a,b,c} Means on the same line with different superscripts differ significantly ($P<.05$).

Means from trial 2 are shown in table 6. No differences among treatments in feed/gain or LER were found. ADG and ADFI were lower ($P<.05$) for the pigs receiving the CSF 8 and CSF 12 diets than for those receiving the control or CSF 4 diets.

TABLE 6. PERFORMANCE OF WEANLING PIGS FED VARIOUS LEVELS OF COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 2

Item	Control	CSF4	CSF8	CSF12
ADG, kg	.35 ^a	.36 ^a	.31 ^b	.32 ^b
Feed/gain	2.04	2.06	2.10	2.11
ADFI, kg	.72 ^a	.73 ^a	.66 ^b	.68 ^b
LER	51.1	50.7	49.6	49.5

^{a,b} Means on the same line with different superscripts differ significantly ($P < .05$).

Published data concerning LCP cottonseed flour in the diets of weanling pigs are not available for comparison with these results. Dyer et al. (1952) and Kornegay et al. (1961) found that CSM was not adequate for use as the only supplementary protein source for weanling pigs. Sewell et al. (1957) and Stephenson et al. (1965) demonstrated that CSM could replace 50% of the SBM in the diet without affecting weight gain. The data presented here indicated that 12.5% of the SBM could be replaced with cottonseed flour without affecting any of the criteria evaluated. However, when 25% of the SBM was replaced with cottonseed flour, ADFI and ADG were reduced in trial 2.

Although the results of experiment 1 indicated that the dietary levels of cottonseed flour used in experiment 2 were acceptable to the weanling pig, ADFI decreased linearly ($P < .01$) with increasing dietary levels of cottonseed flour in both trials. ADG was highly

correlated ($r = .86$ and $.95$ for trials 1 and 2, respectively) with ADFI. These data indicated that reduced consumption was a major factor in reducing gains from pigs fed higher levels of cottonseed flour. These results agree with Kornegay *et al.* (1961) and Clawson *et al.* (1975), who found that CSM or whole ground cottonseed would reduce feed intake of pigs.

Experiment 3

The performance of growing pigs in experiment 3 is shown in table 7. Pigs fed the diet containing 5% cottonseed flour performed as well as pigs on the control diet in all criteria evaluated. The ADG of pigs fed CSF 10 and CSF 15 were lower ($P < .05$) than the gains made by pigs fed the control diet. The LER value for the pigs fed the 10% CSM diet was lower ($P < .05$) than that of any other group. There were no differences in feed/gain or ADFI among treatments.

TABLE 7. PERFORMANCE OF GROWING PIGS FED VARIOUS LEVELS OF COTTON-SEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Item	Diet				
	Control	CSF5	CSF10	CSF15	CSM10
ADG, kg	.72 ^a	.68 ^{ab}	.60 ^{bc}	.54 ^c	.64 ^{ab}
ADFI, kg	2.23	2.14	1.98	1.82	2.20
Feed/gain	3.07	3.16	3.30	3.34	3.44
LER	48.5 ^a	49.4 ^a	49.2 ^a	50.7 ^a	42.8 ^b

^{a,b,c} Means on the same line with different superscripts differ significantly ($P < .05$).

The results of the finishing period of experiment 3 are shown in table 8. Although differences in initial weight existed, analysis of covariance indicated that these differences did not affect the criteria evaluated. No differences existed for ADG, feed/gain or ADFI. LER was higher ($P<.05$) for the pigs fed CSF 4 and CSF 12 than for the control. The LER values for the diets containing cottonseed flour were higher ($P<.05$) than that for the CSM diet.

TABLE 8. PERFORMANCE OF FINISHING HOGS FED VARIOUS LEVELS OF COTTON-SEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Item	Diet				
	Control	CSF4	CSF8	CSF12	CSM8
ADG, kg	.67	.74	.68	.64	.63
ADFI, kg	2.89	2.85	2.82	2.63	2.86
Feed/gain	4.28	3.88	4.14	4.14	4.57
LER	41.9 ^{ac}	48.0 ^b	47.0 ^{ab}	48.8 ^b	38.6 ^c

^{a,b,c}Means on the same line with different superscripts differ significantly ($P<.05$).

Haines et al. (1957) fed diets containing 5, 9.5, 15 and 20% CSM to growing and finishing pigs. These workers found no differences in ADG or feed efficiency for the diets containing 5, 9.5 or 15% CSM. ADG was reduced for the pigs receiving the 20% CSM diet. The results reported here indicated that diets containing 10 or 15% cottonseed flour reduced ADG of growing pigs. During the finishing

period, however, the diets containing 4, 8 or 12% cottonseed flour supported weight gains and feed efficiency which were similar to those produced by the SBM control.

Experiment 4

Gross and microscopic examination of the four control pigs slaughtered revealed only one lesion. This lesion was judged to be a nematode migration scar. Both microscopic and gross lesions were observed in all 16 pigs receiving the cottonseed flour or CSM diets. The lesions, which were confined to the liver, are shown grossly in figure 1 and microscopically in figure 3. The liver from a control pig is shown grossly and microscopically in figures 2 and 4, respectively.

Grossly, the livers of the affected pigs were spotted with yellowish-white, frequently hemorrhagic lesions ranging in size from one to several centimeters. The lesions were smooth with no indentation of the capsular surface.

Microscopically, the lesions varied from an inflammatory infiltration of the portal triads and interlobular septa to an active cirrhosis. The initial infiltration was composed of an inordinately large proportion of eosinophiles, which were distributed evenly throughout the portal triads and the interlobular spaces. Numerous round cells also were evident. Disruption of hepatic cords and of hepatocytes in the periportal and interlobular areas was present. Evidence of degenerative changes in the hepatocytes was observed.

Figure 1. Typical gross lesions observed in the livers of pigs fed cottonseed flour and CSM in experiment 4.

Figure 2. Normal liver from control pigs in experiment 4.

Figure 3. Typical microscopic section of advanced lesions from pigs fed cottonseed flour or CSM in experiment 4.

Figure 4. Microscopic section of normal liver from control pigs in experiment 4.

In the more advanced lesions, some histiocytes were present and what appeared to be the beginning of a fibroblastic proliferation was noted. Fibrosis was marked and hepatocytes were actively being isolated and destroyed by the invading fibroblasts. In addition, indications of bile duct proliferation were observed.

The etiology of the lesions described here is unknown. The occasional parasite migration scars observed were markedly different from the lesions described above. The fact that the lesions were confined initially to the portal triad and interlobular areas and that the eosinophiles were evenly distributed throughout the lesions indicates that the reactions were not due to parasite migrations.

The lesions observed in this experiment also were markedly different from those of gossypol toxicity, as described by Smith et al. (1972). The fundamental hepatic lesion of gossypol toxicity is centri-lobular necrosis, while the lesions observed here were primarily periportal and interlobular in origin. Also, degenerative changes of the heart are described as a symptom of gossypol toxicity. No such changes were observed in the pigs examined here.

Wood and Larson (1961) describe a hepatoma in rainbow trout which was later attributed to feeding CSM (Wolf and Jackson, 1963). Dunbar and Herman (1971) have attributed visceral granuloma, a disease of hatchery-reared brook trout, to the feeding of CSM. Extensive microbiological tests failed to isolate any microorganism. The addition of free gossypol to a synthetic diet failed to produce a significant increase in the incidence of lesions in these trout.

Although some similarities exist between the lesions described in fish and those reported here, it must be noted that comparisons between species so different can only be made with caution. The major significance of the references cited is the indication of toxicity from CSM which appears to be unrelated to gossypol. Further research is necessary to verify these results and to determine the causative agent.

General Discussion

The data from experiments 1, 2 and 3 indicated that the pigs did not always exhibit a preference for the diet which would sustain maximum performance. Although the pigs in experiment 1 showed a preference for a diet containing 10% cottonseed flour, pigs fed a similar diet in experiment 3 had weight gains which were lower than those of the control pigs. ADG and ADFI were lower for pigs fed an 8% cottonseed flour diet than for those fed the control diet in experiment 2, trial 2. These results agree with Wahlstrom et al. (1974).

The analyzed values for lysine content of the diets in trial 1 of experiment 2 and in experiment 3 were different from the calculated values. The lysine values became lower as the level of cottonseed flour in the diet increased. The effects of this relative lysine deficiency are unknown. The results of trial 2 of experiment 2, in which all diets were similar in lysine content, indicated that the differences in lysine content did not affect ADPI

or ADG. The relative lysine deficiency may have affected feed/gain.

Since the effects of the relative lysine deficiency on feed/gain were unknown, another measure of feed efficiency was needed. The availability of lysine is an important factor in determining cottonseed protein quality. The LER was calculated in an attempt to obtain an estimate of the relative availability of lysine from SBM, CSM and cottonseed flour. The LER is a modification of the protein efficiency ratio. Although composition of gain is not considered in this method, the LER should serve as an estimate of the relative availability of lysine for growth. It should be noted that the conditions under which these experiments were conducted did not allow for an accurate measure of feed wastage. Therefore, no attempt was made to adjust for feed wastage.

The data reported here indicated that the lysine from the cottonseed flour used was as available as that from the SBM. In experiment 3, CSM lysine was approximately 90% as available as SBM lysine. Abnormally high feed wastage, which may have affected LER, was observed with the pigs fed the CSM diets.

Nitrogen solubility was determined for the CSM and cottonseed flours. Solubility for the CSM nitrogen was 47.6%, while that of the flours ranged from 92.8 to 99.3%. These results indicated that more bound lysine was present in the CSM than in the flours. These results agree with those obtained by the LER.

When cottonseed flour was used at levels of 4 to 5% of the diet, growth rates and feed efficiencies of weanling, growing and finishing

swine were similar to their respective controls. Finishing hogs fed 8 or 12% cottonseed flour diets grew as fast and were as efficient as the controls. These performance data indicated that cottonseed flour is of acceptable quality to replace a portion of the SBM in swine diets.

The results of experiment 4 indicated that toxicity of CSM and cottonseed flour may still be the most limiting factor in their use in swine diets. The fibrosis of the liver associated with feeding cottonseed products in experiment 3 was severe enough to cause condemnation of the livers. Of more importance to the swine producer is the reduced growth rate and feed efficiency which would be expected from pigs with advanced lesions. Both cottonseed flour and CSM produced lesions in this experiment. The cottonseed flour used failed to meet the specifications for use in human diets. The factors which will cause this failure are: 1) free gossypol content greater than 450 ppm, 2) n-hexane content greater than 60 ppm, 3) arsenic content of greater than .2 ppm and 4) fat content of greater than 1% by weight. Gossypol and arsenic are known to produce hepatic lesions, but the lesions described here do not resemble those attributed to gossypol or arsenic (Smith et al., 1972). Aflatoxins are known to produce fibrosis in swine livers, but this fibrosis is normally accompanied by a centrilobular necrosis (Smith et al., 1972). Centrilobular necrosis was not characteristic of the lesions described here. In view of the fact that cottonseed flour is intended for human consumption, further research is necessary in

order to substantiate these findings and to determine the causative agent.

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APPENDIX

APPENDIX TABLE 1. CONSUMPTION (kg) BY REPLICATE OF EACH DIET
IN EXPERIMENT 1

Treatment	Diet	Replicate			
		1	2	3	4
Trial 1					
A	Control	9.93	5.58	7.03	1.76
	CSF10	14.47	19.68	11.29	10.88
B	Control	23.04	16.92	14.38	13.61
	CSF15	1.00	1.68	5.76	1.00
C	Control	25.49	22.00	13.70	11.61
	CSF18	.73	1.77	4.94	1.09
Trial 2					
D	Control	20.37	1.59	2.04	6.08
	CSF10	4.44	22.81	12.29	8.80
E	Control	14.74	18.87	10.88	9.62
	CSF14	6.67	3.90	6.53	6.89
F	Control	11.97	9.39	12.61	5.80
	CSF18	11.97	14.83	7.57	8.35

APPENDIX TABLE 2. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY GAIN OF WEANLING PIGS FED VARIOUS LEVELS OF COTTON-SEED FLOUR IN EXPERIMENT 2, TRIAL 1

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	20			
Total Reduction	7	0.004919	5.160	
Weight Group	1	0.004033	4.231	0.0948
Treatments	5	0.005273	5.531	0.0419
Remainder	5	0.000953		

APPENDIX TABLE 3. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY
FEED INTAKE OF WEANLING PIGS FED VARIOUS LEVELS OF
COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 1

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	12			
Total Reduction	7	0.019886	17.75	
Weight Group	1	0.019200	17.143	0.0090
Treatments	5	0.022073	19.708	0.0026
Remainder	5	0.001120		

APPENDIX TABLE 4. LEAST SQUARES ANALYSIS OF VARIANCE: FEED/GAIN
RATIO OF WEANLING PIGS FED VARIOUS LEVELS OF
COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 1

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	12			
Total Reduction	7	0.039489	4.53	
Weight Group	1	0.000675	0.078	0.7912
Treatments	5	0.055068	6.363	0.0317
Remainder	5	0.008655		

APPENDIX TABLE 5. LEAST SQUARES ANALYSIS OF VARIANCE: LYSINE EFFICIENCY RATIO OF WEANLING PIGS FED VARIOUS LEVELS OF COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 1

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	12			
Total Reduction	7	30.086829	5.402	
Weight Group	1	0.410700	0.074	0.7968
Treatments	5	41.999893	7.540	0.0224
Remainder	5	5.570080		

APPENDIX TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY GAIN OF WEANLING PIGS FED VARIOUS LEVELS OF COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 2

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	16			
Total Reduction	7	.006039	74.970	
Weight Group	3	.005342	66.310	.0000
Treatments	3	.001742	21.621	.0002
Remainder	9	.000081		

APPENDIX TABLE 7. LEAST SQUARES ANALYSIS OF VARIANCE: FEED/GAIN
RATIO OF WEANLING PIGS FED VARIOUS LEVELS OF
COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 2

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	16			
Total Reduction	7	0.052157	15.699	
Weight Group	3	0.037550	72.271	0.0021
Treatments	3	0.004117	11.303	0.3515
Remainder	9	0.003322	1.239	

APPENDIX TABLE 8. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY
FEED INTAKE OF WEANLING PIGS FED VARIOUS LEVELS OF
COTTONSEED FLOUR IN EXPERIMENT 2, TRIAL 2

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	16			
Total Reduction	7	0.019586	47.641	
Weight Group	3	0.041450	100.824	0.0000
Treatments	3	0.004217	10.257	0.0029
Remainder	9	0.000411		

APPENDIX TABLE 9. LEAST SQUARES ANALYSIS OF VARIANCE: LYSINE
EFFICIENCY RATIO OF WEANLING PIGS FED
VARIOUS LEVELS OF COTTONSEED FLOUR
IN EXPERIMENT 2, TRIAL 2

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	16			
Total Reduction	7	63.959411	29.548	
Weight Group	3	24.414175	11.279	0.0021
Treatments	3	2.519775	1.164	0.3760
Remainder'	9	2.164569		

APPENDIX TABLE 10. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY GAIN OF GROWING PIGS FED VARIOUS LEVELS OF COTTONSEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.013490	13.979	
Weight Group	1	0.033640	34.860	0.0041
Treatments	4	0.010385	10.762	0.0205
Remainder	4	0.000965		

APPENDIX TABLE 11. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE
DAILY FEED INTAKE OF GROWING PIGS FED
VARIOUS LEVELS OF COTTONSEED FLOUR
AND ONE LEVEL OF CSM IN EXPERIMENT
3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.158167	10.423	
Weight Group	1	0.650250	42.850	0.0028
Treatments	4	0.061365	4.044	0.1023
Remainder	4	0.015175		

APPENDIX TABLE 12. LEAST SQUARES ANALYSIS OF VARIANCE: FIELD/GAIN RATIO OF GROWING PIGS FED VARIOUS LEVELS OF COTTONSEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.048590	5.273	
Weight Group	1	0.104040	11.290	0.0283
Treatments	4	0.042875	4.653	0.0828
Remainder	4	0.009215		

APPENDIX TABLE 13. LEAST SQUARES ANALYSIS OF VARIANCE: LYSINE
EFFICIENCY RATIO OF GROWING PIGS FED
VARIOUS LEVELS OF COTTONSEED FLOUR
AND ONE LEVEL OF CSM IN EXPERI-
MENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	16.744073	9.117	
Weight Group	1	21.257640	11.574	0.0272
Treatments	4	18.686140	10.174	0.0226
Remainder	4	1.836640		

APPENDIX TABLE 14. LEAST SQUARES ANALYSIS OF VARIANCE: INITIAL WEIGHT OF FINISHING HOGS FED VARIOUS LEVELS OF COTTONSEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	68.3910	31.611	
Weight Group	1	331.7760	153.352	0.0002
Treatments	4	19.0665	8.813	0.0290
Remainder	4	2.1635		

APPENDIX TABLE 15. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE DAILY GAIN OF FINISHING HOGS FED VARIOUS LEVELS OF COTTONSEED FLOUR AND ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.005527	5.611	
Weight Group	1	0.016810	17.066	0.0145
Treatments	4	0.003785	3.843	0.1103
Remainder	4	0.000985		

APPENDIX TABLE 16. LEAST SQUARES ANALYSIS OF VARIANCE: FEED/GAIN
OF FINISHING HOGS FED VARIOUS LEVELS OF
COTTONSEED FLOUR AND ONE LEVEL OF CSM
IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.096050	3.056	
Weight Group	1	0.049000	1.559	0.2799
Treatments	4	0.124535	3.963	0.0054
Remainder	4	0.031425		

APPENDIX TABLE 17. LEAST SQUARES ANALYSIS OF VARIANCE: LYSINE
EFFICIENCY RATIO OF FINISHING HOGS FED
VARIOUS LEVELS OF COTTONSEED FLOUR AND
ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	26.950667	7.416	
Weight Group	1	4.624000	1.272	0.3224
Treatments	4	39.206000	10.789	0.0204
Remainder	4	3.634000		

APPENDIX TABLE 18. LEAST SQUARES ANALYSIS OF VARIANCE: AVERAGE
DAILY FEED INTAKE OF FINISHING HOGS FED
VARIOUS LEVELS OF COTTONSEED FLOUR AND
ONE LEVEL OF CSM IN EXPERIMENT 3

Source	Degrees of Freedom	Mean Squares	F-Value	Probability of Greater F
Total	10			
Total Reduction	6	0.114593	2.928	
Weight Group	1	0.479610	12.255	0.0249
Treatments	4	0.022285	0.569	0.7006
Remainder ^a	4	0.039135		

APPENDIX TABLE 19. ANALYSIS OF COVARIANCE: INITIAL WEIGHT WITH AVERAGE DAILY GAIN FOR THE FINISHING PERIOD OF EXPERIMENT 3

Source of Variation	df	Sum of Products			Y adjusted for X	F
		x, x	x, y	y, y	Mean Square	
Total	9	416.696	2.8548	.03589		
Blocks	1	331.776	2.3616	.01681		
Treatments	4	76.266	2.3143	.01514		
Error	4	8.654	-1.8211	.00394	-.12642	
Treatments + Error	8	84.920	.4932	.01904		
Treatments Adjusted					.09886	.782

APPENDIX TABLE 20. ANALYSIS OF COVARIANCE: INITIAL WEIGHT WITH AVERAGE DAILY FEED INTAKE
FOR THE FINISHING PERIOD OF EXPERIMENT 3

Source of Variation	df	Sum of Products			Y adjusted for X		
		x,x	x,y	y,y	df	Mean Square	F
Total	9	416.696	15.6618	.72519			
Blocks	1	331.776	12.6144	.47961			
Treatments	4	76.266	2.1718	.08914			
Error	4	8.654	0.8756	.15644	3	.022616	
Treatments + Error	8	84.920	3.0474	.24558	7		
Treatments Adjusted					4	.017	.756

APPENDIX TABLE 21. ANALYSIS OF COVARIANCE: INITIAL WEIGHT WITH FEED/GAIN FOR THE FINISHING PERIOD OF EXPERIMENT 3

Source of Variation	df	Sum of Products			Y adjusted for X	Mean Square	F
		x,x	x,y	y,y			
Total	9	416.696	5.2168	.67284			
Blocks	1	331.776	4.0320	.04900			
Treatments	4	76.266	.7812	.49814			
Error	4	8.654	.4036	.12570	3	.0356257	
Treatments + Error	8	84.920	1.1848	.62384	7		
Treatments Adjusted					4	.1251081	3.512

APPENDIX TABLE 22. CRUDE PROTEIN, GROSS ENERGY AND LYSINE CONTENT OF THE DIETS FED IN EXPERIMENT 2

Trial	Diet	Diet form	Crude protein			Gross energy (Kcal/kg)	Lysine	
			As fed	Dry matter basis			Calculated	Analyzed
1	Control	Meal	20.08	23.14		4310	.96	.89
	CSF8	Meal	20.95	23.98		4205	.96	.84
	Control	Pellet	19.22	21.54		4220	.96	.89
	CSF8	Pellet	21.39	23.96		4272	.96	.84
	CSF16	Pellet	20.07	22.62		4230	.96	.79
	CSF24	Pellet	22.14	24.58		4486	.96	.75
2	Control	Meal	20.55	23.24		4313	.96	1.02
	CSF4	Meal	22.09	24.79		4098	.96	1.02
	CSF8	Meal	22.21	24.91		4098	.96	1.01
	CSF12	Meal	22.69	25.40		4452	.96	1.01

APPENDIX TABLE 23. CRUDE PROTEIN, GROSS ENERGY AND LYSINE CONTENT OF THE DIETS FED IN EXPERIMENT 3

Period	Diet	Crude protein		Gross energy (Kcal/kg)	Lysine	
		As fed	Dry matter basis		Calculated	Analyzed
Growing	Control	17.00	19.47	4405	.70	.67
	CSF5	19.28	21.62	4275	.70	.64
	CSF10	19.77	22.23	4304	.70	.62
	CSF15	16.89	18.91	4362	.70	.59
	CSM10	19.65	22.12	4330	.70	.68
Finishing	Control	15.58	17.74	4360	.57	.56
	CSF4	16.92	19.32	4402	.57	.54
	CSF8	15.36	17.47	4295	.57	.52
	CSF12	15.86	17.94	4348	.57	.49
	CSM8	18.48	21.01	4351	.57	.57

APPENDIX TABLE 24. VITAMIN AND TRACE MINERAL LEVELS SUPPLIED BY
PREMIX USED IN EXPERIMENT 3^a

Vitamins	Units per kg. of diet	Trace minerals	PPM of diet
Vitamin A	2640.0	Iron	50
Vitamin D	352.0	Copper	5
Riboflavin	2.6	Manganese	27.5
Ca pantothenate	10.6	Zinc	100.0
Niacin	7.2	Iodine	.75
Choline	88.0		
Vitamin B ₁₂	11.0		

^aCorresponding values for experiments 1 and 2 were 1.5 times higher.