A STUDY OF THE TOXICITY OF GOSSYPOL

THESIS FOR THE DEGREE OF M. S.

Donald E. Wilcox

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THESIS

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Thesis

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INTRODUCTION

Economical milk production in regions where alfalfa hay is not readily grown depends to a large extent upon the cost of protein concentrates needed to balance the ration. In Michigan, although alfalfa is increasing in favor, much of the hay that is fed is low in protein, and lack of sufficient protein in the ration is very often a limiting factor in milk production.

Ordinarily cottonseed meal is the cheapest high protein concentrate that can be purchased, and the biological value of its protein has been shown by feeding experiments to be relatively high. Its use as a cattle feed has been somewhat limited, however, because its use in fairly large amounts is thought to produce injury. Several experiment stations and some of our leading texts on cattle feeding recommend the use of cottonseed meal only in limited amounts.

Cottonseed meal injury has been attributed to the presence in it of chloline, betaine, pyro-phosphoric acid, bacteria and molds, protein decomposition products and gossypol.

The injury has also been attributed to deficiencies in the rations of which cottonseed meal is a part such as, a lack of vitamin B, vitamin A, iron, calcium, and a factor or factors carried by good quality hay.

Gossypol has been found to be very toxic where fed to some species of animals. There is no direct evidence, however, to show whether or not it is toxic to dairy cattle when fed in fairly large amounts with a ration that is otherwise adequate.

The purpose of this investigation was to study the toxicity of gossypol to growing dairy heifers, when fed, as it naturally occurs, in raw cottonseed kernels. A ration which supplies all of the known factors essential for growth, maintenance and health was fed in conjunction with the raw cottonseed. An attempt was also made, using rats, to determine some facts regarding the physiological effects of feeding gossypol as they are affected by various additions to an otherwise normal ration. A study of the occurrence of gossypol in raw cottonseed and in cottonseed meal is also included.

REVIEW OF LITERATURE

The use of cottonseed and cottonseed products as food dates back approximately one hundred years. The Royal Society of Arts of London (1) in 1783 offered medals to the person who would patent a process for expressing the oil from the seed of cotton and make of the remaining seed, hard and dry cakes as food for cattle. The offer required that one ton of oil should be expressed and 500 pounds of dried cake be obtained to win the gold medal. For the preparation of one half as much material a silver medal was offered. Probably the large quantities required defeated the purpose for although the premiums were offered for six years no process was presented.

The South Carolina Agricultural Society (2) in 1785 offered a premium for the patent of a process for the extraction of oil from cottonseed. However, it was not until 1818 that Daniel Gillet of Springfield, Massachusetts (2) patented a process for preparing food from cottonseed. The following year a process for extracting oil from the seed was patented by George P. Digges of Virginia.

No doubt cottonseed was used as a food for cattle to a limited extent before cottonseed meal was placed on the market. It was not until about 1850, however, that cottonseed products became commercially important as food products. Prior to the Civil War there were but seven cottonseed mills in the south. Since that time the growth of the cottonseed oil and cottonseed meal industry has been very rapid. Cottonseed, formerly almost wholly wasted, now is made up into products having a yearly value estimated at $250,000,000 (3). Approximately one-third of this

amount is obtained from cottonseed oil meal and cottonseed cake.

Manufacture of Cottonseed Meal

The process of manufacture of cottonseed meal may have a marked influence on the presence of gossypol in the finished product due to the effect of heat and pressure in converting the "free gossypol to a product known as "bound gossypol" or "D-gossypol" a substance much less toxic than the original form. For this reason a short description of the process of cottonseed meal manufacture is included here.

The cottonseed on reaching the oil mills is first screened to remove sand, dirt, bolls, and foreign substances, and finally a draft of air is used to complete the cleaning process. The seed is now ready for the linters. These machines are an elaboration of the ordinary cotton-gin; and whatever staple remains upon the seed is stripped off in passing through them. From the linters the seed passes to the huller, a high speed cutting machine which cuts it up thoroughly. The hulls, by screens and heaters are now separated from the meats, which latter are, by screw conveyors, conducted to bins contiguous to roller crushers, and as fast as required are passed through the crushers where the mass is reduced to a uniform consistency, and is known to millmen as "uncooked meal".

In the manufacture of cottonseed meal from this raw product the raw kernels are usually cooked before the oil is expressed, to increase the yield of oil. The details of the cooking vary to a considerable extent in different mills.

A few mills use the "expeller", a cold-press method in which the decorticated raw kernels are usually dried in a pre-heater and are then passed into the expeller proper. In the expeller they are forced by pressure through small orifices, this operation subjecting them to a grinding action which squeezes out the oil. Both the dry heating and the grinding action are important in connection with conversion of gossypol to bound gossypol or D-gossypol in the cottonseed meal.

Some of the mills use the open kettle process in the manufacture of the meal. In this method the decorticated cottonseed kernels are agitated in large steam-jacketed kettles until they are more or less thoroughly comminuted. The time of cooking and the steam pressure (temperature) in the jacket vary greatly. After cooking the kernels pass to a cake former and then to a hydraulic press where the oil is expressed. The press cake is then ground to a fine meal.

The continuous-cooker which is now used by a majority of mills, consists of three to five steam jacketed drums or stacks placed one above the other. The raw kernels enter the upper stack and proceed downward as those below are emptied. There is considerable variation in the time required for the kernels to pass through the stacks and also the steam pressure used in the stacks at the various mills. In either of the latter methods water or steam may be added or a current of air passed through the cooker in order to regulate the moisture content of the kernels.

The variation in method of manufacture of cottonseed meal as well as variations in temperature, pressure and moisture content within one process as used by different mills may have a marked influence on the gossypol content or the condition of the gossypol in the finished cottonseed meal.

Feeding Experiments with Special Reference to Cottonseed Meal Injury

Numerous investigations have been conducted in the past fifty years dealing with the use of cottonseed products as a food. They have included a study of the biological value of its protein, its replacement value in the ration as compared with other feeds, its effect on the quality and quantity of butterfat produced when it has been fed to dairy cows, and also a study of their injurious effect when fed in large amounts.

Only those investigations having a direct bearing on cottonseed meal injury will be reported.

Voelker (4) seems to have given the first published report of the injurious effects which occurred from feeding cottonseed meal. In 1872 he reported cases of injury to sheep and cattle in England which was attributed to the use of cottonseed meal. Other cases were reported in Europe and in England a few years later, but it was not until about 1890 that the problem came under investigation in this country.

<u>Experiments with Cattle</u> Stone (5) in 1889 reported that the practice of feeding cottonseed hulls seems to have begun as early as 1870, soon after the introduction of the oil industry. Feeding hulls and cottonseed meal as an exclusive diet for cattle was started about 1885. No harmful effects from feeding 25 to 30 pounds of hulls and five to eight pounds of cottonseed meal daily had been reported up to the time the report was published.

Wright (6) fed 2000 steers and heifers weighing from 850 to 950 pounds for a period of three months (some of them longer) on a ration composed of cottonseed hulls and cottonseed meal. Eight pounds of meal and 25 pounds

of hulls were fed per day. Hay was fed once a week and oftener if scouring developed. The average gain in weight was about 75 pounds per month. About one per cent of the cattle scoured and three of the animals died. Death was attributed to pleure pneumonia.

Lloyd (7) fed six lots of 10 cows each for 12 weeks (11 weeks on experiment) on rations in which cottonseeds, cottonseed meal, and corn were fed in conjunction with Bermuda hay and mixed hay. The cows used were "natives" and grade Jerseys. The feed eaten and the milk produced is summarized below:

	Lot I. Bermuda Hay Cottonseed	Lot II. Mixed Hay C. S.	Lot III. Bermuda Hay C.S.M.	Lot IV. Mixed Hay C.S.M.	Lot V. Bermuda Hay Corn	Lot VI. Mixed Hay Corn
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Hay	9.4	8.5	14.2	12.0	12.2	9.8
Cotton Seed	8.6	9.8	-	-	-	-
C.S. Meal	-		9.7	9.9		
Corn					9.9	9.75
Milk Produced	8.5	7.9	10.9	9.9	8.8	8.5

The milk production is very low in all cases but is probably in part due to lack of producing ability on the part of the native cows. During the 12 weeks that the cows were fed on these rations no injurious results were noted.

Chamberlain (8) fed four steers for an 84 day feeding period on a ration of cottonseed hulls, "ad libitum" (15-20 lbs. per day) and cottonmeal 3-5 pounds per day. At the end of the trial they were still growing and appeared healthy but there were some indications that the digestion of the animals had been impaired.

Tietze (9) in 1893 reported investigating several cases in which the death of calves was attributed to the feeding of cottonseed meal. The calves received three heaping liters per day of cottonseed meal in addition to skim milk, hay, and linseed cake.

Emery (10) one year later reported an investigation in which three calves were fed skim milk to which one-fourth to one-half pound of cottonseed meal was added. The three calves died in from four to six weeks. The death of two of the calves was attributed to a toxic principle in the cottonseed meal.

Gautier and Larsen (11) in 1895 reported a number of cases of poisoning of calves which were directly traceable to the eating of cottonseed meal.

Emery and Kilgore (12) fed four oxen on a ration composed entirely of cottonseed meal and cottonseed hulls. Large amounts of both cottonseed meal and cottonseed hulls were consumed. At the end of 92 and 135 days of feeding there was no injury noticeable. Fairly good gains were made as is shown in the summary below.

No. of	Cottonseed Meal Consumed per day	Cottonseed Hulls Consumed per day	Period days	Gain in Weight pounds
1	9.3	17.2	92	150
2	18.0	15.6	92	155
3	9.6	14.1	135	185
4	8.3	12.4	135	240

Connell and Kyle (13) of the Texas Station reported in 1899 that steers which were fed 20 pounds of cottonseed hulls and 4.5 pounds of cottonseed meal for a period of 100 days made fair gains and showed no

injurious effects. They reported also the results of a large number of farmers and feeders on the use of cotton seed and cottonseed hulls. No injurious effects were reported but the majority of feeders fed the cotton seed only for short periods of time.

Rust (14) in 1905 reported observing peculiar toxic symptoms in draft oxen which were receiving two pounds of cottonseed meal per day. He describes the condition as follows:

"Edematous swelling appeared at the extremities, the appetite being undisturbed. In later stages weakness of the hind quarters appeared and in single animals disturbances of equilibrium. Four of 15 affected oxen showed disturbances of vision. Apparently they became completely blind as was evidenced by their groping gait, and by their colliding with other animals and surrounding objects. Examination of the eyes revealed no special lesions except marked protrusion of the eye ball and abnormal enlargement of the pupil. The most severely affected animals were slaughtered, and the remainder recovered after administration of laxatives and alteration of the diet including discontinuance of the use of cottonseed meal."

Michels and Burgess (15) after reviewing the literature bearing on cottonseed meal feeding concluded that when the health of cows is unfavorably influenced it is in all probability due to one of the following causes:

(1) Feeding the meal in conjunction with unsuitable roughage; (2) feeding it in a stale or musty condition; and (3) feeding it in excessive quantity. They believed that derangements of the animals' system results from feeding cottonseed meal with cottonseed hulls where the latter forms an exclusive roughage. Such a ration they point out is unbalanced, con-

taining an excess of nitrogenous matter, and this one-sidedness combined with the woody indigestible nature of the hulls is responsible for the ill health that sometimes follows its use. They observed no ill effects from feeding cottonseed meal as one-half or three-fourths of the grain mixture when the roughage used was corn or sorghum silage. Five to six pounds per day of cottonseed meal was sometimes fed.

McNutt (16) noted the unthrifty condition of four calves fed on a grain mixture of cottonseed meal, oats, and wheat bran as contrasted with another lot of calves fed on a grain mixture of corn, oats, and wheat bran. Both lots received the same amount of corn silage and hay. The calves which appeared unthrifty were under ten months of age. In the same lot two other calves made normal gains. These results were held to indicate that cottonseed meal should not be fed to calves under ten months of age.

In connection with the same investigation a bull calf was fed on an exclusive diet of cottonseed meal and skim milk. One-fourth of a pound of cottonseed meal was fed at the start and this amount was gradually increased until 1.5 pounds was being fed. The calf died 71 days after the feeding of cottonseed meal was started.

Dinwiddie and Short (17) in 1911 reported an investigation in which two, four hundred pound Jersey steers were fed on a ration of cottonseed meal and cottonseed hulls. The cottonseed meal was fed at the rate of two pounds per day at the start of the experiment and this amount was increased to four pounds during the second month. After 70 days on the experiment the animals lost their appetite and started to lose weight. Symptoms of injury noted were; an unsteady and reeling gait; the defects of locomotion appearing in all four limbs. Blindness occurred in both

steers and one animal died. The erythrocyte count of the animal that died was below normal, 3,400,000 per cubic mm. The hemoglobin was 50 per cent normal.

Moore (18) in 1914 started an investigation to determine whether or not the use of cottonseed meal and the use of cottonseed hulls were injurious to dairy cows if fed in large amounts and if so the nature and extent of the injuries. Three lots of five heifers each were used in the experiment.

Lot I received a heavy ration of cottonseed meal with very little other grain feed. Hay formed the roughage portion of the ration. No cottonseed hulls were fed.

Lot II received no cottonseed meal but cottonseed hulls were fed as a roughage.

Lot III received no cottonseed products.

The cows never received more than 25 pounds of cottonseed hulls nor more than five pounds of cottonseed meal per day.

In Lot I which received nearly five pounds of cottonseed per day throughout six lactations there were 14 cases of garget, one cow lost two quarters of her udder and two others lost one quarter each; there were three cases of retained afterbirth; one calf was born dead and another was very weak at birth. In Lot II which received approximately 15 pounds of cottonseed hulls per day there was one case of garget, one quarter of an udder was lost, and there was one abortion.

The animals in Lot III except for two slight cases of garget were normal throughout the experiment and produced normal calves.

This investigator concluded that the feeding of five pounds of cottonseed meal for a considerable length of time was injurious to the dairy cow, causing inflammation of the udder, difficult breeding, and probably having a tendency to cause retention of the afterbirth. The use of cottonseed hulls as a roughage where cottonseed meal was not fed did not seem to have as pronounced injurious effects. No mention was made of the kind or quality of hay used in this experiment but it was concluded in this experiment that where silage is fed in the winter and where good pastures are available in the summer that as much as four pounds per day of cottonseed meal can be safely fed to dairy cows.

At the North Carolina Station (19) an extensive series of investigations were carried out in which it was shown that the heavy feeding of cottonseed meal to dairy cows does not produce injurious effects if it is fed in conjunction with hay and silage. This series of investigations is reported in detail under the section "Theories of the Cause of Cottonseed Meal Injury".

The work of Huffman and co-workers (20) (21) at the Michigan Station where the heavy feeding of cottonseed meal with a good quality of roughage has proven very successful is reported in detail under the same head.

Moore and Huffman (22) have shown that cottonseed meal is as laxative as linseed oil meal when fed as the principle source of protein to milking cows. It had formerly been supposed that cottonseed meal was constipating in effect.

<u>Experiments with Sheep</u> Voelker's (4) report of the death of 5,006 sheep and lambs that was alleged to have been caused by eating decorticated cottonseed cakes is the earliest report noted with regard to

feeding sheep cottonseed products and the possible injury of such a practice.

Von Nathausius (23) observed for several years that the uterus of ewes, which had been fed considerable quantities of cottonseed meal, immediately after lambing became highly inflamed, and the sheep soon died. It is stated that only those animals eating American cottonseed were affected and when the use of the meal was stopped the trouble soon disappeared.

Esser (24) reported the death of about 100 fattening lambs after a few days feeding on 250 grams of cottonseed meal used as an auxiliary food. The meal seemed of good quality and was fed to oxen without injury.

Ladd (25) reported that four mature sheep which were started on a feeding experiment in which the grain ration was a mixture of equal parts wheat bran and cottonseed meal died after six weeks of feeding. No mention was made of the type of roughage used.

More recent experiments by Jones and Dickson (26) have shown that one-half pound or more of cottonseed meal with cottonseed hulls as the roughage can be fed to fattening lambs for a period of 70 days without producing any ill effects.

Gray and Ridgway (27) reported having fed 65 pregnant ewes through the winter (from 63 to 210 days) on a ration of cottonseed meal and cottonseed hulls. No impairment in health or in reproduction was observed except for one case of blindness which occurred.

Cox (28) reports a series of investigations from the New Mexico Station where fattening lambs were fed on rations containing various amounts of cottonseed meal and cottonseed hulls. These investigations cover three years' work which is summarized below:

50 Lambs per Lot 95 days

		Lot I. Corn $\frac{3}{4}$ lb. C.S.M. $\frac{1}{4}$ lb. Alfalfa Hay	Lot II. C.S.M. $\frac{1}{2}$ lb. C.S.Hulls	Lot III. C.S.M. $1\frac{1}{4}$ lb. C.S.Hulls	Lot IV. C.S.M. 1 lb. C. S. Hulls	Lot V. C.S.M. 1 lb. Cane Stover
Feed						
Deaths Attributed to feeding	1st year	0	0	1	0	0
	2nd "	0	1	2	2	0
	3rd "	0	0	1	1	0
	total	-	1	4	3	0

Feed		C.S.M. Corn Stover	C.S.M. Alfalfa Hay
Deaths Attributed to feeding	1st year	1	0
	2nd "	0	1
	3rd "	0	0
	total	1	1

It will be seen that 70 per cent of the losses occurred in the lots that were receiving one pound per day of cottonseed meal fed with cottonseed hulls. These animals became sick about 70 days after the start of the experiment. They became droopy and refused to eat. The eyes often became glazed in appearance. After the second day of the sickness the animals gave indications of considerable abdominal pain. In several instances lambs which showed these symptoms were removed from the experiment and placed on alfalfa pasture or fed alfalfa hay. Recovery was complete in every case.

Post mortem examinations of animals that died showed an accumulation of serum in the abdominal cavity, inflammation and congestion in the abomasum and small intestine. Also, congestion in lymph glands, kidneys, and liver. The urine was extremely acid.

Experiments with Horses and Mules   Emery (10) in 1894 reported that two pounds daily of cottonseed meal could be fed to horses with good success. Although the feeding of cottonseed meal to horses was not generally practiced then, no injurious results had been reported.

Connell and Kyle (13) report three instances where the continued use of cotton seeds and cottonseed meal for horses and mules ultimately had bad effects. These reports were from farmers in Texas. Other farmers reported that they had used it in limited amounts with fair success.

Burkett (29) in a series of experiments at the North Carolina Station found that two pounds of cottonseed meal could be included in the rations of horses without producing any ill effects and that under North Carolina conditions it was a very economical feed.

Templeton (30) carried on investigations in which he supplemented the rations of working mules with cottonseed meal at the rate of 1.1 pounds per 1000 pounds of live weight. Some difficulty was experienced in getting the mules to eat the cottonseed meal, but its inclusion in the ration had no effect on the health, spirit, or endurance of the animals to which it was fed.

Bell and Williams (31) conducted an experiment in which they fed cottonseed meal to nine work horses in a grain mixture of oats, bran, and cottonseed meal. The roughage used was oat hay. Seven team mates of these horses received the same ration except that no cottonseed meal was fed. The amount fed varied somewhat. One pound daily for every 1000 pounds live weight was found to be the most satisfactory level. No reproductive troubles were noted in the mares which received the cottonseed meal. They became safe with foal readily and dropped normal healthy foals.

One mare that received three pounds of cottonseed meal died after 46 days on the experiment. Post mortem examination showed an inflamed condition of the stomach and intestines. These indications seemed to point to cottonseed meal as the causative agent of the injury.

It was noted that the mares thrived better and ate their ration containing cottonseed meal more readily on pasture than they did when they were stall fed.

Experiments with Swine   Curtis and Carson (32) in 1892 reported that during trials the year previous the fatalities due to feeding cotton seed were numerous. Six to seven weeks after the pigs were placed on trial they died of cottonseed meal injury. Boiling the raw seed before feeding seemed to reduce the toxicity somewhat as indicated by fewer deaths. They describe the symptoms as follows: The first sign of sickness appearing in from six to eight weeks after cottonseed meal is added to the ration, is a moping dullness of the animal with loss of appetite and tendency to lie apart. Within the course of 12 to 36 hours, often within shorter time, the animal becomes restless, staggering in his gait; breathing, labored and spasmodic; bare skin showing reddish inflammation; sight defective, and both nervous and muscular systems feeble and abnormal in action. The fatal cases all show "thumps", spasmodic breathing, and in many instances the animal will turn in one direction only. Those pigs which do not die become stunted and do not grow.

Emery (10) reports a case where two pigs which had received skim milk were placed on a cottonseed meal experiment. One pig received cottonseed meal and wheat bran, the other received corn meal and wheat bran. After six weeks on these rations the cottonseed meal was increased to two pounds per day and the pig receiving this amount soon lost appetite and

refused to eat. Replacing the cottonseed meal with corn resulted in the pig's recovery.

Carrcevin (33) observed that hogs which were fed Egyptian cottonseed and cottonseed cake were poisoned very severely. He injected a cold water extract of the seed into a dog and caused death in 18 hours. He observed that the kernels were much more poisonous than the hull and that commercial cottonseed oil was harmless.

Connell and Kyle (13) in 1899 reported the experience of a number of Texas farmers who had fed cotton seed or cottonseed meal to hogs. Where uncooked cotton seed was fed in dry lot the results were in almost every case disastrous. The only favorable results which were reported were in cases where cooked seed was used or where the hogs were allowed to run on pasture.

Dinwiddie (34) fed cottonseed meal in combinations with corn, with bran, with cane and turnips, and with bran, corn and cow pea hay. Three pigs receiving cottonseed meal one part and corn three parts, died after 38 days. Each pig at the time of death had consumed 23 pounds of cottonseed meal. The ration having turnips in addition to corn and cottonseed meal did not prove successful. Where bran was substituted for corn there was a marked improvement. Where bran was fed with cottonseed meal as much as 1.4 pounds per day was fed without producing harmful results. A sow was fed for the last 80 days of her gestation period on a ration containing cottonseed meal. She received 1.3 pounds per day without harm to herself or her litter.

Crude cottonseed oil was fed to three pigs for 20 weeks in amounts exceeding that which is contained in a fatal ration of cottonseed meal. No harmful effects were noted.

Characteristic post mortem findings of pigs dying from cottonseed meal injury were in all cases an acute dropsy of the pleural and heart sacs with intense congestion of the liver and kidneys. The immediate cause of death was thought to be due to suffocation from compression of the lungs.

Georgson, Burtis and Otis (35) used cottonseed meal with corn meal as one-fourth and one-half of the ration. The results in both cases were disastrous, the pigs dying in from three to eight weeks after going on trial. The larger pigs seemed the more resistant. In all cases post mortem examination revealed severe inflammation and congestion of the intestines, lungs, and heart. Up to the time that the symptoms of cottonseed meal injury set in the gains had been very good.

Curtis (36) fed cottonseed meal with corn and cob meal and butter milk to pigs weighing about 100 pounds. The pigs made good gains for about six weeks and then became fatally sick. In every case the amount of cottonseed meal consumed before death occurred was from 27 to 33 pounds. Pigs following cattle that were fed from four to seven pounds of cottonseed meal showed no injurious effects in 17 weeks, the duration of the trial.

Duggar (37) found that .25 to .61 pounds of cottonseed meal daily per 100 pounds of live weight caused sickness and death in pigs in five to seven weeks. Yellow corn formed 75 per cent of the ration. Green pasture tended to alleviate the toxicity somewhat.

Marshall (38) reported experiments in which he mixed cottonseed meal with corn chops and after adding water allowed the mixture to ferment until it was thoroughly sour. The fermented mixture was fed to fattening pigs weighing from 100 to 130 pounds. Where the ration con-

tained one-third cottonseed meal the pigs did well for 74 days. After that time one animal died and one other refused to eat well. One lot which received fermented cottonseed meal as one-half of the ration did well for 60 days but after that time three hogs out of ten died and another became chronically sick and lost weight. Up to seven pounds of the mixture of corn and cottonseed meal was fed daily, or 3.5 pounds of cottonseed. It was concluded that fermenting cottonseed reduces but does not entirely eliminate its toxicity.

Curtis (39) fed fermented cottonseed meal to pigs as 20 per cent of the ration for 90 days with very good results. After that time the gains were not as good as they had been previously but the animals apparently remained in good health for a month longer, when the trial was finished. An average of .62 pounds of cottonseed meal was fed during the entire period.

Walker (40) in 1916 reported that the addition of copperas to a ration of three parts of corn to one part of cottonseed meal prevented injury to pigs.

Withers and Brewster (41) had previously shown that iron was effective as an antidote to cottonseed meal poisoning in rabbits.

Withers and Carruth (42) later reported that the addition of iron in the form of $Fe\ cl_2$ or copperas to a ration containing cottonseed meal greatly reduced the fatal effects. Out of a total of 18 pigs getting the iron, four died while out of 18 not getting iron 13 died.

Rommel and Vedder (43) in 1915 and Wells and Ewing (44) in 1916 reported evidence to show that cottonseed meal injury in pigs is due to the feeding of a deficient diet. These investigations will be reviewed later in the section "Theories of the Cause of Cottonseed Meal Injury."

Goldberg and Maynard (45) reported an experiment in which 12 pigs previously innoculated against cholera weighing from 30 to 50 pounds each were fed on a ration made up of 60 per cent corn meal, 10 per cent wheat middlings, 25 per cent cottonseed meal and five per cent molasses. A mineral mixture was fed "ad libitum".

Nine of the pigs died within eight to 12 weeks from the beginning of the trial. The usual symptoms observed were failure to eat; vomiting; rapid, short shallow breathing; anemia, and a weakness of the leg muscles. A very complete post mortem report was included in the investigations. Particular emphasis was placed on a study of the lesions found. They found a generalized edema throughout the body. The initial lesion in cottonseed meal injury they concluded is ascites.

Barnett and Goodell (46) fed three pigs a ration of two parts corn to one part cottonseed meal for 28 days without producing any noticeable injury. 2.3 pounds of cottonseed meal was consumed daily per pig.

Warren and Williams (47) fed cottonseed meal with milo chops and with milo chops and tankage for a period of 120 days. In one lot of nine pigs where cottonseed meal formed 11 per cent of the ration there was one death from cottonseed meal injury on the 70th day of the experiment. The other pigs showed no symptoms of injury at any time.

Bohstedt and co-workers (49) at the Ohio Station reported that a pig receiving 20.2 per cent cottonseed meal in its ration died on the 120th day of the experiment. Two pigs receiving 25 per cent cottonseed meal died on the 67th and 78th days of the experiment. Post mortem examination in all cases showed marked hemorrhagic condition of the mesentery.

Hale (48) has reported a series of investigations on cottonseed meal for swine which have been carried on at the Texas Station. It is concluded from the results obtained that where not more than nine per cent of the ration is made up of cottonseed meal it can be fed safely to brood sows, boars, growing pigs, and suckling pigs without producing any harmful results. Cottonseed meal fed at a 15 per cent level resulted in a loss of three pigs out of 20 which on post mortem examination showed the characteristic symptoms of cottonseed meal injury. There were no losses from cottonseed meal injury in a lot of 20 pigs fed a ration containing 12 per cent cottonseed meal but they did not look as thrifty nor make as good gains as pigs which received only nine per cent cottonseed meal in their ration. Ground raw cottonseed proved very unsuccessful when used as a protein supplement for 54 pound pigs. As 40 per cent and as 25 per cent of the ration it proved unpalatable and caused scouring on the 18th day of the experiment. After 20 days on the trial the pigs were removed but two of the five pigs died from cottonseed poisoning on the 26th and 28th days after the start of the experiment.

<u>Experiments with pigs following steers receiving cottonseed meal</u>

Georgeson, Burtis and Otis (35) report a case where 40 pigs following steers fed on cottonseed meal died in six or seven weeks showing the same symptons of poisoning as those which were fed cottonseed meal directly.

Soule and Fain (50) reported that of 12 hogs following steers which received 3.23 pounds of cottonseed meal per day, one died. The cause of death was not definitely found to be cottonseed meal poisoning, however. The remaining eleven made fairly good gains over a period of 180 days.

Experiments with Poultry   Roberts and Rice (51) in 1897 observed that the eggs from hens fed on a nitrogenous ration one-third of which was cottonseed meal were small in size, had a dark colored yolk, and a red watery albumin. The eggs were also observed to have poor keeping qualities as compared with eggs from hens which were receiving a carbonaceous ration which contained no cottonseed meal.

Thompson (52) fed cottonseed meal to laying hens as 50 per cent of a ration composed of oats, bran, and cottonseed meal. The health of the hens was not affected as far as could be seen. About eight per cent of the eggs from hens in their second laying year were found to have cottonseed meal spots. Eggs from pullets were not affected.

The cottonseed meal spot is described as, "a brown spot with a reddish tinge varying from the size of a pin point to a quarter of an inch in diameter. The spot floats on the surface of the yolk and can readily be seen when the egg is candled. Often there are several spots in one egg. The fertility and hatchability of the eggs are not affected."

It was concluded from this investigation that laying hens are not injured by the consumption of fairly large amounts of cottonseed meal although it is not recommended that it be fed as the sole protein supplement.

Sherwood (53) fed laying hens cottonseed meal in varying amounts and studied the storage quality of the eggs produced. None of the eggs showed any serious discoloration of yolk or white when first laid and little or no change in color was noted until after four weeks in cold storage. After four weeks in cold storage the eggs from hens receiving 20 and 32 per cent cottonseed meal in their mash began to deteriorate

in color. Eggs from hens receiving nine per cent cottonseed meal in their mash seemed to keep a good color in cold storage. Milo and kafir were fed in addition to the mash in these lots. Where an all mash ration was used the presence of nine or 12 per cent cottonseed meal caused a deterioration in the color of the eggs kept in cold storage after four weeks. When cottonseed meal constituted three to six per cent of the all mash ration the color of the eggs remained good for as long as 28 weeks in storage.

The feeding of a fresh, succulent green feed will improve the storage qualities of eggs where hens are fed a ration containing a fairly large amount of cottonseed meal.

Experiments with Rats Symes and Gardner (54) in 1915 in connection with their study of the toxicity of sodium pyrophosphate found that cottonseed meal proved fatal to rats when fed as an exclusive diet; even five grams of cottonseed meal per rat per day when supplemented with oats and bran were fatal. Meal which was partially extracted with alcohol and ether was found to be much less toxic although death did result after a few weeks.

McCollum, Simmonds and Pitz (55) while studying the vitamin A content of several vegetable oils found that ether extracted cottonseed oil was toxic to rats but commercial cottonseed oil extracted by hot pressing was not injurious. Cottonseed oil was found to be very low in its vitamin A content.

Richardson and Green (56) fed rats rations containing from 35 to 70 per cent of cottonseed meal and cottonseed flour. No cottonseed meal poisoning was produced although some of the animals died, probably from

inadequate diets. The addition of butterfat to a diet composed of lard, starch and cottonseed did not improve growth. It is probable that the diet used was inadequate in minerals because no mineral supplement was used and the addition of whole milk powder produced normal results.

Richardson and Green (57) in a later paper reported that diets composed of 50 per cent cottonseed flour, supplemented with protein free milk and butterfat produced normal growth. No toxic effect was observed when this ration was used for four successive generations; reproduction and growth were normal.

Osborne and Mendel (58) fed cottonseed meal and cottonseed flour in a ration which contained in addition starch and lard. No injurious results were noticed and in one case a normal litter was reared. In several of the successful cases butterfat was added as a supplement and improved gains were noted. Raw cottonseed kernels were used in the rations for rats with the result that the rats died in from six to 12 days. In a series of experiments where the kernels were subjected to different treatments it was found that steaming the kernels for two hours destroyed the toxicity while heating in an electric oven at 110° C. or steaming for one hour did not.

Nevens (59) used 23.9 per cent cottonseed meal in an adequate diet for rats over a period of seven weeks. No symptoms of toxicity were noted.

Gallup (60) fed cottonseed meal at various levels in rations for rats, in determining the digestibility of the cottonseed protein. Up to 51.8 per cent of the ration was cottonseed meal. No injurious effects were noted. The addition of gossypol to the rations did not lower the digestibility.

Bethke, Bohstedt and co-workers (48) fed cottonseed meal to rats as 24.2 per cent of the ration and observed no injurious results.

Theories of the Cause of Cottonseed Meal Injury

Since Voelker (4) in 1873 published his report on the injurious effect accruing from the feeding of cottonseed meal, investigators have studied the effects of cottonseed meal feeding and a number of theories and speculations have arisen to account for the injury which has sometimes occurred in connection with its use.

Choline and Betaine Böhm (61) made one of the earliest attempts to solve the problem of "cottonseed meal injury". He isolated a large preparation of choline from an alcoholic extract of a cottonseed cake which had been fed to young cattle in Germany with fatally injurious results. He concluded that there was but one alkaloidal substance present in cottonseed cake.

Ritthausen and Weger (62) one year later in 1882 isolated betaine from cottonseed cake.

Goehtgens (63) had previously shown that .3 gram of choline injected into a vigorous cat had caused an immediate paralysis and that .5 gram was sufficient to cause instant death. He believed that betaine was non-poisonous.

Maxwell (64) showed that betaine is present in much larger amounts than choline in cottonseed cake and that together they constitute about .24 per cent of the cake.

Von Hoeslin (65) in 1906 reported a series of experiments which showed that when choline is fed by way of the mouth its toxicity is very

limited. Even chlolin hydrochlorate which was found to be more toxic than chloline, could be given in two gram doses to a rabbit without producing serious results.

The possibility that choline may be converted to more toxic substances such as neurin or muscarin has been suggested. Frieman (66) reported that ptomaine substances are probably formed from the nitrogen containing components of lecithin in cottonseed meal. He also suggested that the unsaturated fatty acids present in the fat extraced from cottonseed exert some influence on its toxicity.

Parasitic Organisms and Decomposition Products of Bacterial Action Von Nathausius (23) attributed the toxic effect of cottonseed meal to parasitic organisms associated with the meal.

Edgerton and Morris (67), however, found that meal infected with the molds that usually grow on cottonseed were even less toxic when fed to rabbits and guinea pigs than meal made from uninfected seeds.

König, Spieckermann and Olig (68) made a study of the decomposition products formed by various micro-organisms working on cottonseed meal. They found that all of the organisms investigated gave similar physiological effects and were affected by the composition of the meal and the air supply. A number of cleavage products were caused by the bacteria which attack the meal, some of them being toxic but at no time during the decomposition were the injurious substances present in sufficient amounts to manifest themselves in physiological symptoms.

High Protein Content: Tietze (9) investigated several cases of young calves which were thought to be poisoned by cottonseed meal feeding. These calves received three heaping liters of cottonseed meal per day

in addition to skim milk, hay, and linseed cake. He believed the injury was due to the high protein content of the cottonseed cake.

It has also been supposed by some that cottonseed meal injury was caused by its fiber content and also by the oil it contains.

Phosphoric Acid Hardin (69) in 1892 advanced the theory that the presence of meta and pyro phosphoric acid which he found in the aqueous solution of the meals he examined accounted in part at least for the injurious effect which occurred from feeding cottonseed meal.

Crawford (70) continuing the work of Hardin, by a number of pharmacological tests on rabbits came to the final conclusion that the chief poisonous property of cottonseed meal is a salt of pyro phosphoric acid. The fact that some meals were more toxic than others was thought to be due to the conversion of ortho into the meta and pyro phosphoric acids by heat during the process of manufacture.

Edgerton and Morris (67) two years later presented evidence to show that pyrophosphoric acid had nothing to do with cottonseed meal injury.

Anderson (71) isolated the organic phosphoric acid in cotton seed which he found to be similar to phytin but he found no evidence to show that it is the poisonous principle present in cotton seed.

Withers and Ray (72) found that a pepsin pancreatin extract of cottonseed was not toxic to rabbits in the amounts in which it is usually fed and that the residue was more toxic than the extract. They also showed by feeding pure sodium pyro phosphate in amounts corresponding to that in the meal that it was not the substance responsible for the injury and they further proved that the toxic effect may occur in the meal when the pyrophosphoric acid salts are not present at a toxic level.

Symes and Gardner (54) later corroborated the work of Withers and Ray when they administered sodium pyrophosphate in food to rabbits, rats, and cats. The tolerance of rats for sodium pyrophosphate was seven times the amount found in the cottonseed meal which had proved fatal.

Acidosis Forbes (73) found that in 100 grams of cottonseed meal the excess of acid over base forming elements was equivalent to 7.7 cc. of normal acid.

Wells and Ewing (74) believed that cottonseed meal injury might be due to acidosis for they found an excess of acid over base forming elements in 100 grams of cottonseed meal equal to 8.2 cc. of normal. Later work by these investigators on swine did not substantiate the theory and they came to the conclusion that acidosis is not the cause of cottonseed meal injury.

Toxic Principle in the Protein Molecule Withers and Brewster (41) in 1913 put forth the theory that the toxic principle of cottonseed meal was a constituent group of the protein molecule containing loosely bound sulphur and that the toxic effect of the meal was due to the action of this group upon the iron of the blood. The addition of iron in the form of iron citrate to the rations of rabbits receiving cottonseed meal alleviated the symptoms of injury and when fed before the symptoms appeared, prevented their development, although the rabbits ate five times the amount of meal which proved fatal to rabbits which received the unsupplemented ration.

Withers and Carruth (42) in a later investigation used pigs as experimental subjects. Iron in the form of $Fe\ cl_3$ and copperas was used. Out of a total of 18 pigs getting the iron in addition to a ration containing cottonseed meal four animals died while in a lot of 18 which did

not receive iron 13 died. It is suggested that iron salts facilitate oxidation of the toxic substance.

Deficiency Disease Theories   Wells and Ewing (44) using six week old pigs, fed cottonseed meal in a ration with starch, sugar and skimmed milk. When cottonseed meal was fed at the rate of 25 grams per kilo of body weight per day with five cc. of skim milk, death resulted in from 30 to 50 days. When gluten flour replaced the cottonseed meal the result was also fatal. The addition of 70 cc. of skim milk a day assured maintenance and a fair amount of growth. Failure of both cottonseed meal and gluten flour on a ration balanced from the standpoint of carbohydrates, fats, and proteins was held to indicate a deficient diet rather than the presence of a definitely toxic substance.

Vitamin B   Rommel and Vedder (43) in 1915 advanced the theory that the cottonseed meal injury noted in pigs is a deficiency disease analogous to the disease known as beri beri in man, if not identical with it. They based their hypothesis upon the similarity in post mortem findings and upon experimental work with pigs. They fed pigs upon polished rice and a disease resembling beri beri in man developed. The post mortem examinations on these pigs showed a great similarity to those of pigs which had died of cottonseed meal poisoning. They point out that acute "injury" corresponds to wet beri beri in man and chronic injury corresponds to the dry form in man.

Withers and Carruth (75) were unable to substantiate the work of Rommel and Vedder. Pigs to which they fed a ration of cottonseed meal supplemented with milk and green feed died, while pigs on cottonseed kernels from which gossypol had been extracted with ether developed normally.

Bechdel, Eckles and Palmer (76) reported that the requirement of the dairy calf for vitamin B is either very small or that the vitamin is synthesized by the animal.

Bechdel and co-workers (77) have since shown that vitamin B can be synthesized in the rumen of the cow, and a cow on a vitamin B free ration does not develop symptoms which result from a vitamin B deficiency.

Macy and Mendel (78) reported that cottonseed meal contained some vitamin B but much less than is found in yeast.

Richardson and Green (79) found that cottonseed meal furnished a considerable amount of the water soluble vitamins.

Stevens (80) in recent work has shown that cottonseed meal is an excellent source of both vitamin B and vitamin G. Ten per cent cotton seed meal in a vitamin B free ration contained enough of both factors of the complex to produce approximately optimum growth.

Iron Deficiency McGowan and Chricton (81) have advanced the theory that cottonseed meal injury is associated with an iron deficiency in the ration. They found that the symptoms of poisoning are similar to those which occur in suckling pigs on iron deficient diets and they believe that the injurious results usually occur in pigs which have suffered in the suckling stage from an iron deficiency. They added iron in the form of ferric oxide to pigs on a ration containing a large amount of cottonseed meal and were able to delay the onset of the injury.

Halverson and Sherwood (19) have reported work from the North Carolina Station which shows that the addition of iron to the ration in the form of copperas used at a .5 per cent level failed to show any benefit in delaying the onset of the symptoms of cottonseed meal injury.

Gallup (62) reported that iron salts delayed the onset of cottonseed meal injury in swine.

Factor or Factors Carried by Hay Reed, Huffman and Addington (20) in 1928 showed that a close relationship exists between the symptoms of cottonseed meal injury in cattle and the injury produced when too much concentrates are fed in proportion to roughage or where a roughage of poor quality is fed. They produced a condition of blindness and stiffness of gait in two calves on a ration of wheat straw, as the roughage, and corn gluten and corn distillers grain as the chief sources of protein. No cottonseed meal was fed. Calves receiving cottonseed meal as a chief source of protein with wheat straw as a roughage developed the same symptoms of injury, later having convulsions. The animals died at 275 and 350 days of age. Where timothy hay and silage were fed as a source of roughage heifers receiving cottonseed meal as the chief source of protein developed normally.

Huffman and Moore (21) continuing the work just cited, fed five cows which had been raised from 90 days of age on a ration having cottonseed meal as the chief source of protein, on a ration of timothy hay, corn silage, yellow corn, and cottonseed meal during two lactation periods. Cottonseed meal was fed in sufficient amounts to meet the entire protein requirements for maintenance and lactation. As much as 11 pounds per day was fed during the early months of lactation in the case of some of the cows. During the first lactation two of the cows received an average of over seven pounds of cottonseed meal per day throughout the entire period. These cows produced approximately 10,000 pounds of milk in 10 months.

None of the five animals developed any symptoms of cottonseed meal injury. There was no more udder infection in the case of the animals fed cottonseed meal than there was in the case of animals fed on linseed oil meal. A bull calf 90 days old fed cottonseed from the same lot that was fed to the cows, but getting wheat straw as a roughage developed scours when about 270 days old.

As a result of these investigations Huffman and his associates came to the conclusion that cottonseed meal injury in cattle is due to a lack of a factor or factors carried by hay.

At the North Carolina Experiment Station (19) a series of investigations on cattle were started in 1915, which have recently been summarized by Halverson and Sherwood (19).

In the early trials it was found that when cottonseed meal was fed with corn silage less injury was observed and better gains were made than when cottonseed hulls were used as the roughage. Also a limited amount of pasture fed with cottonseed meal and hulls produced a fair amount of growth. The addition of copperas to the ration at a 0.5 per cent level and the substitution of 50 per cent of the cottonseed meal for corn did not relieve the symptoms of cottonseed meal injury as indicated by unthriftiness and impaired vision.

In 1920 a more extensive investigation was started which included trials on 20 cows. Rations restricted to cottonseed meal and cottonseed hulls resulted in the development of symptoms of cottonseed meal injury in approximately the same time in all cases. Characteristic symptoms which developed were: impaired appetite, staggering gait, affected eyesight (in many cases blindness), and spasms.

The substitution of 50 per cent of the cottonseed meal by 50 per cent oats did not alleviate the injury.

When the roughage contained corn silage or silage and corn stover the symptoms of injury were much milder and spasms and total blindness did not occur. When beet pulp was used the symptoms were similar to those which developed when cottonseed hulls furnished the roughage portion of the ration.

Various supplements were added to the basal ration of cottonseed meal and cottonseed hulls after the symptoms of injury had set in.

The addition of yeast at the rate of one pound weekly to a cow suffering from spasms and anorexia resulted in a marked improvement. Her appetite improved and no more spasms occurred. She gained steadily in weight for 278 days. Another cow in a critical condition was fed two pounds of yeast daily and recovered sufficiently to produce a living calf. At the time the yeast was added to the ration the cow was receiving $Ca\ Co_3$ and butterfat in addition to the basal ration but probably they were present in insufficient amounts.

The addition of casein as 9.93 per cent of the ration did not alleviate the injury.

The ration was known to be very low in its calcium content but the addition of $Ca\ Co_3$ alone in amounts as high as 2.5 per cent of the ration was not beneficial.

Alfalfa leaf meal of excellent quality fed as five per cent and as 10 per cent of the ration did not adequately supplement the ration.

Butterfat fed as 1.94 per cent of the ration did not prevent the injury even when the calcium content of the ration was made normal.

By using the preventitive treatment (adding the supplement before injury set in) the amounts of supplements and the combinations needed to prevent injury were obtained. They are summarized below.

Supplements Used in Cottonseed Meal Rations Which Proved to be Approximately Adequate

Basal Ration - Cottonseed Meal and Cottonseed Hulls

Per cent in Concentrations

Ration No.	Calcium Carbonate	Steamed Bone Meal	Butter fat	Cod Liver Oil	Yeast	Wheat Embryo	Crude Casein
17	4.24	-	-	1.99	6.42	-	-
16	2.92	-	-	1.94	-	9.72	-
13	1.95	-	-	1.94	.87	7.81	-
10	1.92	-	3.41	-	4.26	4.26	-
10 A	1.80	-	3.21	-	6.01	8.02	-
9	1.92	-	1.71	1.71	1.71	6.82	8.52
Basal Ration - Cottonseed Meal and Silage							
11	2.10	-	1.86	-	1.86	-	9.32
12	-	3.48	-	1.74	4.35	4.35	-
Basal Ration - Cottonseed Meal, Corn, C.S.Hulls, Silage and Stover							
22	2.89						
23	2.75						

It appears that all of the rations which were adequately supplemented contained approximately two per cent or more of calcium carbonate, six per cent or more of yeast or wheat embryo and nearly one and three-quart-

ers per cent of cod liver oil or three and one-quarter per cent of butterfat.

In a more recent investigation in the series it has been shown that feeding of meals from other high oil bearing seeds other than cottonseed, with poor quality roughage resulted in the development of the same symptoms in dairy heifers that characterize cottonseed meal injury. Linseed oil meal, peanut oil meal and soybean oil meal were used with wheat straw as a ration for dairy heifers. A staggering gait, swollen legs and joints, blindness and convulsions developed in about the same length of time that they did when cottonseed meal was fed with wheat straw.

Vitamin A assays of the livers of heifers which died on these rations showed that .3605 grm. of dried liver per day did not contain a sufficient amount of vitamin A to cure opthalmia in rats. A similar amount of dried liver from normal animals was effective in curing opthalmia.

A similar assay showed that beet pulp and cottonseed hulls fed at the rate of one gr. daily to rats affected with opthalmia contained insufficient vitamin A to improve the condition. Wheat straw was found to have a limited amount of vitamin A.

In a recent project with milking cows it has been shown that when the ration consisted of one-third cottonseed meal and a mineral mixture and two-thirds roughage made up of equal parts of cottonseed hulls, corn silage and alfalfa hay the cows suffered no ill effects over a period of 175 days. However, when dried beet pulp replaced the silage, poor health and failure in five of 10 milking cows resulted.

In discussing these results Halverson and Sherwood concluded that cottonseed meal injury is due to a deficiency in the ration and not to the presence of a toxic substance. They believe that the data suggests

a deficiency of vitamin A and inadequate calcium. They do not account for the improved results which were obtained when yeast was fed. They also suggest that vitamin D may not be present in sufficient amounts to insure good calcification of bones and teeth.

Bechdel, Honeywell, and Dutcher (83) described the symptoms of vitamin A deficiency in heifer calves which had been on a ration low in vitamin A for six to seven months. Edema appeared first in the legs of the animals and then gradually spread to the neck and shoulders. One heifer went completely blind and another had fit like spasms. Two calves were born prematurely to the heifers on the deficient ration. Post mortem examinations showed the tissues were infiltrated with a watery fluid in the region where edema existed. These symptoms agree fairly closely with those which occur in cottonseed meal injury.

Jones, Eckles, and Palmer (84) described the symptoms of vitamin A deficiency in calves as loss of appetite and failure to grow, later scouring developed. Zerophthalmia set in and respiratory troubles occurred. Post mortem findings revealed edema of the kidneys, atrophy of the intestines, necrosis of the rumen and sclerosis of the liver. These investigators found that wheat straw is a fairly good source of vitamin A as indicated by curative treatments with rats.

McCollum, Simmonds and Pitz (55) have reported that cottonseed oil is very low in its vitamin A content. Alfalfa leaves were found to be an excellent source of this factor, however.

Steenbock and Gross (85) found that five per cent of immature alfalfa leaf added to a vitamin A free diet had sufficient vitamin A to promote normal growth and the rearing of some young.

In view of the high vitamin A content attributed to alfalfa and also because of its high calcium content it is difficult to explain the failure of the five cows on the North Carolina experiment whose ration consisted of approximately 25 per cent alfalfa hay.

Gossypol Withers and Ray (85) in 1912 extracted cottonseed meal with gasoline and then further extracted it with a hot alcoholic solution of Na OH for two hours. The extracted meal when fed to rabbits at the level which caused death in the case of the unextracted cottonseed meal did not produce any apparent injury although the rabbits lost weight. They concluded that the beneficial effect was due to hydrolysis, to the formation of a sodium salt, or to some other change not yet determined.

Withers and Carruth (87) two years later separated from cottonseed a substance which appeared identical with one isolated from cottonseed by Marchlewski in 1899 which he had named gossypol. It was administered to rabbits in various ways and was found to be toxic in every case. They found as did Marchlewski that gossypol was readily oxidized in an alcoholic solution of NaOH, the oxidation apparently rendering it non toxic. This fact would explain their earlier work.

Withers (88) in 1915 published the results of a more extensive investigation on the toxicity of gossypol. Raw cottonseed kernels were extracted with petroleum ether (in which gossypol is insoluble) and the extracted kernels were fed to rabbits. Fatal injury resulted. Kernels of raw cottonseed which had been extracted with petroleum ether were further extracted with ethyl ether, and the residue which amounted to about 2.5 per cent of the original weight of the material was administered to rabbits. It was found to be toxic when injected intra peri-

toneally when fed in small doses or when fed through a catheter. Death occurred in the case of injection in three to 13 hours. In the case of catheter feeding in 12 - 25 hours and when fed orally death occurred in from eight to 15 days.

Crude gossypol acetate precipitated from an ethyl ether extract by addition of glacial acetic acid was found to be more toxic than the crude residue from ether extraction.

Oxidizing the gossypol acetate with alcoholic NaOH apparently completely eliminated its toxicity.

Characteristic post mortem examination of the rabbits which died from the injury showed; reddish serum in the abdominal cavity, liver congested, lungs slightly congested and endamatous. There was some intestinal inflammation and some hemorrhage at the pyloric end. The kidneys were also somewhat congested.

Schwartze and Alsberg (89) made a study of the toxicity of gossypol on cats and rabbits. Metabolism studies with cats showed that they were often in negative nitrogen balance when on a ration of meat, butterfat and whole milk powder. Ninety mgm. of gossypol were administered daily. Gossypol was noticed to have a paralytic effect on cats and rabbits not merely upon the neuro muscular apparatus of striated muscle but also upon that of smooth muscle. It was also found that gossypol is a circulatory depressant. It produces a fall in blood pressure immediately after injection. for some time after injection the heart shows skipped or weak beats.

Post mortem examination showed that edema may follow gossypol injection as well as gossypol feeding. There is local edema of the lungs

which may be hemorrhagic and there is also effusion in the serous cavities.

Schwartz and Alsberg (90) working with rats found that the threshheld of toxicity for gossypol is about .0675 per cent of the ration. Reproduction at this level was poor, however. Above this level the incidence of death was large. They found that in general the toxicity of cottonseed is indicated by its gossypol content but in a few varieties the toxicity in biological assays was found to be less than the gossypol content would indicate.

Menaul (91) administered gossypol through the diet and found that it is very slowly absorbed. In rabbits death was produced in about 14 days when .1 gram per day was fed. When introduced directly into the blood stream the effects are noticeable at once and death results quickly. Small quantities of gossypol mixed with fresh blood reduced its oxygen carrying capacity materially. Gossypol was also found to exert a hemolytic effect on the erythrocytes.

The anatomical effects of the poisoning were found to be a passive hyperemia and edema of the lungs and some hydro thorax. Intestinal inflammation was noted in rabbits fed the free gossypol.

Withers and Carruth (92) compared the toxic effect of gossypol and cottonseed products in rats, rabbits, chickens and swine. Rats were fed a ration containing up to 50 per cent cottonseed meal without producing injurious results. Long cooked cottonseed meal and ether extracted raw kernels were found to be practically non toxic. Well cooked cottonseed meal with milk as a supplement proved very successful.

Rabbits were found to be much more sensitive to injury than rats and were poisoned by long cooked cottonseed meal which had been found to be non-toxic to rats.

Hens fed on cottonseed meal had a diminished egg production when fed excessive amounts of meal but they sustained no other noticeable injury. When raw kernels of cottonseed were fed a discoloration of the egg yolk was noted.

Pigs were found to be very susceptible to cottonseed meal injury and were poisoned even by thoroughly cooked cottonseed meal fed at 25 per cent level with cracked corn and wheat bran. Soybean meal and peanut meal fed at the same level produced good gains. Supplementing long cooked cottonseed meal and the basal ration with butter, meatscrap, milk powder, and a salt mixture did not prevent death. These investigators came to two conclusions: that there is marked species difference in susceptibility to gossypol poisoning or "gossypol tolerance" as they call it and further, that in the cooking process the gossypol is converted to a less toxic substance which they call D-gossypol.

Withers and Carruth (93) have suggested that while most of the gossypol disappears in the cooking process due to an oxidation some may become bound through condensing with amino and carboxyl groups of protein molecules.

Clark (94) further elucidated this hypothesis. During the heating and pressing to which the seeds are subjected the resin glands containing the gossypol are disrupted and possibly much of the gossypol is dissolved in the oil present. It thus comes in intimate contact with the protein of the seeds and in this condition favored by heat and pressure,

probably condenses with free amino groups of the protein molecules as it does with many primary amines, forming substances similar in type to dianiline gossypol (which is readily obtained by condensing pure gossypol with aniline).

Clark (95) has shown that aniline D-gossypol is identical with dianiline gossypol obtained by condensing gossypol with aniline and further that upon hydrolysis of aniline D-gossypl a substance was obtained which was shown to be identical both chemically and physiologically with analytically pure gossypol.

These facts were held by Clark to render untenable the hypothesis that D-gossypol is an oxidation or hydrolytic product of gossypol as had been suggested by Carruth (93) and Sherwood (96) and point to a mechanism such as that suggested in which gossypol is condensed with an amino group of the protein molecule.

Jones and Waterman (97) had previously shown that peptic and tryptic digestion of casein and cottonseed globulin in vitro was reduced 15 per cent by the presence of gossypol as one per cent of the weight of either of the proteins. They believe that incomplete digestion (83 per cent) of cottonseed globulin by animals may be explained as due to the inhibiting action of gossypol. This fact indirectly lends support to the hypothesis that gossypol in cottonseed meal is bound with a group in the protein molecule.

Osborne and Mendel (58) in 1917 had shown that rats on rations composed of cottonseed meal or cottonseed flour (41 per cent) plus starch and lard and in most cases butterfat had made good gains and showed no injurious effects. Raw cottonseed kernels proved unsuccessful, the rats dying in a very few days. However, when the raw kernels were subjected to steam heat for two hours the toxicity was destroyed. Steaming the raw kernels for one hour or heating in an electric oven at 110° did not destroy the poisonous property.

Dowell and Menaul (98) autoclaved cottonseed meal at 15 pounds for 20 minutes in a damp condition and then air dried it. It was fed at the rate of 1.33 per cent of the body weight to young pigs averaging 28.5 pounds apiece. A basal ration of dorso, skimmed milk and alfalfa was used. The pigs made fairly good gains. Pigs on a similar ration containing unautoclaged meal showed no ill effects for three weeks but after that they failed to grow normally, at 78 and 83 days, two of the pigs on the unautoclaved meal died. It was concluded by these investigators that autoclaving destroys the poison in cottonseed meal but whether it is the effect of high temperature or oxidation by oxygen of the air during drying was not discovered.

Gallup (99) autoclaved damp cold pressed cottonseed for one hour at 20 pounds pressure. He also steamed some of the cottonseed meal for one hour by passing steam directly into the meal. The treated meal was dried and fed as 1.5 per cent of the body weight of pigs weighing approximately 30 pounds. The basal ration was made up of corn chops and alfalfa. In the case of the pigs fed the autoclaved and the steamed meal the gains were rather small but they were considerably larger than the gains made by pigs in a check lot fed untreated cold pressed meal. It is stated, however, that the untreated meal was sparingly eaten and no account of this apparent difference in palatability was taken in interpreting the results. Food consumption was not included. Pigs receiving

tankage instead of cottonseed meal made even poorer gains than the pigs fed on untreated meal.

Cotton seeds untreated, and cotton seeds autoclaved for one hour and for two hours were fed to white rats as 35 per cent of the ration. The ration was adequate from other standpoints. Autoclaving for one hour was found to completely destroy the toxicity of the seeds for rats. Cottonseed meal when fed to rats as 25 per cent of the ration produces good results but 35 per cent caused injury as indicated by failure to grow. Steaming the meal produced normal results. The addition of six per cent butterfat, five per cent yeast, and one per cent $CaCo_3$ apparently did nothing to overcome the toxic effect of either cotton seeds or cottonseed meal.

The Chemistry of Gossypol

Kuhlman (100) in 1861 while attempting to recover fatty acids from the "foots" of cottonseed oil purification found in his residue after distillation a greenish blue mass which he called "cottonseed blue" and gave to it the empirical formula $C_{17}H_{24}O_4$.

Longmore (101) separated a very impure product from cottonseed oil which he describes as a "light brown powder of pungent odor". It was found to be quite insoluble in water but soluble in alcohol and alkalies. It was probably gossypol in a very impure state.

Marchlewski (102) probably first isolated gossypol in a fairly pure form while engaged in the purification of cottonseed oil. He concluded that it was a phenol derivative and named it gossypol from Gossyp (ium phen) ol. He proposed the formula $C_{13}H_{14}O_4$ or $C_{32}H_{34}O_{10}$. His prepara-

tion contained loosely bound acetic acid and had a melting point of 179-180° C. He described its properties as: "A beautiful crystalline yellow colored dihydroxy phenolic substance, easily soluble in alcohol, benzene, chloroform, ether, acetone and glacial acetic acid. Insoluble in water, soluble in concentrated sulphuric acid with a magnificent red color, easily soluble in alkalies, the solution for the first second being yellow and after a short time becoming a beautiful violet and then fading, the fading being due to oxidation." Marchlewski made no crystalline derivatives but he showed that it was not a glucoside and that in all probability it contained no alkoxyl groups.

Withers and Carruth (87) isolated gossypol acetate from cottonseed in 1914 but they reported no study of its chemical properties.

Carruth (103) in 1918 made the first extensive study of the chemistry of gossypol. He prepared gossypol acetate by treating an ether extraction of decorticated seeds with 80 per cent acetic acid. The substance was purified by dissolving the crude gossypol acetate in ether and acetic acid and distilling until enough ether was driven off to allow the gossypol to precipitate. Crystalline gossypol was obtained from the acetate by dissolving the latter in ether and adding water. The ether was distilled off leaving the gossypol as crusts floating on the water which contained all of the acetic acid. The free gossypol was then crystallized from alcohol.

Molecular weight studies were made using a method whereby the acetic acid that was taken up by water from an ether solution of the gossypol acetate was titrated with standard alkali. From this data the amount of acetic acid present was obtained, and finding it to be 10.0 per cent

Carruth calculated the molecular weight of gossypol to be 592 if the empirical formula is $C_{30}H_{28}O_9$ or 594 if its formula is $C_{30}H_{30}O_9$. Molecular weight determinations were made by direct titration of free gossypol with standard alkali, it being assumed that gossypol neutralises two equivalents of NaOH. By this method the values obtained were 544 and 532. The fact that the disodium salt of gossypol is neutral to phenolphthalein in aqueous solution gives an indication of its relative strength as an organic acid. Analytical data give results which indicate that the empirical formula is $C_{30}H_{28}O_9$ or $C_{30}H_{30}O_9$.

Complete acetylation of the gossypol was found to be difficult but Carruth concluded that there are four or five hydroxyl groups in gossypol. Marchlewski had supposed there were only two.

Aniline readily combines with gossypol to form a bright orange yellow compound. It was thought to be a simple dianiline compound but nitrogen determinations showed that it contained too much nitrogen to make this appear logical. 4.65 per cent nitrogen was found which would indicate a compound containing two molecules of gossypol to five molecules of aniline. The compound is very insoluble and therefore can be used to determine the gossypol content of cotton seeds. Its insolubility also probably accounts for its non toxic properties since it is probably not appreciably absorbed in the intestinal tract.

Carruth (103) found that the substance which had been named D-gossypol which is present in quite large amounts in cottonseed meal and is insoluble in ether could be extracted from the meal with hot aniline. From the aniline extract an orange-yellow compound crystallises out. D-gossypol was obtained from it by dissolving the substance in alcoholic

potash and pouring into water, oxidation being prevented by adding a pinch of sodium hyposulfite. D-gossypol was then precipitated in amorphous flakes by adding acid. The substance was then crystallized from ether and alcohol. A yellow crystalline substance was obtained which had no definite melting point but darkened and softened around 256° C.

Attempts to get a clue to the constitution of gossypol failed through inability to split the substance into simpler known substances. The fact that several flavone pigments occur in the cotton plant and the fact that gossypol has 30 carbon atoms suggests that it may be derived by condensation and subsequent reduction of two molecules of flavone. The acidic properties are thought to be due to carboxyl and hydroxyl groups arranged as in flavonals rather than to carboxyl groups. These substances are sufficiently acid to form salts from an alkali acetate. The presence of nine oxygen groups may readily be accounted for by the presence of five hydroxyls, two carboxyl groups and two bridge oxygen atoms, all of which types occur in flavonals.

Schwartz and Alsberg (90) made an optical crystallographic study of gossypol acetate under the microscope. They found that in ordinary light the substance consists of bright yellow flakes, often rather acutely rhombic in outline, or sometimes approximately hexagonal. Two or more acute crystals are sometimes grown together to form a twin with a deep reëntrant angle at one end. The crystal system is apparently triclinic.

Clark (104) in recent work has repeated some of Carruth's earlier investigations on gossypol and has studied several additional derived compounds. Gossypol was prepared by the method of Carruth. The crude

gossypol acetate obtained was found to have a melting point of 184 - 187° C. The acetic acid was removed from the gossypol also by the method of Carruth. To purify the substance for analysis the gossypol was recrystallized from ether several times. The purified gossypol was found to have a molecular weight of 511. The analytical data obtained upon it gave results which indicate the empirical formula $C_{30}H_{30}O_8$. This differs from the formula given by Carruth in having one less oxygen. It is possible that Carruth was working with a compound that was not entirely pure. The melting point of the purified gossypol was found to be 214° C.

When gossypol was heated to its melting point it lost two molecules of water producing a new crystalling body, anhydrogossypol $C_{30}H_{26}O_6$. This is probably the same compound which Carruth called B-gossypol.

Oximation of gossypol produced a colorless dioxime which seemed to indicate that the chromofares are quinone groups.

Acetylation of gossypol showed the presence of six hydroxyl groups. Two of these hydroxyl groups behave differently from the remaining four, being much more acidic and requiring drastic treatment for the hydrolysis of their acetyl derivatives.

Of the eight carbon atoms present two are present as carbonyl groups and the other six as hydroxyl groups.

Clark (95) has also shown that Carruth's aniline D-gossypol is identical with dianiline gossypol prepared by condensation of the pure gossypol with aniline, and upon hydrolysis of aniline D-gossypol, a substance identical in its properties with pure gossypol is formed. This seems to indicate that the structure of the gossypol molecule is not changed in the heating and pressing process.

Clark (105) oxidized gossypol with alkaline permanganate and obtained formic, acetic and iso butyric acids as reaction products. Under the conditions of the experiment the presence of iso butyric acid as a decomposition product of the gossypol molecule indicated a side chain consisting of at least the iso butyl group.

Clark (105) in a later investigation treated gossypol with 40 per cent sodium hydroxide at the temperature of the steam bath for one-half hour. Formic acid and apogossypol, a new phenolic substance, were formed. It has the formula $C_{28}H_{30}O_6$ and is formed by the elimination of the two carbonyl groups of gossypol as formic acid. The substance is a colorless crystalline material having no definite melting point. It is less toxic than gossypol when fed to white rats. It causes acute injury only, differing somewhat from gossypol in this respect.

Clark (107) (108) had made further studies on the action of chronic acid and also the action of boiling hydriodic acid on the derivatives of gossypol. However, no results have been obtained which elucidate the structure of gossypol.

Formation and Occurrence of Gossypol in the Cotton Seed

Carruth (103) refers to gossypol as occurring in internal "resin glands" or "oil glands" of the cottonseed.

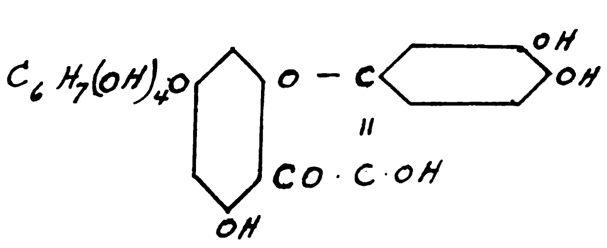
Stanford and Viehoever (108) have made a study of the formation and the secretions of the internal glands of the cotton plant. These glands were found to be constantly present and definitely arranged throughout the genus gossypuim.

In the seed the glands were found directly beneath the palisade layer into which they often project. They are oblate-spheroidal in form, with long axis perpendicular to the cotyledon surfaces. Smaller glands are found in the cortex of the radicle, covered by a few parenchymal layers.

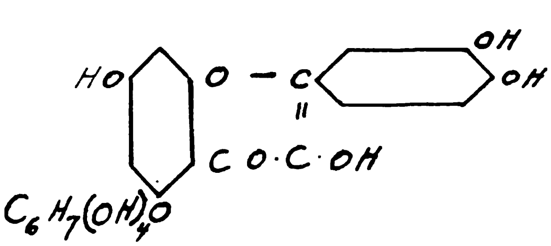
The glands in portions of the plant which are exposed to light are surrounded by an anthocyan-bearing envelope of the flattened cells, contain quercetin probably partly or wholly in the form of its glucosides. The glands not normally exposed to light are surrounded by a layer of flattened cells containing no anthocyans. They contain gossypol. Gossypol is formed in the glands of the developing corolla. On their exposure to light it is replaced by quercimeritrin. Gossypol in the unfolding cotyledons is changed, probably through oxidation, without the formation of quercimeritrin. As gossypol occurs in the glands of the flower before opening and quercimeritrin after opening the former may give rise to the latter. The exact nature of the change remains uncertain. Where the glands are not exposed to light the gossypol apparently remains unchanged.

Perkins (110) in 1909 had isolated quercimeritrin from Egyptian cotton flowers. It was isolated. It has glistening bright yellow plates which on hydrolysis yielded glucose and quercetin. He concluded that one of the following formulas indicated its structure.

I



II



Viehoever (111) in 1918 showed the quercimeritrin also occurred in upland cotton grown in this country.

Gallup (112) studied the gossypol content of the cotton seed during different stages of development. Seeds from bolls which were mature and about to crack had an average gossypol content of .048 per cent. Seeds from bolls which were just open had a gossypol content of .428 per cent, after the bolls were fully opened there was a very slow increase reaching a maximum of .551 per cent in mature seeds several months later.

Gallup (113) in a later study found that the development of oil and gossypol in the cottonseed occurred at about the same time and during a very short and well defined period of growth, just as the bolls were opening. He concluded that apparently gossypol is associated in some way with the formation of oil in the seed.

Schwarts and Alsberg (114) made gossypol determinations on cotton seeds of different varieties grown in widely separated regions. They also made oil determinations on the same seeds. The results are given below.

Variety	Place Grown	Year Grown	Moisture	Ether Extract	Nitrogen	Gossypol Found
Lone Star	Greenville, Tex.	1917	5.93	31.46	6.19	.4137 .3833 .3869
" "	Yuma Valley, Cal.	1919	5.64	33.06	5.88	.4636 .4649
" "	Greenville, Tex.	1919	5.65	33.97	6.30	.5307 .5684
" "	" "	1919	-	33.40	6.40	.5288 .5076
" "	Texas	1919	5.45	34.22	5.87	.6322 .6461
" "	Arkansas	1919	5.84	35.36	5.84	.6849 .6770
" "	Manchester, N.C.	1919	5.66	35.45	5.62	.6592 .6899

Variety	Place Grown	Year Grown	Moisture	Ether Extract	Nitrogen	Gossypol Found		
Lone Star	Courtland, Ala.	1919	5.11	38.28	5.45	.7392	.7410	
" "	" "	1919	6.04	37.15	5.20	.7970	.7982	
" "	Bakersfield, Cal.	1919	5.68	35.92	5.60	.8781	.8761	
" "	Elizabeth City, N.C.	1919	5.82	38.46	4.91	.9676		
Durango	Courtland, Ala.	1919	5.88	37.16	5.27	.7176	.6970	
"	Bakersfield, Cal.	1919	5.66	37.01	4.82	.8574	.8709	
"	Columbia, S. C.	1919	5.70	36.06	5.85	.8736	.9075	
"	Yuma Valley, Cal.	1919	5.45	37.53	5.15	.8846	.9312	.8831
"	" " "	1919	5.69	37.40	4.77	.9552	.9319	
"	Georgia, N.C.&S.C.	1919	5.58	38.87	5.19	.9811	.9849	
"	Columbia, S. C.	1918	5.93	38.97	4.94	.9843	.9833	
"	Columbia, S. C.	1917	4.81	40.60	4.88	1.0603	1.0348	
Trice	Bells, Tenn.	1918	6.12	28.87	6.42	.3970	.4250	
"	" "	1917	5.45	32.51	6.28	.5776	.5797	
"	" "	1919	6.55	35.84	5.75	.8893	.9061	
"	Tennessee	1919	-	-	-	.9426	.9524	
"	Eastern States	1919	-	-	-	1.0590	1.1832	
Acola	Clarksville, Tex.	1918	5.44	33.69	6.04	.4374	.4560	
"	" "	1919	6.21	34.56	5.71	.5554	.5557	
"	Oklahoma	1919	4.92	35.41	5.70	.8976	.9094	.9067
"	Bakersfield, Cal.	1919	5.25	40.98	4.40	.9639	.9512	
Meade	Charleston, S. C.	1918	5.52	37.05	4.97	.5856	.5741	
"	Ware County, Ga.	1918	5.06	37.87	5.08	.6455	.6446	

Variety	Place Grown	Year Grown	Moisture	Ether Extract	Nitrogen	Gossypol	Found
Glixi	Eastern States	1919	5.72	38.29	5.00	1.0185	
"	Florence, S. C.	1919	5.74	39.53	4.66	1.0305	1.0366
Columbia	Easley, S. C.	1918	5.46	38.89	4.85	1.1105	1.1162
"	South Carolina	1919	-	-	-	1.0064	1.0278
Egyptian	Sacatone, Ariz.	1918	5.66	36.68	4.73	1.1832	1.1758
"	Bakersfield, Cal.	1919	5.59	36.08	5.34	1.1745	1.1847
King	Richmond, Va.	1919?	5.24	38.35	5.14	.9219	.9185
Cleveland	St.Mathews, S.C.	1919?	6.49	35.20	5.25	.7127	.6892
Sea Island	Blackshear, Ga.	1918	-	-	-	.9446	

From these data Schwartz and Alsberg found a positive correlation between the ether extract content and the gossypol content (Correlation Coef.;γ equals .75 ± .03. There was a negative correlation between the nitrogen content and the ether extract. There is no relationship between the variety and the gossypol content. The gossypol content seems to vary considerably with the season in which it is grown and also with the region where it is grown. The seeds from the southwest have a tendency to be low in oil and gossypol, those from the southeast tend to be higher, and those from the Pacific coast tend to be still higher.

Garner, Allard and Roubert (115) concluded that under practical conditions climate is a more potent factor than variety or soil type in controlling the size of the seed and its oil content. They found as did Schwartz and Alsberg that the oil content of the seed tended to vary with the region in which it is grown.

Sherwood (96) analyzed 40 samples of cottonseed meal for their gossypol and D-gossypol content. These data together with other information which he obtained is given in the following table.

1921 - Open Kettle

Sample	Soil on Which Grown	Variety of Cotton	Water Added During Cooking	Time of Cooking Min.	Temp. of Seed Cooker (°F.)	Steam Pressure in Jackets lbs.	Color	Moisture %	Gossypol	D-Gossypol
1	Sandy loam	mixed	no	24	210	-	B	14.92	.045	.874
2	" "	Cleveland Big Boll	yes	48	-	60	Y B	6.66	.154	.755
3		mixed	steam	12	300	90	Y	6.32	.160	.669
4	Black	"	no	20	-	50	Y B	7.52	.019	.919
5	Red	"	no	25	210	-	Y	6.36	.021	.881
6	"	"	no	22	70	-	Y B	6.66	.143	.963
16	Sandy	mixed	no	7 (1) 7 (2) 7 (3) 7 (4)	64 79 100 107	100 100 100 100	Y B	5.21	.020	.754
17	Clay	mixed	no	20 (1) 20 (2) 20 (3) 20 (4)	210		Y B	6.04	.026	1.019
		probably in error								
18			2%	20 (1) 20 (2) 20 (3) 20 (4)	220		Y B	7.75	.009	.875
19			2%	20 (1) 20 (2) 20 (3) 20 (4)	220		Y B	7.75	.009	.878

1921 - Expeller Process

Sample	Soil on Which Grown	Variety of Cotton	Water Added During Cooking	Time of Cooking Min.	Temp. of Seed Cooker (° F.)	Steam Pressure in Jackets lbs.	Color	Moisture %	Gossypol	D-Gossypol
									raw kernels	
20	red & gray	Cleveland Big Boll					G Y		.58	.14
38)					162			7.18	.026	.333
39)1918					170			7.54	.020	.425
40)					160			6.39	.023	.348
Open Kettle Process										
21				40	135-250			7.75	.033	.910
22				33		60		7.38	.051	.758
23				20	202	60		8.29	.111	.858
24				22.5		40		10.35	.078	.544
1921 - Continuous Cooker										
7	loam	mixed	no	18 (1) 18 (2)		40 60	Y B	7.58	.034	.902
8	sandy	mixed	no	20 (1) 13 (2)		18	Y	8.08	.228	.789
9	Coastal plain	short staple	8%	38	218	80-90	Y	6.43	.048	.843
10	sandy loam	Cooks Improved	no	20 (1) 20 (2) 20 (3)	175 175 175	40 75 90	Y	5.74	.022	.848
11			2%	20 (1) 20 (2) 20 (3) 20 (4)	170 200 215 220	90 60 40 20	Y B	6.20	.023	.811
12	Norfolk loam	mixed	5%	20 (1) 20 (2) 20 (3) 20 (4)	180 200 220 220	75 60 50 30	B D	5.72	.016	.808

1921 - Continuous Cooker

Sample	Soil on Which Grown	Variety of Cotton	Water Added During Cooking	Time of Cooking Min.	Temp. of Seed Cooker (°F.)	Steam Pressure in Jackets lbs.	Color	Moisture %	Gossypol	D-Gossypol
				20 (1)		60				
13			Steam	20 (2)		60	Y B	7.17	.040	.773
				20 (3)		40				
				20 (4)		10				
				20		5				
14	loam	mixed	small	20	218	10	Y B	5.83	.025	.820
			spray	20	to	70				
				20	220	65				
				23 (1)	220	60				
15	sandy	improved	yes	23 (2)	220	60	Y B	5.61	.328	1.014
		small		23 (3)	220	60				
		seeds		23 (4)	220	25				
25				10-12		80		9.73	.152	.584
26				20		60-80		6.97	.089	.575
27				20				8.01	.078	.932
28				20		40		7.74	.089	.754

1918 - Continuous Cooker

Sample	Soil on Which Grown	Variety of Cotton	Water Added During Cooking	Time of Cooking Min.	Temp. of Seed Cooker (°F.)	Steam Pressure in Jackets lbs.	Color	Moisture %	Gossypol	D-Gossypol
29				22	240	25		8.46	.037	1.076
30				60	210	5-45		8.30	.058	.832
31				24		15-35		10.12	.044	.696
32				120	226	35		12.05	.062	.984
33				72	212	15-20		8.10	.010	.751
34				27		50		9.05	.018	.843
35				96		25-100		10.60	.058	.633
36				27		50		8.09	.007	.999
37				75	200-230	20-60		7.21	.007	.888

THE EFFECT OF VARIOUS SUPPLEMENTS IN COUNTERACTING THE INJURIOUS EFFECTS OF TOXIC SUBSTANCES

Mention has previously been made of the work of Withers and Brewster (41), Withers and Carruth (42), and Gallup (82) which showed the value of iron salts in counteracting or delaying the onset of the symptoms of cottonseed meal injury in rabbits and swine. It was not specifically stated by these investigators that iron has an antidotal effect on gossypol poisoning, although their results point to such an interpretation.

Gallup (116) has recently published work in which he shows that rats on a diet containing 40 per cent cottonseed meal failed to grow normally after 60 to 90 days on the ration. However, the addition of iron in the form of $Fe\ So_4.\ 7\ H_2O$ as .5 to one per cent of the ration produced normal growth and made possible the reproduction and rearing of normal litters. Very marked individual variation in susceptibility to gossypol poisoning was noted by Gallup in this investigation.

Agahur (117) in a series of articles (1926-1929) reported toxic effects from cod liver oils and showed that the toxicity was greatly affected by the composition of the basal ration particularly its content of vitamin B and C.

Slagswold (118) previously reported cases of poisoning among cattle in Sweden which received cod liver oil. The symptoms of which are comparable to those which develop in the case of cottonseed meal injury.

Light, Miller, and Frey (119) reported that the acute symptoms of vitamin D overdosage can be counteracted at certain levels by the liberal feeding of yeast.

Morris and Church (120) in 1930 published work in which they showed that certain cod liver oils are toxic due to their content of a poisonous base, iso-amylamine. They isolated this substance and fed it with a normal cod liver oil. When the basal ration contained 10 per cent yeast the toxic symptoms developed but when 18 per cent yeast was added no indications of poisoning were observed.

High protein diets are known to increase intestinal putrefaction with consequent increased ptomaine formation. Hassan and Drummond (121) in 1927 showed that the feeding of yeast and yeast extracts were beneficial in counteracting the effect of an extremely high protein diet.

The beneficial effects of massive yeast feeding in counteracting the effect of toxins in the rations suggests that perhaps the symptoms of gossypol poisoning may be delayed or offset by properly reinforcing the diet, not only by the addition of iron salts but also by the use of other supplements which may have a direct effect on the digestive apparatus.

DISCUSSION OF REVIEW OF LITERATURE

A critical analysis of the investigations pertaining to cottonseed meal injury suggests beyond any doubt that gossypol is the toxic principle present in cottonseed and to a lesser extent in cottonseed meal, responsible for the injurious effect produced in swine, rabbits, guinea pigs, and rats. The marked species difference with respect to susceptibility to gossypol poisoning is an interesting phenomenon. Swine apparently are extremely susceptible to the poisoning from gossypol and perhaps also from D-gossypol. Rabbits and guinea pigs are also very easily poisoned by small doses of gossypol. Rats are much more resistant to the effects of gossypol poisoning than rabbits and swine; however, when gossypol occurs in any appreciable amount in their diet they are poisoned.

With regard to the effects of feeding gossypol to ruminants there is very little direct evidence to show that it is poisonous when it is fed in the amounts in which it normally occurs in cottonseed meal. "Cottonseed meal injury" of cattle has been reported commonly and a belief has grown up that cottonseed meal cannot be fed at a very high level without producing injurious results. However, the recent investigations at the Michigan and North Carolina Agricultural Experiment Stations have shown quite definitely that a deficiency in the ration exists when the so-called "cottonseed meal injury" occurs and that when a ration complete in minerals and vitamins is used in conjunction with cottonseed meal no injurious results are obtained.

The chemistry of gossypol is not yet clearly enough elucidated to permit a deduction as to the structure of the gossypol molecule and it

is difficult therefore to explain what kind of a reaction is responsible for the formation of Carruth's D-gossypol in the manufacture of cottonseed meal. It appears that D-gossypol or "bound gossypol" as it exists in cottonseed meal is much less toxic than free gossypol as it naturally occurs in cotton seed.

EXPERIMENTAL WORK

Object

Cottonseed meal furnishes a cheap source of protein for dairy cattle rations. It has not been used extensively because it has apparently produce injurious effects with several classes of live stock.

This injurious effect has been attributed to the presence in cottonseed meal of a phenolic substance called gossypol. It has been proven that gossypol is extremely toxic to some species of animals but its effect on dairy cattle is not well known.

The object of this experiment is to determine whether or not gossypol as it occurs in raw cottonseed meal is toxic to dairy heifers where an adequate ration is used, and to study, with rats, the effect of adding various supplements to a normal ration in alleviating the injury caused by gossypol.

ORIGINAL PLAN OF EXPERIMENT WITH DAIRY HEIFERS

Procedure

Animals Used

Five grade Holstein heifer calves will be used in this investigation. Animals G 20, G 21, and G 22 will constitute lot I. Animal G 17 will constitute lot II. Animal G 16 will constitute lot III.

Age

The animals will be placed on experiment at 6 months of age and will continue on experiment through first calving time or longer if the results up to that time indicate a need for a continuation of the experiment.

Health

Only healthy growing heifers which have no observable abnormalities will be used in this experiment.

Choice of Rations

Milk The calves of all three lots will receive whole milk until 45 days of age. From 45 days to 180 days they will receive skim milk in amounts not to exceed 12 pounds per day.

Grain The heifers in lot I will receive all of the raw crushed cottonseed of known gossypol content that they will readily consume and up to a level which will furnish all of the protein necessary for maintenance and growth according to the Armsby Feeding Standard (122). Lot II will receive choice cottonseed meal in sufficient quantities to furnish all the protein needed for maintenance and growth. Lot III will receive linseed oil meal in similar amounts. Lots I and II will receive .4 pounds of yellow corn per day in addition to cottonseed and cottonseed meal.

Roughage Timothy hay of good quality will be fed in whatever amounts the heifers will consume. Corn silage of good quality will be fed with the hay. It will be offered in whatever amounts the heifers will readily consume but it will not be fed at a level high enough so that the amount of hay consumed is limited.

Minerals Two per cent of special steamed bone meal and one per cent salt will be mixed with all of the grain fed to the heifers in the three lots.

Management

Water The animals in all of the lots will receive water at least once daily until they are 15 months of age. When they are running in an outside enclosure during the day, they will have free access to water. After they become 15 months old they will be placed in stanchions equipped with drinking cups.

Shelter The calves will be housed in the College Experimental Barn until they are 18 months of age. From 18 months to the time of parturition they will be kept in the Experimental Barn annex. In either case the conditions of housing will be similar to those commonly found on Michigan farms. Calves will be kept in individual box stalls until they become large enough to be tied in individual stalls. At 15 months of age they will be placed in stanchions.

Sunlight The animals will be exposed to direct sunlight during the time that they are allowed to run outside. During the spring and summer months this will amount to several hours each day. During the winter months the amount of sunlight that they will receive will be very limited due to natural conditions of cloudiness and also to the fact that they will be confined inside most of the time. Exposure to not more than one to two hours of sunshine per week will be the usual condition in the months of December, January, and February.

Care The animals will be under the care of a competent feeder who will have charge of the experimental barn where the heifers are housed.

Feeding Methods

The animals will be fed twice daily. Silage and grain will be fed in the morning and hay will be fed at night. The mangers will be so arranged that losses in feeding will be reduced to a minimum and feed refused will be weighed back.

COLLECTION OF DATA

Feed Records

Feed Consumed All feed fed during the experiment will be weighed and feed refused will be weighed back. A daily record of all feed consumed will be kept. This will be totaled for 30 day periods and placed in table form.

Nutrients Required The nutrients required for maintenance and growth will be calculated for each 30 day period from the Armsby Feeding Standard. The weight to be used in figuring the nutrients required will be obtained from the monthly weight records.

Nutrients Consumed The nutrients consumed will be calculated from the average daily consumption of each 30 day period. These will be arranged in table form.

Per cent of Raw Cottonseed Meats in the Ration The per cent of raw cottonseed meats in the ration will be computed on the basis of the total food intake by 30 day periods. All feeds will be converted to a dry basis using the Henry and Morrison (123) tables for moisture content.

Amount of Gossypol Ingested The amount of gossypol consumed per day will be calculated using the per cent of gossypol found in samples of the raw cottonseed meats fed.

Per cent of Gossypol in the Ration The per cent of gossypol in the ration will be calculated for each 30 day period for the heifers in lot I.

Milligrams of Gossypol per Kilo of Body Weight The milligrams of gossypol ingested per kilo of body weight per day will be calculated from the data obtained, using the weight for the end of each period as the basis for computing the kilograms of body weight, for each of the animals in lot I.

Reproduction

Oestrual Periods The date of the appearance of the first heat period and each subsequent heat period will be recorded.

Breeding Data The date or dates upon which each animal is bred will be recorded. A competent veterinarian will examine each animal to determine whether conception has occurred. Any abnormalities in the reproductive organs as found by the veterinarian will be noted.

Ease of Parturition Notes will be made at the time of calving on the ease with which the calf is delivered.

Placenta A record will be made of the time after parturition at which the cow delivers the placenta. In case the placenta is retained more than 24 hours it will be removed by a competent veterinarian. In case of an abortion a part of the placenta will be plated by a bacteriologist to determine whether or not a causative organism is present.

Recovery The condition of the animal after parturition will be carefully watched for several days. Any abnormality in health or condition will be recorded.

Offspring The weight, strength, and general physical condition of the offspring will be recorded at the time of parturition. The eyes of the calf will be tested to determine whether or not the vision is impaired.

Observations

Weight The animals in all the lots will be weighed at 10 day periods until they are 15 months of age. After that time they will be weighed at 30 day intervals. The weights used for computing nutrients required will be taken from an average of three weighings taken on consecutive monrings of each 30 day period. The animals will be weighed in the morning before they are fed. Increase in weights will be shown by graphs compared to Eckles normal.

Height at Withers The height at withers will be determined every 30 days. An average of three measurements will be taken with the animal being moved between each determination. The increase in height at withers will be shown in graphical form compared to the Eckles normal.

Consistency of Feces The consistency of feces will be determined for a 14 day period on each animal by the method developed by Moore and Huffman.

Health Any abnormalities in the health of the animals will be investigated by a veterinarian and the results of his findings will be recorded.

Appetite The appetite of the animals during the experiment will be watched by the feeder in charge. He will record any indication of loss of appetite.

Blindness The animals will be watched very carefully for any indication of blindness, and the condition if found will be recorded in detail.

Stiffness and Abnormalities in Gait The heifers will be observed by the feeder in charge while they are in the outside yard. Any condition of stiffness or irregularity in gait will be recorded.

Oxygen Carrying Capacity of the Blood The oxygen carrying capacity of the blood will be determined on each animal every month after the heifers are 15 months old. The gasometric method of Van Slyke (124) will be used. These analyses will be made by the Department of Experiment Station Chemistry.

Photographs Photographs will be made of the heifers at six months, 15 months, and at the time of parturition.

PROCEDURE

Animals Used

The animals were used as originally planned in this experiment.

Age

The heifers in lot I were started on experiment at eight months of age instead of at six months as was originally planned.

Health The heifers were all in good health at the start of the experiment.

Choice of Rations

Milk Milk was fed as originally planned.

Grain Grain was fed as originally planned. Raw cottonseed containing an average of .8 per cent "free gossypol" was obtained for the experiment and was used throughout.

Roughage Roughage was fed as planned. The quality of the silage fed during the latter part of July and the first part of August, 1930, was rather poor. The quality of silage was reduced at that time due to a shortage in supply.

Minerals Minerals were supplied as originally planned.

Management

Water Water was supplied as originally planned, except that from October of 1930 until the completion of the trial, when the animals were housed in the experimental barn annex, which is not equipped with a watering system, the water was supplied in an outside tank.

Shelter The shelter used was as originally planned.

Bedding Wood shavings and sawdust were used throughout the trial for bedding.

Exercise Exercise was allowed as planned.

Sunlight The animals were allowed the benefit of whatever sunlight there was during the time when weather conditions permitted their being out doors.

Care Care was given as originally planned.

Feeding Method The feeding method originally planned was followed throughout the experiment.

DATA

Feed Record

Feed Consumed The record of feed consumed by 30 day periods by all animals is included in Tables I and V.

Nutrients Required The nutrients required as computed from the Armsby Feeding Standard is included with the tables of feed consumed.

Nutrients Consumed The amount of nutrients consumed is included with the above data in Tables I to V. Digestible crude protein and net energy values from Armsby's tables were used in computations of nutrients consumed except in the case of raw cottonseed meats. In the case of the raw cottonseed the digestible crude protein value was obtained by multiplying the nitrogen found on analysis by 6.25 and taking 70 per cent of the protein as digestible, (Henry and Morrison (123b)). A value of 17.15 per cent crude digestible protein was thus obtained. The net energy value was assumed to be approximately 115 therms, considering the raw cottonseed meats to contain 36 per cent oil which was found to be the case on analysis.

Per cent of Raw Cottonseed Meats in the Ration The per cent of raw cottonseed meats in the ration computed on the amount of dry material in the ration for each 30 day period after the animals in lot I were placed on trial is shown in Tables V to VIII.

Amount of Gossypol Ingested The amount of gossypol ingested with the raw cottonseed meats by the animals in lot I is included in Tables V to VIII. Computations of the amount of gossypol ingested is on the basis of the average per centage of gossypol found in samples of the raw cottonseed meats used. This amounted to .8 per cent.

Per cent of Gossypol in the Ration The per cent of gossypol in the ration of the heifers in lot I is included with the above data.

Milligrams of Gossypol per Kilo of Body Weight The milligrams of gossypol per kilo of body weight per day is included with the above data.

Reproduction

Oestrual Periods The occurrence of the first oestrual period is recorded and each consequent period is noted in Table IX for all of the animals in all lots.

Breeding Data The dates on which the heifers were bred is included in Table IX. None of the heifers in either of the three lots have freshened at the time that these data were assembled, hence the observations covering parturition cannot be included.

Observations

Weight The weight of the individual animals in each lot, taken by 30 day periods compared to the Eckles normal, is shown in graphs I to V. The weight by 30 days periods is also shown in tabular form in Table X.

Height at Withers The growth in height at withers of all the animals in the three lots compared to the Eckles normal is shown in graphs I to V. The height at withers taken by 30 days periods is also included in Table X.

Consistency of Feces The consistency of feces of the animals in the three lots over a period of 14 days is shown in Table XI.

Health The health of the animals in all of the three lots was good throughout the experiment. There was no apparent difference in health of the animals in lot I as compared to the two check animals. They have maintained a good condition of flesh since the start of the experiment, and it was impossible to find any evidence of malnutrition in their general appearance.

Appetite The appetite of the two check animals in lot II and lot III was good throughout the experiment except for one instance when G 17 went off feed for a period of a few days. Its appetite returned to normal without treatment, however, and it has continued to eat normally.

The heifers in lot I have had a fairly good appetite during the experiment but have on a few occasions gone off feed. It was found impossible to feed as high a level of raw cottonseed meats as was originally planned, because the unpalatable nature of the meats caused the animals to eat them sparingly on several occasions.

Blindness There was no indication of impaired vision in any of the animals used in this investigation.

Stiffness and Abnormalities in Gait The animals in lot I have shown no stiffness in their joints, no swelling in their legs, nor any noticeable abnormalities in gait at any time during the experiment.

Oxygen Carrying Capacity of the Blood Table XII shows the oxygen carrying capacity of the blood as indicated by its hemoglobin content for all of the animals on experiment.

Photographs The photographs of the animals taken at the start of the experiment and at 15 months of age are included in plates I to VIII.

EXPERIMENTS WITH RATS

While a very marked species difference is known to exist in susceptibility to gossypol poisoning, as has been previously pointed out it was thought desirable to study the effects of gossypol poisoning on rats, using raw cottonseed meats from the same lot of material that was used in feeding the dairy heifers. The toxicity of the raw cottonseed meats could thus be compared to a known value, for raw cottonseed kernels as well as gossypol have been fed to rats by several investigators.

It is also possible in working with rats to study the antidotal effect of various supplements to a basal ration which contains a sufficient amount of gossypol to be injurious to the health of the animals. The amount of time and expense connected with such an investigation in large animals practically prohibits the use of dairy cattle.

The object of the feeding trials with rats was to check the toxicity of the cottonseed meats used in the ration of the dairy heifers by feeding the material directly and by isolating the gossypol from it and feeding it in an otherwise normal diet. It was further desired to study the effect of various supplements to the basal diet used in protecting the animals against poisoning from gossypol.

Animals Used

The rats used in this series of trials were albino and piebald animals. No animals which were known to be cross bred were used in any of the trials.

Age The animals were started on experiment when they were approximately 28 days of age. Up to the time that they were 28 days of age they had been left with the mother and were allowed to eat the same simple ration which she received.

Weight The animals averaged from 50 to 60 grams in weight when started on the trials except in the case of series I in which heavier animals were used. In each series the lots were so arranged that the average weight of each lot was nearly the same as the average for all of the lots.

Sex Both male and female animals were used in these trials. Whenever growth weights were compared animals of the same sex were used. All graphs showing increase or decrease in weight will be labeled with the sex of the animal.

Management

Cages Used Wire cages about 10 inches in diameter and 12 inches high with screen bottoms which allowed the feces and urine to pass through were used in all of the trials.

Method of Feeding Feed was kept before the animals at all times. No attempt was made to measure accurately feed consumption but the appetite of the animals was observed by the relative amount of food eaten daily and by the tendency of some animals to waste feed when the appetite became impaired.

Water Distilled water was kept before the animals at all times.

Diets Used

Series I This series was run to check the toxicity of the ration which was being fed to the dairy heifers on the trial previously reported. The possibility that alfalfa hay carried a factor or factors which might alleviate the injurious effects of gossypol was studied. Raw cottonseed meats taken from the same lot of feed as that fed to the dairy heifers was used as 25 per cent and 50 per cent of the diet. The diets used were:

G 4

raw cottonseed meats	25
alfalfa meal	20
yellow corn	53
steamed bone meal	1
salt	1

G 5

raw cottonseed meats	25
yellow corn	73
steamed bone meal	1
salt	1

G 6

raw cottonseed meats	25
rolled oats	73
steamed bone meal	1
salt	1

G 7

raw cottonseed meats	25
alfalfa	20
rolled oats	53
steamed bone meal	1
salt	1

G 8

linseed oil meal	25
alfalfa meal	20
rolled oats	53
steamed bone meal	1
salt	1

<u>Series II</u> This series was run to determine the effect of feeding cod liver oil and large quantities of yeast with rations containing .2 per cent and 0.1 per cent gossypol acetate. The basal ration used was assumed to be adequate for good growth and the supplements added were in excess of those demanded for an adequate diet which contained no toxic substances.

G W 1. Positive control - basal diet

yellow corn	44
whole wheat flour	40
alfalfa meal	4
casein	10
Ca Co_3	1
salt	1

G W 2. Negative control

Basal diet plus .2 per cent gossypol acetate

G W 3. Negative control

Basal diet plus .1 per cent gossypol acetate

G W 4.

Basal diet plus .2 per cent gossypol acetate plus two per cent cod liver oil.

G W 5.

Basal diet plus .1 per cent gossypol acetate plus two per cent cod liver oil.

G W 6.

Basal diet plus .2 per cent gossypol acetate, 15 per cent whole wheat replaced by 15 per cent yeast.

G W 7.

Basal diet plus .1 per cent gossypol acetate, 15 per cent whole wheat replaced by 15 per cent yeast.

Series III This series was a study of the effect of yeast, cod liver oil and butterfat when added to an adequate diet in alleviating the toxicity of gossypol. Raw cottonseed meats containing approximately .8 per cent gossypol were used at various levels.

G C 1. Positive control - basal diet

rolled oats	84
casein	10
alfalfa meal	4
$Ca\ Co_3$	1
NaCl	1

G C 2. Negative control

rolled oats	74
raw cottonseed	10
casein	10
alfalfa meal	4
$Ca\ Co_3$	1
NaCl	1

G C 3.

Diet G C 2 plus 2 per cent cod liver oil

G C 4.

Diet G C 2 with 10 per cent rolled oats replaced by 10 per cent powdered yeast.

G C 5. Negative control

rolled oats	69
raw cottonseed meats	15
casein	10
alfalfa meal	4
Ca Co_3	1
NaCl	1

G C 6.

Diet G C 5 plus two per cent cod liver oil

G C 7.

Diet G C 5 with 10 per cent rolled oats replaced by 10 per cent powdered yeast

G C 8.

Diet G C 5 with 15 per cent rolled oats replaced by 15 per cent powdered yeast

G C 9.

Diet G C 5 with 10 per cent rolled oats replaced by 10 per cent butterfat

G C 10. Negative Control

rolled oats	64
raw cottonseed meats	20
casein	10
alfalfa meal	4
Ca Co_3	1
NaCl	1

G C 11.

Diet G C 10 with 10 per cent rolled oats replaced by 10 per cent yeast.

G C 12.

Diet G C 10 with 15 per cent rolled oats replaced by 15 per cent yeast.

G C 13.

Diet G C 10 plus two per cent cod liver oil. After one week on this diet the animals were changed to a diet in which 15 per cent rolled oats was replaced by 15 per cent yeast. Two per cent cod liver oil was added as before.

G C 14. Negative control

rolled oats	59
raw cottonseed meats	25
casein	10
alfalfa meal	4
$Ca\ Co_3$	1
NaCl	1

G C 15.

Diet G C 14 with 10 per cent rolled oats replaced by 10 per cent yeast.

G C 16.

Diet G C 16 with 15 per cent rolled oats replaced by 15 per cent yeast.

Collection of Data

Weight The animals were weighed individually when they were placed on experiment and were weighed every seven days thereafter as long as the trial was continued.

Growth The amount of growth measured by increase in weight from week to week is shown in graphical form in graphs VI to XXXII.

Health The growth curve has been used entirely as a measurement of gossypol injury, although there have been other indications of poisoning. Loss of appetite has been one of the first indications of injury. At high levels of intake the injury was acute and death resulted before other symptoms set in. Where chronic injury developed, there was usually extreme irritability. An emaciated condition developed accompanied by a loss of hair on the neck and shoulders.

Determination of Gossypol in Samples of Cottonseed Meal and Raw Crushed Cottonseed Meats

Collection of Samples Five southern oil companies located at various places in the southern states collected samples from the regular mill run of the 1929 crop. Twelve samples of cottonseed meal and 12 samples of raw crushed cottonseed meats, ready for the cooker were collected in the Spring of 1930. The samples of meal and the sample of meats are not in all cases from corresponding "batches". The analysis of the samples was made in the summer of 1930.

Method of Analysis The samples of raw crushed cottonseed meats and cottonseed meal were ground finely enough so that the material would

pass through a 10 mesh sieve. The gossypol content was determined by the aniline method of Carruth (103).

Fifty grams of the material was extracted with ethyl ether in a continuous extractor, of the Soxhlet type, until the distillate was perfectly colorless. The ether was then driven off and the oil which remained was diluted with three volumes of petroleum ether and thoroughly mixed. This solution was allowed to stand in cylinders until the insoluble resinous materials settled out. A 75 per cent aliquot of the liquid was removed from the cylinder and placed in a small precipitating bottle. Three cubic centimeters of aniline were added and the material was set aside for two weeks to allow complete precipitation to take place. The precipitated Dianiline gossypol was recovered on a gooch crucible, washed thoroughly with petroleum ether and dried at 100° C. The weight of the Dianiline derivative multiplied by .775 was taken as the amount of gossypol present in the aliquot of the oil. The amount of gossypol present in the cottonseed meal and in the raw crushed cottonseed meats was expressed in terms of per cent by weight.

Results   The data recorded are shown in Table XIII.

Because of the fact that the cottonseed meal samples analyzed were not from the same "batch" as the raw cottonseed meats no attempt has been made to correlate gossypol content in the finished meal with gossypol content in the original seeds. However, a close observation of these data indicates that there is probably a relationship between the gossypol content of the finished meal and the conditions under which it was processed. The mill using cottonseed meats having the lowest gossypol content of any

analyzed, furnished samples of meal having a higher gossypol content than the average of meals from all of the mills.

It will be observed that none of the samples of cottonseed meal analyzed had a gossypol content of more than five one hundredths of one per cent and there are but eight samples having a gossypol content over four one hundreths of one per cent. This consistently low gossypol content in samples of meal from various regions and different mills indicates that there is almost no possibility of getting a lot of cottonseed meal that will contain more than six per cent of the amount that has been present in the raw crushed cottonseed meats which have been fed without injury to growing dairy heifers.

Discussion of Experimental Results

A study of Tables I to V showing feed consumed, nutrients required and nutrients consumed shows that the animals in lot I, G 20, G 21, and G 22, received less digestible protein than the amount required according to the Armsby Standard. During several thirty day periods the deficiency in amount of protein as compared with the requirement was considerable. This deficiency in amount of protein was due largely to the fact that the heifers would not eat a sufficient amount of raw cottonseed meats to furnish the necessary protein. The raw cottonseed meats were unpalatable having a rather bitter residual taste and being very high in oil. The energy furnished by the ration fed to the animals of lot I was above the requirements during most of the thirty day periods included in the experiments. The rations of the heifers in lot II and lot III furnished an excess of protein and a small excess of energy throughout the experiment.

Despite the fact that the heifers in lot I received less digestible protein than those in Lot II and lot III their growth was very comparable. All of the heifers were below the Eckles normal in weight and height at withers during a part of the growing period. At two years of age, however, all of the animals were normal or above, as compared with the Eckles standard, and there was no significant difference between the heifers of lot I and the two check animals in growth.

The ration of the heifers in lot I contained on the average from 20 to 25 per cent raw cottonseed meats during the experiment as Tables VI, VII, and VIII indicate. This amount of raw cottonseed meats represented approximately .2 per cent of gossypol in the ration. The amount of gossypol in the ration was three times the amount found to be toxic to rats by Schwartz and Alsberg (90) and four times that found to be toxic to rats by Clarke, yet the heifers showed no indication of injury. In view of the fact that rats were found relatively much less susceptible to gossypol poisoning than rabbits and swine, this level of two per cent of gossypol in the ration of growing heifers indicates a very high species tolerance for gossypol.

On the basis of milligrams of gossypol per kilo of body weight consumed daily the heifers in lot I had a lower intake of gossypol than was found to be the threshhold of toxicity for rats by Schwartz and Alsberg. The average for all of the heifers in lot I throughout the experiment was 31.8 milligrams of gossypol per kilogram of body weight, which is about one-half the amount found to be toxic to rats by the above cited experimentors.

There was no significant differences between the heifers in lot I and the two check animals in the oxygen carrying capacity of the blood as indicated by its hemoglobin content.

In the experiments with rats series I, a diet similar to the ration fed the heifers was used in which a 25 per cent level of raw cottonseed meats was used. As graphs VI to IX show, the rats lost weight steadily and died in from 10 to 28 days or were killed because of their extremely emaciated condition. A diet high in vitamin A (G 4 and G 7) showed no superiority over one low in vitamin A (G 6) in lessening the injurious effects produced by the cottonseed meats. Vitamin A was furnished by alfalfa hay which was used as 20 per cent of the diets of G 4 and G 7.

The failure of the heifers to show any injury in response to high gossypol feeding may be accounted for on two premises. First, that in the digestive tract of the ruminant conditions exist such that gossypol is not absorbed, or if it is absorbed, is changed chemically so as to be less toxic, or second, that the gossypol intake per unit of body weight was not high enough in this experiment to reach the threshhold of toxicity. In view of the fact that swine, rabbits, and guinea pigs are poisoned by very small quantities of gossypol per unit of body weight and because there are not clear cut cases of cottonseed meal injury in ruminants which can be directly traced to gossypol poisoning it seems quite likely that the failure of the heifers to show any injury from gossypol feeding is due to conditions along the digestive tract, peculiar to the ruminant. What these conditions may be cannot readily be suggested.

The results of gossypol analysis on samples of cottonseed meal which show a consistently low content of gossypol in the meal from various mills indicates that there is practically no possibility of the dairyman getting a meal which contains enough gossypol to be toxic to dairy cattle even though it be fed in excessively large amounts.

Throughout the experiment the feces of the heifers in lot I were much harder in consistency than those of the two check animals. Whether or not this difference is due to the presence of gossypol in the ration is not known but it seems likely that the condition causing the hard consistency may be associated with the presence of a large amount of cottonseed oil which with its predominance of fatty acids of high molecular weight may upon saponification form heavy soapy material that tends to make hard well formed feces.

This condition which arises from the feeding of raw crushed cottonseed meats having a high oil content may in part explain why cottonseed meal has in the past been considered costive although its effect as has been shown by Huffman and Moore is actually laxative.

The results with rats, series II and series III, indicate clearly that where gossypol is fed at a comparatively low level but at a level which still causes injury, the addition of yeast as 15 per cent of the diet decreases the injurious effect to such an extent that the animals grow normally. Cod liver oil used as two per cent of the diet relieved the injury to a lesser extent but the beneficial effect did not appear to be permanent.

The effect of yeast cannot be attributed to bolstering a vitamin B

deficient diet. The diet used in series II and series III was probably not deficient in vitamin B. The effect of yeast on the intestinal tract is far reaching. It is well known that it changes the intestinal flora and in the review of literature it was pointed out that it is beneficial in counteracting the injury occurring from the feeding of high protein diets which cause intestinal putrefaction with consequent formation of ptomaines. It was also shown that it is effective in counteracting the toxic effect of iso amylamine in the diet. The mechanism of its beneficial effect has not been explained in these cases nor can it be explained in this connection but it seems likely that its effect is restricted to conditions along the intestinal tract.

The temporary beneficial effect of cod liver oil in relieving the injurious effect produced by gossypol feeding cannot readily be explained.

Summary

1. Raw crushed cottonseed meats (gossypol content eight tenths per cent) were fed in amounts verying from 20 to 25 per cent of a ration containing silage and good quality hay without producing injury in growing dairy heifers. No lameness, swollen legs or impaired vision resulted. The hemoglobin content of the blood was not decreased.

2. Gossypol analysis of cottonseed meal samples showed that a maximum of .05 per cent gossypol may normally occur. This is but six per cent of the amount present in the raw crushed cottonseed meats used in the experiment.

3. The feeding of raw cottonseed meats to dairy heifers resulted in the formation of hard well formed feces, an effect distinctly different from that produced by the feeding of cottonseed meal.

4. Rats died in 10 to 28 days when fed a diet containing the same proportions of raw cottonseed meats that were fed without injury to heifers. Equivalent amounts of crude gossypol acetate produce similar results.

5. The addition of massive doses of yeast (15 per cent) to a diet containing an injurious amount of gossypol, alleviated the injurious effect and allowed for practically normal growth in rats.

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A P P E N D I X

Table I. Animal G 20 - Feed Consumed by 30 Day Periods from Birth to Two Years of Age. Average Daily Requirements According to the Armsby Standard and the Consumption of Digestible Protein and Energy.

	Feed Consumed								Nutrients Required			Nutrients Consumed		
Age For Period	Weight	Whole Milk	Skim Milk	Cod Liver Oil	Corn and Oats	Alfalfa Hay	Silage	Raw C.S.	Dig. Crude Protein	Energy	Protein from C. S.	Total Protein	Protein Intake Compared with Standard	Energy
	Lbs.	Lbs.	Lbs.	grs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Therms.	Lbs.	Lbs.	Lbs.	Therms.
19	75.5	102		160								.180		1.557
49	108.0	204	83	300	5.0	3.5			.411	3.169		.351	-.060	2.526
79	149.0		360	300	37.0	6.0			.497	3.493		.562	.065	2.662
109	195.0		360	70	77.0	40.0			.591	3.736		.798	.207	3.980
139	255.0		360	Gr.Mix.	102.5	53.5			.672	4.009		.920	.248	4.733
169	293.0		360	67	28.0	24.0 Timothy 52.5			.718	4.212		.990	.272	5.019
199	307		165	72.5		101.0			.732	4.296		.666	-.066	3.992
229	360			120.	Corn	141.0	36		.773	4.731		.762	-.011	5.122
259	388			30.	9.2	150.0	97	36.0	.792	4.992	.206	.565	-.222	5.033
289	400.3				12.0	141.0	132	57.1	.800	5.104	.326	.503	-.297	5.252
319	449.7				12.0	112.0	150	64.2	.816	5.528	.367	.562	-.254	5.203
349	496.7				12.0	165.0	194	69.0	.832	5.944	.394	.658	-.174	6.380
379	501.0				12.0	160.0	240	58.2	.833	5.975	.333	.608	-.225	6.138
409	540.0				12.0	137.0	249	52.9	.843	6.217	.302	.558	-.285	5.754
439	590.0				12.0	153.0	186	69.0	.85	6.720	.394	.643	-.207	6.166
469	615.0				10.2	150.0	362	80.4	.85	6.912	.460	.766	-.084	7.442
499	620.0				12.0	149.0	289	85.8	.85	6.96	.490	.753	-.097	7.264
529	670.0				12.0	159.0	436	90.0	.85	7.360	.515	.861	.011	8.382
559	661.0				12.0	165.0	417	67.0	.85	7.288	.383	.729	-.121	7.485
589	712.0				12.0	180.0	480	80.0	.85	7.672	.452	.841	-.009	8.533
619	744.0				12.0	180.0	480	90.0	.85	7.864	.515	.898	.048	8.916
649	761.0				12.0	169.0	457	81.0	.85	7.960	.463	.827	-.023	8.391
679	796.0				12.0	180.0	401	65.5	.85	8.170	.375	.729	-.121	7.648
709	844.0				12.0	180.0	428	75.0	.85	8.400	.429	.795	-.055	8.155
739					12.0	180.0	480	75.0	.85		.429	.868	-.018	8.462

Table II. Animal G 21 - Feed Consumed by 30 Day Periods from Birth to Two Years of Age. Average Daily Requirements According to the Armsby Standard and the Consumption of Digestible Protein and Energy.

		Feed Consumed							Nutrients Required		Nutrients Consumed			
Age for Period	Weight	Whole Milk	Skim Milk	Cod Liver Oil	Corn and Oats	Alfalfa Hay	Silage	Raw Cotton Seed	Dig. Crude Protein	Energy	Protein from R. C. S.	Total Protein	Protein Intake Compared With Standard	Energy
	Lbs.	Lbs.	Lbs.	cc.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Therms	Lbs.	Lbs.	Lbs.	Therms
19*	70	102										.177		1.557
49	107.3	204	84	160	4.5	4.5			.410	3.160		.354	-.056	2.530
79	150		360	300	50.0	14.0			.500	3.500		.628	.128	3.058
109	196.7		360	300	77.0	43.0			.594	3.745		.808	.214	4.012
139	255.0		360	70	102.5	80.5			.672	4.009		1.014	.342	4.695
				Gr.Mix.		24.0								
169	293.0		360	63	28.0	Tim.Hay			.718	4.212		.968	.250	4.890
						50.3								
199	309.0		165	72		101.0			.734	4.308		.666	-.068	3.992
229	355			120	Corn	141.0	26.0		.770	4.684		.760	-.010	5.025
259	383			50	9.5	150.0	97.0	35.0	.788	4.945	.200	.561	-.227	5.010
289	425.7				12.0	150.0	130.0	64.4	.808	5.329	.368	.595	-.213	5.630
319	446				12.0	120.0	150.0	64.2	.815	5.502	.367	.570	-.245	5.318
349	491				12.0	159.0	194.0	69.0	.830	5.892	.394	.652	-.178	6.294
379	535				12.0	180.0	240.0	69.0	.845	6.269	.394	.690	-.155	6.839
409	564				12.0	158.0	242.0	77.0	.85	6.512	.440	.714	-.136	6.844
439	599				12.0	138.0	366.0	84.0	.85	6.792	.480	.780	-.070	7.480
469	623.3				12.0	120.0	368.0	84.0	.85	6.984	.480	.763	-.093	7.238
499	651.7				12.0	149.0	289.0	86.4	.85	7.056	.493	.775	-.075	7.297
529	676.7				12.0	158.5	424.0	85.7	.85	7.408	.490	.832	-.018	8.146
559	681.7				12.0	167.0	446.0	61.5	.85	7.456	.352	.710	-.140	7.657
589	722.3				12.0	180.0	480.0	83.5	.85	7.732	.477	.861	.011	8.677
619	759.				12.0	180.0	480.0	90.0	.85	7.948	.514	.898	.048	8.916
649	789				12.0	180.0	480.0	90.0	.85	8.128	.514	.898	.048	8.916
679	832.3				12.0	180.0	404.0	90.0	.85	8.380	.514	.870	.020	8.513
709	879				12.0	180.0	428.0	90.0	.85	8.680	.514	.879	.029	8.640
739	913				12.0	180.0	480.0	90.0	.85	8.892	.514	.898	.048	8.916

*First period 19 days

Table III. Animal G 22 - Feed Consumed by 30 Day Periods from Birth to Two Years of Age. Average Daily Requirements According to the Armsby Standard and the Consumption of Digestible Protein and Energy.

	Feed Consumed								Nutrients Required			Nutrients Consumed		
Age for Period	Weight	Whole Milk	Skim Milk	Cod Liver Oil	Corn and Oats	Alfalfa Hay	Silage	Raw C.S.	Dig. Crude Protein	Energy	Protein from C. S.	Total Protein	Protein Intake Compared with Standard	Energy
	Lbs.	Lbs.	Lbs.	cc.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Therms.	Lbs.	Lbs.	Lbs.	Therms.
13	98.7	86		110	0.5	.5			.400	3.100		.218	-.182	1.919
43	127.0	313		300	6.0	8.0			.438	3.331		.390	-.048	3.255
73	167.0	24	336	Gr.Mix.	72.5	23.0			.553	3.609		.723	.170	3.807
103	214.0		360	3	74.0	64.0			.617	3.823		.702	.085	4.239
133	253.0		360	74		Timothy 71.0			.670	4.000		.877	.207	4.529
163	285.0		360	61		112.0			.710	4.164		.853	.143	4.801
193	325.0		228	44		150.0	36		.750	4.404		.660	-.090	4.036
223	338			60	Corn	148.0	96		.759	4.525		.487	-.272	4.085
253	385.3			22	7.6	150.0	120	25.0	.790	4.964	.143	.465	-.325	4.530
283	412.3				12.0	150.0	128	48.0	.804	5.208	.278	.503	-.301	5.072
313	450.0				12.0	165.0	156	69.0	.816	5.528	.394	.644	-.172	6.178
343	505.3				12.0	180.0	180	69.0	.835	6.009	.394	.668	-.167	6.521
373	543.0				12.0	206.0	184	77.0	.848	6.338	.440	.741	-.107	7.271
403	557.7				12.0	138.0	366	84.0	.85	6.464	.480	.780	-.070	7.490
433	588.3				12.0	147.0	356	84.0	.85	6.704	.480	.779	-.071	7.407
463	605.3				12.0	150.0	287	84.0	.85	6.840	.480	.763	-.087	7.233
493	665.3				12.0	158.5	421.6	89.2	.85	7.320	.510	.854	-.004	8.268
523	697.6				12.0	174.0	471.0	90.0	.85	7.584	.515	.890	.04	8.793
553	720.0				12.0	180.0	542.0	99.5	.85	7.720	.569	.976	.126	9.609
583	765.7				12.0	180.0	534.0	105.0	.85	7.990	.600	1.000	.150	9.590
613	768.3				12.0	180.0	458	102.0	.85	8.002	.583	.960	.110	9.259
643	803.3				12.0	180.0	404	100.0	.85	8.218	.572	.928	.078	8.883
673	828.3				12.0	180.0	426	90.0	.85	8.368	.515	.879	.029	8.680
703	902.3				12.0	169.0	408	78.0	.85	8.810	.446	.793	-.057	7.916

Table IV. Animal G 17 - Feed Consumed by 30 Day Periods from Birth to Two Years of Age. Average Daily Requirements According to the Armsby Standard and the Consumption of Digestible Protein and Energy.

	Feed Consumed							Nutrients Required		Nutrients Consumed			
Age for Period	Weight	Whole Milk	Skim Milk	Corn and Oats	Timothy Hay	Silage	C. S. Oil Meal	Dig. Crude Protein	Energy	Protein from C. S. M.	Total Protein	Protein Intake Compared with Standard	Energy
	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Therms	Lbs.	Lbs.	Lbs.	Therms
38*	107	306		2	.5			.410	3.160		.343	-.067	3.014
68	139	84	276	17.5	8.5			.463	3.427		.483	.020	2.667
98	175		360	54.0	15.0			.564	3.645		.605	.041	3.082
128	210		360	26.0 Corn 21.0	54.0		8.0	.611	3.804	.099	.709	.098	3.918
158	258		318	30.0	106.0	52.0	12.0	.677	4.022	.148	.724	.047	4.529
188	308		294	30.0	141.0	62.0	22.8	.733	4.302	.281	.867	.134	5.315
218	333			15.6	150.0	90.0	52.8	.755	4.479	.651	.870	.115	4.716
248	350			12.0	150.0	90.0	62.0	.767	4.637	.765	.975	.209	4.900
278	374			12.0	150.0	90.0	63.0	.783	4.861	.777	.988	.205	4.932
308	401			12.0	143.0	143.0	65.0	.800	5.112	.802	1.025	.225	5.175
338	443			12.0	180.0	192.0	66.0	.814	5.476	.814	1.092	.278	5.996
368	462			12.0	180.0	210.0	66.0	.820	5.641	.814	1.099	.279	6.091
398	517			12.0	180.0	210.0	68.0	.839	6.113	.844	1.128	.280	6.166
428	552			12.0	180.0	210.0	67.1	.85	6.416	.828	1.112	.262	6.125
458	593			12.0	180.0	297.0	69.0	.85	6.744	.851	1.168	.318	6.645
488	651			12.0	180.0	428.0	69.0	.85	7.208	.851	1.216	.366	7.339
518	683			12.0	180.0	461.0	69.0	.85	7.464	.851	1.228	.378	7.514
548	718			12.0	186.0	504.0	69.0	.85	7.708	.851	1.249	.399	7.828
578	720.3			12.0	210.0	470.0	69.0	.85	7.720	.851	1.261	.411	7.992
608	726.3			12.0	169.0	254.7	69.0	.85	7.756	.851	1.141	.291	5.863
638	794.0			12.0	207.5	526.1	69.0	.85	8.158	.851	1.279	.429	8.254
668	819.0			12.0	224.0	563.3	69.0	.85	8.314	.851	1.309	.459	8.687
698	849.0			12.0	239.0	598.0	69.0	.85	8.560	.851	1.337	.487	9.066
728	890.0			12.0	240.0	600.0	69.0	.85	8.740	.851	1.339	.489	9.111

*First Period 38 days

Table V. **Animal G 16 - Feed Consumed by 30 Day Periods from Birth to Two Years of Age. Average Daily Requirements According to the Armsby Standard and the Consumption of Digestible Protein and Energy.**

	Feed Consumed							Nutrients Required		Nutrients Consumed			
Age for Period	Weight	Whole Milk	Skim Milk	Corn and Oats	Timothy Hay	Silage	Linseed Oil Meal	Dig. Crude Protein	Energy	Protein from L.O.M.	Total Protein	Protein Intake Compared with Standard	Energy
	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Therms	Lbs.	Lbs.	Lbs.	Therms.
27*	97	191						.400	3.100		.210	-.1899	1.850
57	125.3	216	132	5.0	4.0			.435	3.314		.414	-.0211	2.890
87	169		360	41.0	31.0			.555	3.618		.683	.1279	3.138
117	200.0		360	87.0	42.0			.598	3.759		.729	.1316	4.386
147	260.0		360	30.5 Corn 20.0	90.0		9.5	.679	4.031	.100	.757	.0774	4.407
177	282.0		102	30.0	150.0	50	15.0	.707	4.146	.159	.518	-.1888	4.180
207	328.0			30.0	167.0	86	66.3	.752	4.432	.701	.968	.2161	5.584
237	360.0			5.0	173.0	113	74.4	.773	4.731	.786	1.012	.3390	5.337
267	390.0				180.0	120	77.0	.793	5.010	.814	1.038	.2447	5.402
297	423.0				180.0	120	78.0	.807	5.303	.824	1.048	.2411	5.430
327	443.0				195.0	172	84.0	.814	5.476	.888	1.146	.3320	6.091
357	484.0				210.0	223.	84.0	.827	5.831	.888	1.179	.3522	6.584
387	515.0				210.0	240	81.0	.838	6.096	.856	1.148	.3097	6.499
417	567.0				210.0	240	81.0	.85	6.536	.856	1.148	.3097	6.499
447	600.0				210.0	240	81.0	.85	6.800	.856	1.148	.3097	6.499
477	625.0				210.0	336	81.0	.85	7.000	.856	1.189	.3344	7.090
507	695.0				210.0	432	81.0	.85	7.560	.856	1.224	.3742	7.599
537	702.0				210.0	466	81.0	.85	7.612	.856	1.237	.3868	7.779
567	736.0				210.0	504	81.0	.85	7.816	.856	1.251	.4007	7.981
597	760.0				212.0	470	81.0	.85	7.954	.856	1.237	.3874	7.790
627	780.0				240.0	385	81.0	.85	8.074	.856	1.237	.3871	7.780
657	833.0				240.0	600	81.0	.85	8.400	.856	1.316	.4659	8.879
687	851.0				240.0	600	82.6	.85	8.560	.873	1.333	.4828	8.974
717	899.0				240.0	600	84.0	.85	8.790	.888	1.348	.4976	9.005
747	932.0				240.0	600	84.0	.85	9.000	.888	1.348	.4976	9.005

*First Period 27 days

Table VI. Animal G 20 - Per cent of Raw Cottonseed and Per cent of Gossypol in the Ration. Milligrams of Gossypol per Kilogram of Body Weight, 30 Day Periods.

Age for Period	Weight Lbs.	Weight Kilos	Total Food Consumed Dry Basis Lbs.	Raw Crushed Cottonseed Consumed Lbs.	Per cent Raw Cottonseed in Total Ration Per cent	Amount of Gossypol Consumed Lbs.	Per cent Gossypol in Total Ration Per cent	Grams of Gossypol Consumed per day Grams	Mg. of Gossypol per Kilo of Body Weight
259	388.0	176.0	198.93	32.6	16.39	.288	.1448	4.35	24.72
289	400.3	181.6	221.78	51.72	23.32	.457	.2060	6.91	38.05
319	449.7	204.0	207.35	58.35	28.14	.514	.2477	7.76	38.04
349	496.7	225.3	270.13	62.51	23.14	.552	.2043	8.35	37.06
379	501.0	227.2	277.03	52.73	19.03	.466	.1681	7.03	30.94
409	540.0	244.9	245.16	47.93	19.55	.423	.1726	6.40	26.13
439	590.0	267.5	257.42	62.51	24.28	.552	.2144	8.35	31.21
469	615.0	278.8	309.77	72.84	23.51	.643	.2076	9.39	33.68
499	620.0	281.2	296.19	77.73	26.24	.686	.2317	10.37	35.01
529	670.0	303.4	347.49	81.54	23.46	.720	.2072	10.89	35.89
559	661.0	299.7	326.97	60.70	18.56	.536	.1639	8.10	27.03
589	712.0	322.8	368.58	72.48	19.66	.640	.1736	9.68	29.99
619	744.0	337.5	377.64	81.54	21.59	.720	.1907	10.89	32.27
649	761.0	345.5	353.72	73.39	20.75	.648	.1832	9.80	28.36
679	796.0	361.1	334.75	59.43	17.76	.524	.1565	7.92	21.93
709	844.0	382.8	350.37	67.95	19.39	.600	.1712	9.07	23.69
739			363.86	67.95	18.67	.600	.1649	9.07	

Table VII. Animal G 21 - Per cent of Raw Cottonseed and Per cent of Gossypol in the Ration. Milligrams of Gossypol per Kilogram of Body Weight, 30 Day Periods.

Age for Period	Weight Lbs.	Weight Kilos	Total Food Consumed Dry Basis Lbs.	Raw Crushed Cottonseed Consumed Lbs.	Per cent Raw Cottonseed in Total Ration Per cent	Amount of Gossypol Consumed Lbs.	Per cent Gossypol in Total Ration Per cent	Grams of Gossypol Consumed per day Grams	Mg. of Gossypol per Kilo of Body Weight
259	383.0	173.72	198.12	31.71	16.00	.280	.1413	4.23	24.35
289	425.7	193.09	235.88	58.35	24.74	.515	.2181	7.79	40.34
319	446.0	202.30	214.43	58.16	27.12	.514	.2395	7.76	38.36
349	491.0	222.7	264.83	62.51	23.60	.552	.2084	8.34	37.45
379	535.0	242.7	294.49	62.51	21.27	.552	.1874	8.34	34.36
409	564.0	255.8	273.58	69.77	25.50	.616	.2252	9.31	36.40
439	599.0	271.7	305.09	76.10	24.94	.672	.2003	10.16	37.39
469	623.3	282.7	288.96	76.10	26.34	.672	.2355	10.16	35.94
499	631.7	286.5	296.75	78.29	26.38	.691	.2329	10.45	36.47
529	676.7	306.9	340.00	77.64	22.83	.686	.2016	10.36	33.76
559	681.7	309.2	331.49	55.72	16.80	.492	.1484	7.44	24.06
589	722.3	327.6	371.75	75.65	20.35	.668	.1797	10.03	30.67
619	759.0	344.3	377.64	81.54	21.59	.720	.1906	10.89	31.63
649	789.0	357.9	377.64	81.54	21.59	.720	.1906	10.89	30.43
679	832.3	377.5	357.65	81.54	22.80	.720	.2013	10.89	28.85
709	879.0	398.7	363.96	81.54	22.39	.720	.1978	10.89	27.31
739	913.0	414.1	377.64	81.54	21.59	.720	.1906	10.89	26.30

Table VIII. Animal G 22 - Per cent of Raw Cottonseed and Per cent of Gossypol in the Ration. Milligrams of Gossypol per Kilogram of Body Weight, 30 Day Periods.

Age for Period	Weight Lbs.	Weight Kilos	Total Food Consumed Dry Basis Lbs.	Raw Crushed Cottonseed Consumed Lbs.	Per Cent Raw Cottonseed in Total Ration Per cent	Amount of Gossypol Consumed Lbs.	Per cent Gossypol in Total Ration Per cent	Grams of Gossypol Consumed per day Grams	Mg. of Gossypol per Kilo of Body Weight
253	385.3	174.8	193.61	22.65	11.70	.200	.1033	3.02	17.28
283	412.3	187.0	220.49	43.49	19.72	.384	.1741	5.81	31.07
313	450.0	204.1	260.14	62.51	24.03	.552	.2122	8.35	40.91
343	505.3	229.2	279.71	62.51	22.35	.552	.1973	8.35	36.43
373	543.0	243.3	310.99	69.76	22.43	.616	.1980	9.31	38.27
403	557.7	253.0	305.09	76.10	24.94	.672	.2203	10.16	40.16
433	588.3	266.8	310.42	76.10	24.52	.672	.2165	10.16	38.08
463	605.3	274.6	294.92	76.10	25.80	.672	.2279	10.16	37.00
493	665.3	301.8	352.55	80.82	22.92	.714	.2024	10.79	35.75
523	697.6	316.4	379.97	81.54	21.46	.720	.1895	10.89	34.42
553	720.0	326.6	402.56	90.15	22.39	.796	.1977	12.03	36.83
583	765.7	347.3	405.43	95.13	23.46	.840	.2072	12.70	36.57
613	768.3	348.5	382.72	92.41	24.15	.816	.2132	12.34	35.41
643	803.3	364.4	366.71	90.60	24.71	.800	.2182	12.10	33.21
673	828.3	375.7	363.44	81.54	22.44	.720	.1981	10.89	28.99
703	902.3	409.3	338.31	70.86	20.95	.567	.1675	9.62	23.50

Table IX. Breeding Record Lot I

	Animal G 20				Animal G 21				Animal G 22			
	Date	Age Days	Oestrual Cycle Days	Bred	Date	Age Days	Oestrual Cycle Days	Bred	Date	Age Days	Oestrual Cycle Days	Bred
Born	4-15-29	0			4-15-29	0			5-21-29	0		
Placed on Experiment	12- 7-29	236			12- 7-29	236			1-10-30	234		
1st Oestrual Period	5-20-30	400		No	6- 8-30	419		No	8-29-30	465		Yes
2nd Oestrual Period	6-13-30	424	24	No	6-18-30	490	71	Yes	10- 6-30	503	38	Yes
3rd Oestrual Period	7-10-30	451	27	Yes	9-11-30	514	24	Yes	11- 7-30	545	32	Yes
4th Oestrual Period	8- 3-30	475	24	Yes	9-23-30	526	12	Yes	12-12-30	580	35	Yes
5th Oestrual Period	10- 3-30	536	61	Yes	11-24-30	588	62	Yes	1- 4-31	603	23	Yes
6th Oestrual Period	12-31-30	625	89	Yes								
	Examined - Found Pregnant 4-10-31				Examined - Found Pregnant 4-10-31				Examined - Found Pregnant 4-10-31			

Table IX. (Cont.) Breeding Record Lots II and III

	Lot II				Lot III			
	Animal G 17				Animal G 16			
	Date	Age Days	Oestrual Cycle Days	Bred	Date	Age Days	Oestrual Cycle Days	Bred
Born	12-27-28	0			12- 9-28	0		
Placed on Experiment	7-13-29	198			7- 3-29	207		
1st Oestrual Period	4- 4-30	463		Yes	2-13-30	432		No
2nd Oestrual Period	5- 3-30	492	29	No	3- 8-30	455	23	No
3rd Oestrual Period	5-22-30	511	19	No	3-28-30	475	20	Yes
4th Oestrual Period	6-10-30	530	19	Yes	4-23-30	501	26	Yes
5th Oestrual Period	6-29-30	549	19	Yes	5-14-30	522	21	Yes
6th Oestrual Period	9-15-30	637	88	Yes	6- 4-30	543	21	Yes
7th Oestrual Period	10-22-30	657	22	Yes	6-25-30	564	21	Yes
8th Oestrual Period	11-19-30	685	28		7-31-30	600	36	Yes
	Examined - Found Pregnant 4-10-31				Examined - Found Pregnant 10-27-30			

Table X. Showing Weight and Height at Withers of All Animals on Experiment as Compared with Eckles Normal

Age Mos.	Eckles Normal Weight	Eckles Normal Height at Withers	Animal G 20 Weight	Animal G 20 Height at Withers	Animal G 21 Weight	Animal G 21 Height at Withers	Animal G 22 Weight	Animal G 22 Height at Withers	Animal G 17 Weight	Animal G 17 Height at Withers	Animal G 16 Weight	Animal G 16 Height at Withers
	lbs.	cm.	lbs.	cm.	lbs.	cm.	lbs.	cm.	lbs.	cm.	lbs.	cm.
0	90	71.8	63	71.0	70	69.0	93		82	71.0	88	73
1	121	76.8	108	76.0	107	75.7	127	82	107	74.0	97	76
2	157	82.2	149	83.3	150	83.0	167	87	139	77.0	125	80
3	200	87.0	195	88.0	197	90.0	214	97.7	175	82.0	169	83.5
4	249	92.3	255	94.0	255	94.0	253	100.0	210	85.0	200	86.0
5	302	96.9	293	98.0	293	97.7	285	103.0	258	89.0	260	91.0
6	349	101.2	307	100.7	309	100.0	325	106.0	308	96.0	282	93.0
7	389	104.4	360	100.0	355	101.7	338	107.0	333	100.0	328	99.7
8	425	107.4	388	104.0	383	104.7	385	109.7	350	103.0	360	103.0
9	466	109.4	400	107.0	426	107.0	412	110.7	374	103.0	390	106.0
10	501	111.4	450	108.0	446	108.0	450	112.7	401	105.0	423	108.0
11	529	112.8	497	111.7	491	112.0	505	118.0	443	107.0	443	109.0
12	558	114.3	501	113.0	535	112.0	542	117.7	462	110.0	484	110.0
13	574	116.0	540	114.7	564	115.0	558	117.7	517	110.0	515	111.3
14	596	117.7	590	115.7	599	116.0	588	118.7	552	112.0	567	115.3
15	612	119.1	615	117.7	623	117.7	605	120.0	593	114.0	600	115.0
16	643	120.5	620	118.0	632	118.0	665	121.5	651	115.0	625	116.7
17	660	121.2	670	118.2	677	119.8	698	125.0	683	118.7	695	117.7
18	686	121.7	661	120.0	682	120.0	720	126.7	718	119.7	702	119.0
19	715	122.6	712	121.2	722	122.2	765	127.7	720	120.2	736	122.0
20	746	123.7	744	122.3	759	123.7	768	128.3	726	122.0	760	123.7
21	774	124.2	761	124.5	789	124.3	803		794	122.7	780	125.0
22	796	124.9	796	124.8	832	125.0	828	128.3	819	122.2	833	122.2
23	824	125.7	844	125.7	879	124.7	902	129.7	849	125.7	851	122.3
24	841	126.3		127.8	913	127.2			890	125.7	899	126.3

Table XI. Consistency of Feces of the Heifers in all Lots for a Fourteen Day Period

	Lot III	Lot II	Lot I		
Date	G 16 Linseed Oil Meal	G 17 Cottonseed Meal	G 20 Raw Cotton seed	G 21 Raw Crushed Cottonseed	G 22 Raw Crushed Cottonseed
March 29	.65	.80	.35	.30	.25
30	.85	.50	.50	.25	.20
31	.60	.55	.35	.25	.25
April 1	.90	.80	.30	.30	.15
2	.55	.55	.30	.25	.15
3	.70	.80	.40	.25	.20
4	.70	.70	.35	.25	.40
5	.40	.50	.45	.35	.20
6	.80	.70	.65	.30	.30
7	1.10	.70	.40	.30	.30
8	.80	.65	.40	.35	.20
9	.70	.55	.35	.30	.20
10	.65	.40	.30	.25	.20
11	1.00	.65	.45	.40	.35
Ave. for 14 days	.743	.632	.396	.293	.239
Ave. for Lot I	.309				

Standard

1.5 very soft	.75 average
1.25 medium soft	.50 medium hard
1.00 soft	.25 very hard

.15 exceedingly hard

Table XII. Hemoglobin Content of the Blood of All the Animals for Last Nine Months on Experiment

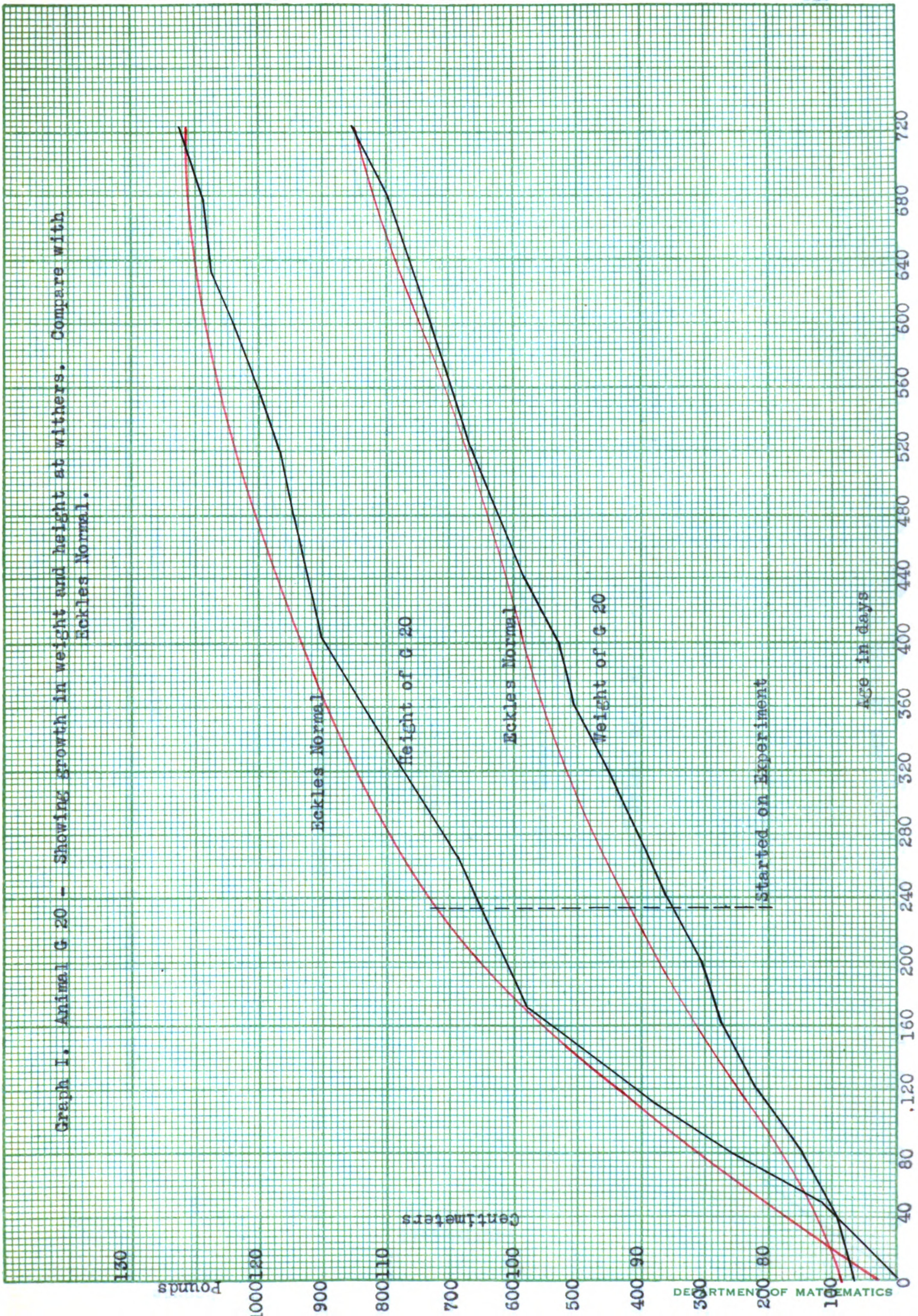
	Animal G 20	Animal G 21	Animal G 22	Animal G 17	Animal G 16
	hemoglobin per cent	hemoglobin per cent	hemoglobin per cent	hemoglobin per cent	hemoglobin per cent
8- 6-30	77.8	82.9	79.2	75.6	80.7
9- 8-30	74.4	71.5	63.8	74.6	82.7
10-15-30	55.0	79.7	73.6	73.3	81.0
11-19-30	86.4	80.2	81.2	91.0	85.7
12-11-30	84.6	90.0	88.7	89.7	101.1
1-14-31	86.5	86.5	99.0	90.7	96.5
2-11-31	89.1	87.0	85.0	88.5	94.7
3-11-31	89.0	77.8	81.9	95.3	73.0
4-24-31	86.9	92.6	92.6	96.2	90.9

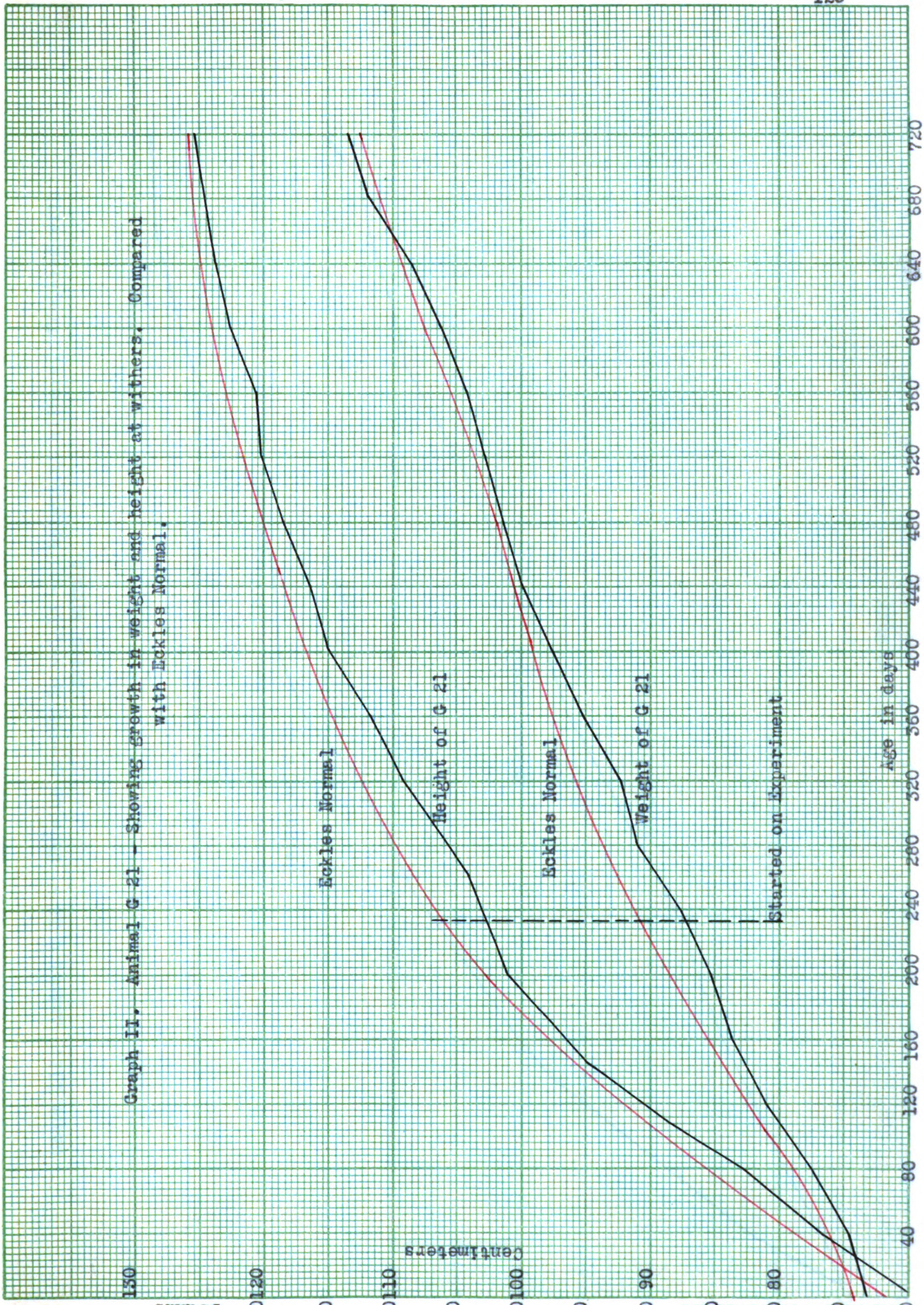
Table XIII. Per cent of Gossypol in Samples of Cottonseed Meal and Raw Crushed Cottonseed Meats

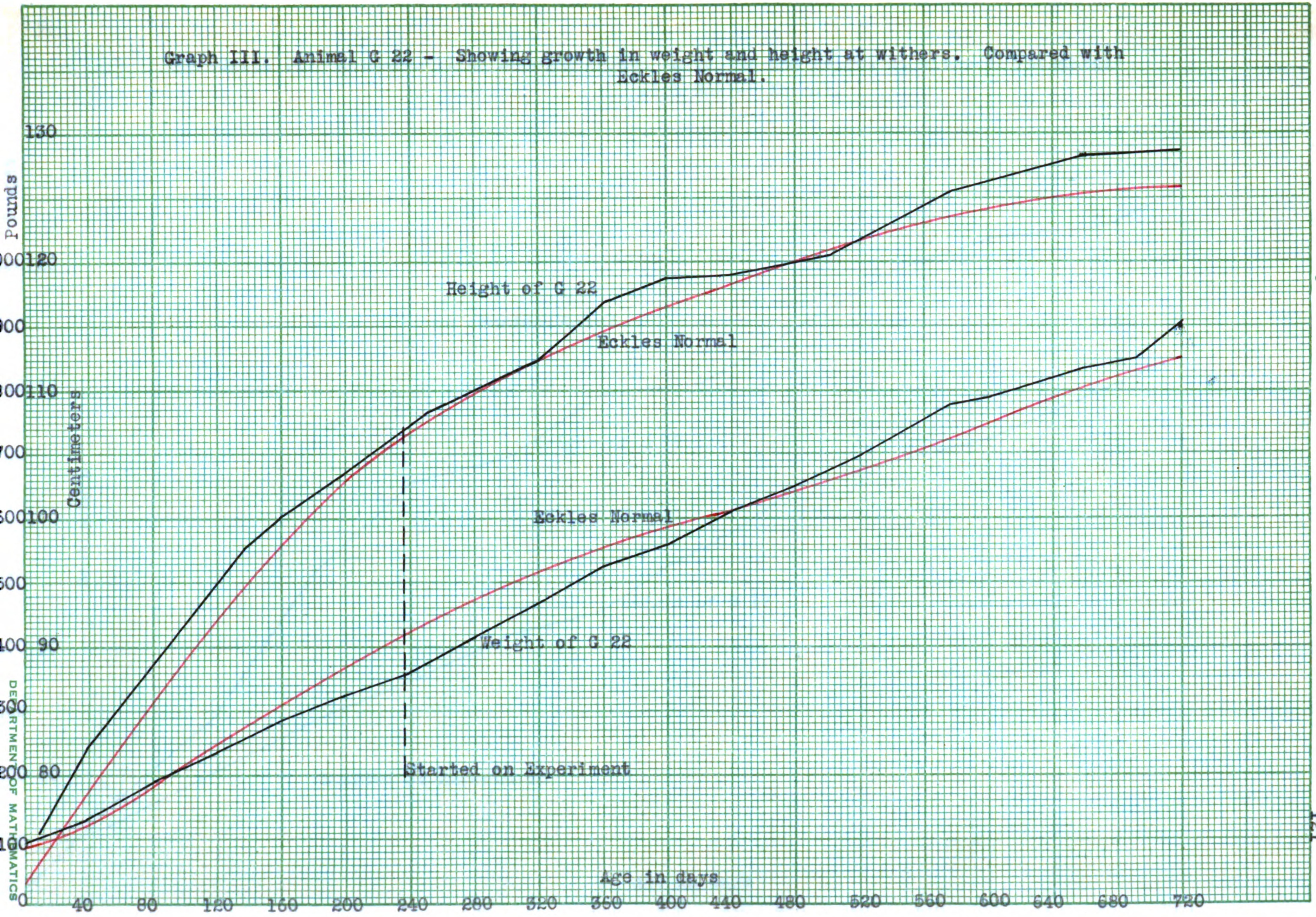
Region from which Samples were Obtained	Sample No.	Per cent of Gossypol in C. S. M.	Sample No.	Per cent of Gossypol in Raw Crushed Cottonseed Meats
North Carolina	1	.0244	1	.621
" "	2	.0139	2	.560
" "	3	.0233	3	.815
" "	4	.0155	4	.681
" "	5	.0159	5	.684
" "	6	.0025	6	.592
" "	7	.0223	7	.840
" "	8	.0195	8	.664
" "	9	.0154	9	.764
" "	10	.0218	10	.871
" "	11	.0239	11	.859
" "	12	.0250	12	.661
South Carolina	13	.0415	13	.807
" "	14	.0375	14	.778
" "	15	.0293	15	.716
" "	16	.0202	16	.749
" "	17	.0191	17	.836
" "	18	.0326	18	.768
" "	19	.0295	19	.782
" "	20		20	.779
" "	21	.0239	21	.696
" "	22	.0288	22	.847
" "	23	.0425	23	.804
" "	24	.0435	24	.761
Mississippi	25	.0301	25	.657
" "	26	.0254	26	.607
" "	27	.0344	27	.763
" "	28		28	.711
" "	29	.0321	29	.676
" "	30	.0381	30	.642
" "	31	.0295	31	.723
" "	32	.0202	32	.643
" "	33	Trace	33	.592
" "	34	.0392	34	.575
" "	35	.0414	35	.730
" "	36	.0245	36	.658
Oklahoma	37	.0158	37	.585
"	38	.0169	38	.497
"	39	.0162	39	.500
"	40	.0230	40	.541
"	41	.0248	41	.636
"	42		42	.585

Table XIII. (Continued)

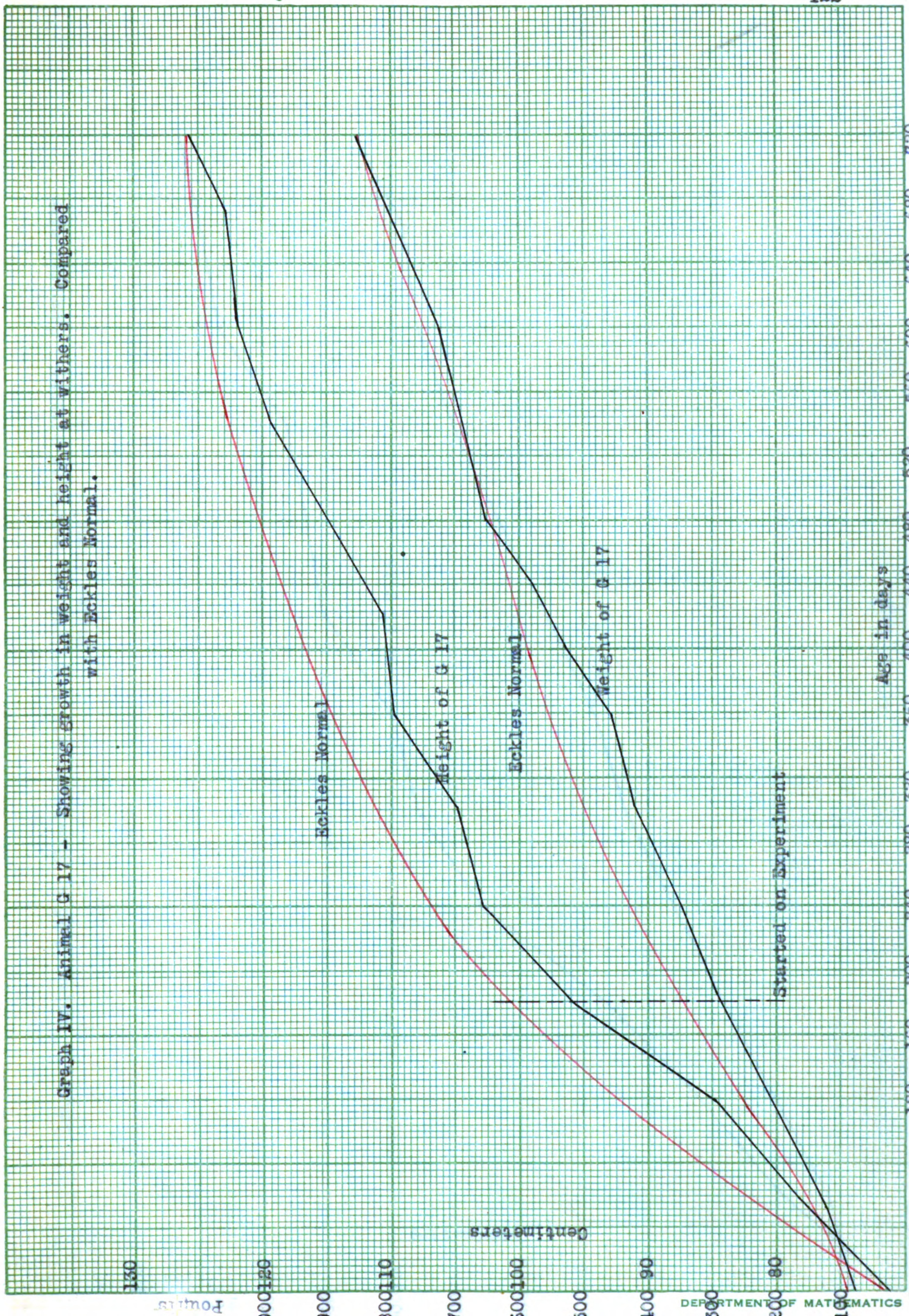
Region from which Samples were Obtained	Sample No.	Per cent of Gossypol in C. S. M.	Sample No.	Per cent of Gossypol in Raw Crushed Cottonseed Meats
Oklahoma	43	.0096	43	.620
"	44	Trace	44	.602
"	45	.0211	45	.568
"	46	.0204	46	
"	47	.0263	47	.542
"	48		48	.612
Texas	49	.0447	49	.343
"	50	.0499	50	.263
"	51	.0472	51	.357
"	52		52	.342
"	53	.0368	53	.277
"	54	.0316	54	.280
"	55	.0379	55	.304
"	56	.0428	56	.231
"	57	.0335	57	.245
"	58	.0396	58	.246
"	59	.0344	59	.301
"	60	Trace	60	.242


Graph I. Animal G 20 - Showing growth in weight and height at withers. Compare with Eckles Normal.
Eckles Normal
Height of G 20
Eckles Normal
Weight of G 20
Started on Experiment
Age in days
Centimeters
Pounds
130
120
110
100
90
80
1000
900
800
700
600
500
400
300
200
100
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
680
720

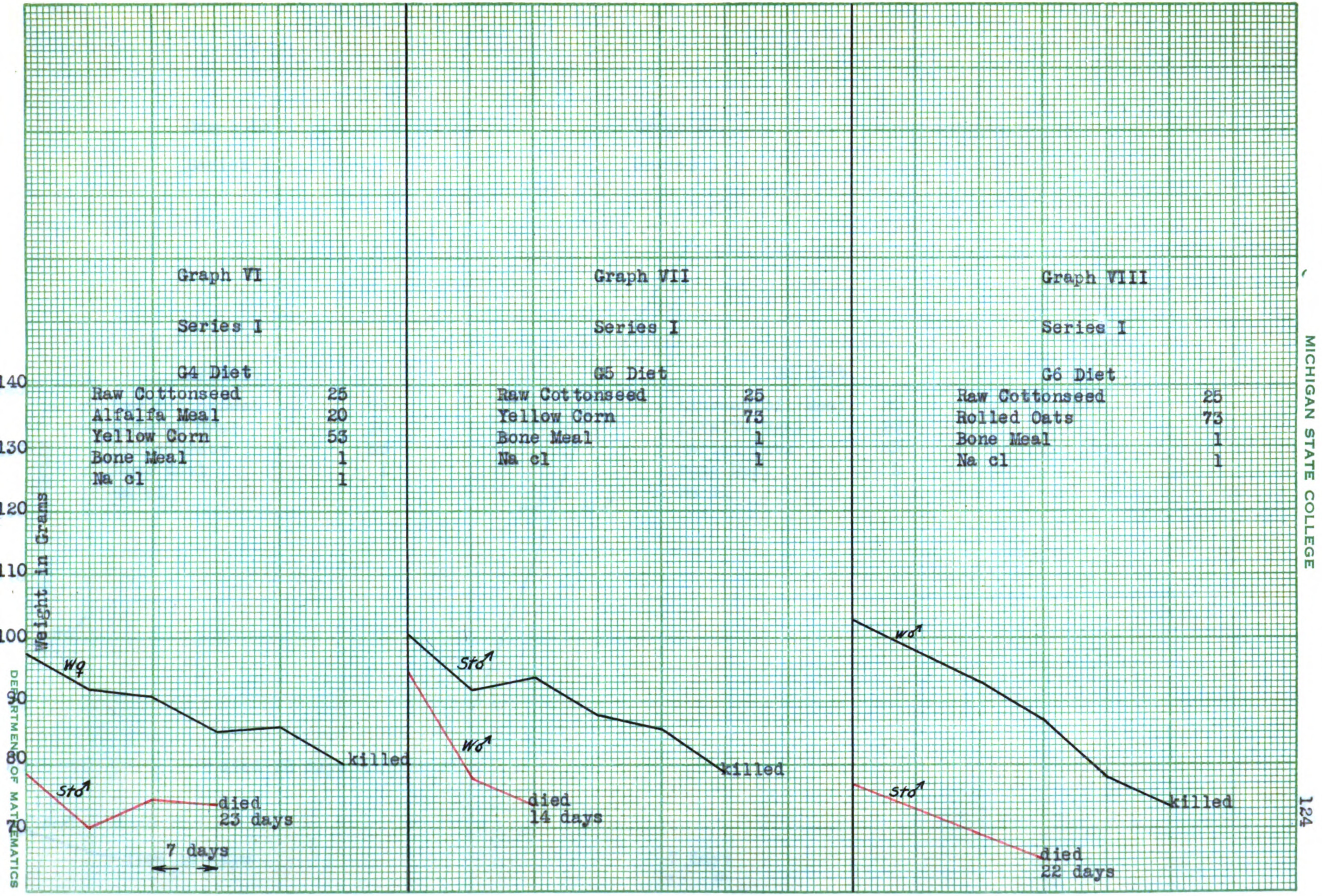

Graph II. Animal G 21 - Showing growth in weight and height at withers. Compared
with Eckles Normal.
Eckles Normal
Height of G 21
Eckles Normal
Weight of G 21
Started on Experiment
Age in days
Centimeters
Pounds
130
120
110
100
90
80
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
680
720

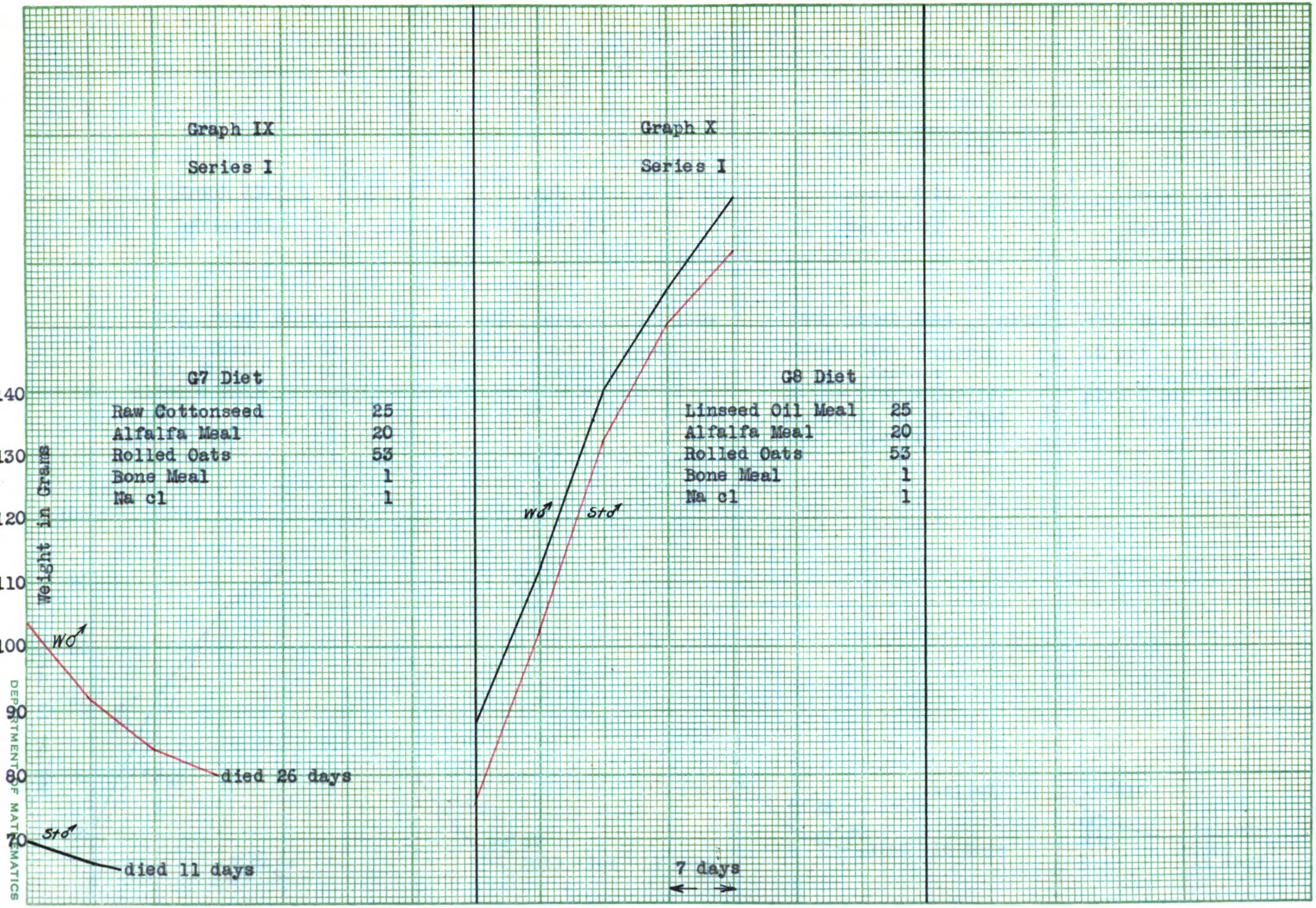


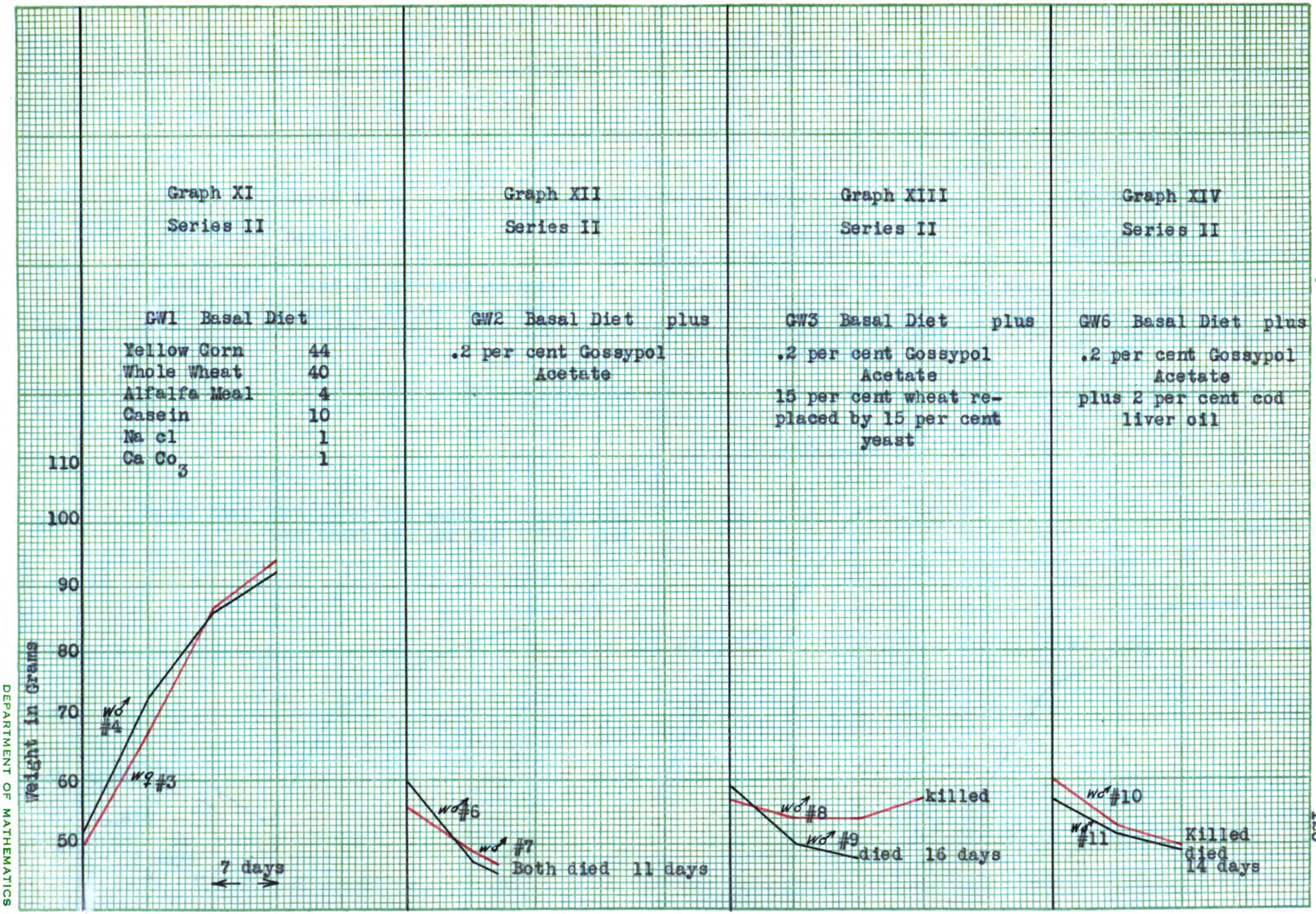
Graph III. Animal G 22 - Showing growth in weight and height at withers. Compared with Eckles Normal.

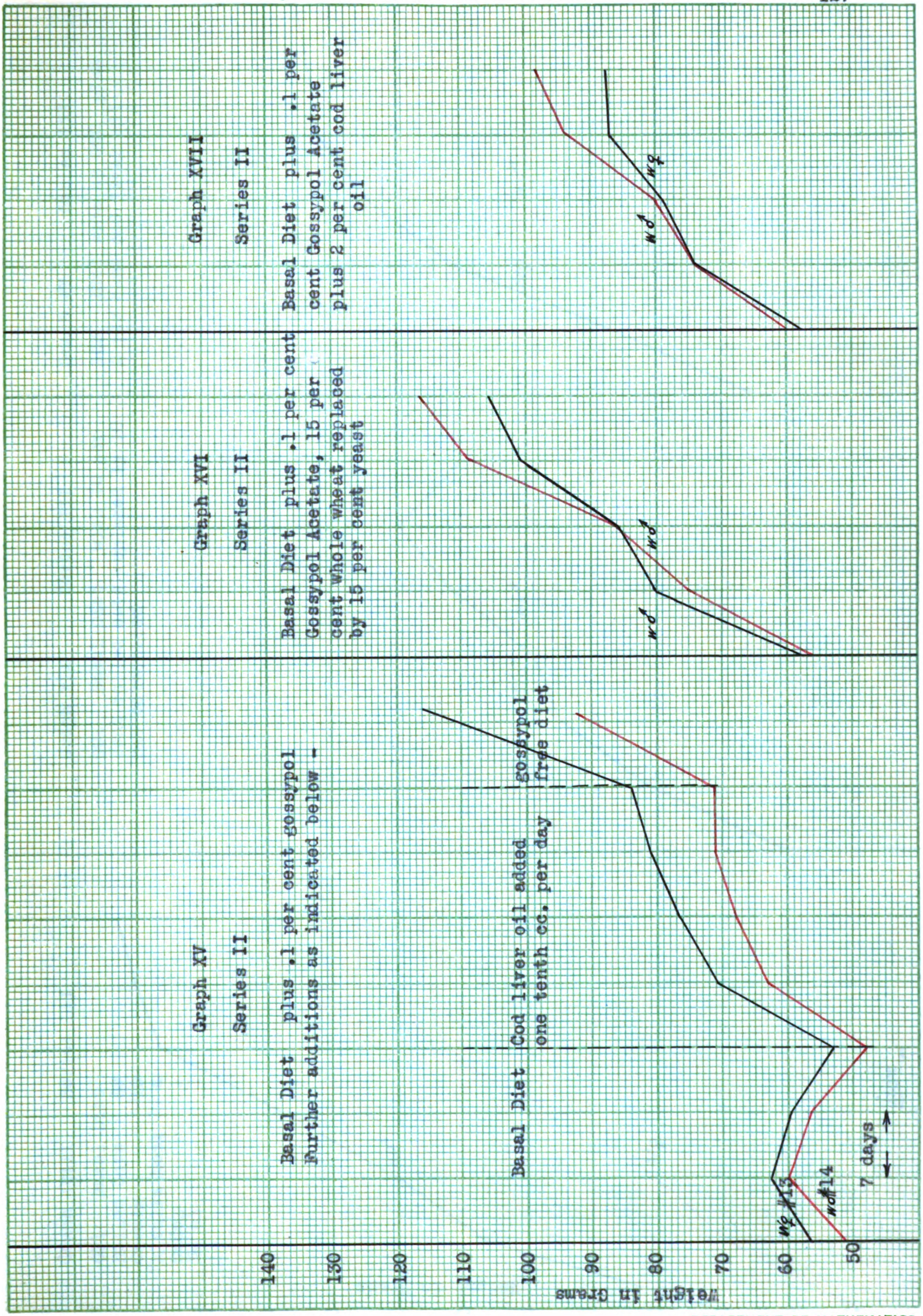

Graph IV. Animal G 17 - Showing growth in weight and height at withers. Compared
with Eckles Normal.
Eckles Normal
Height of G 17
Eckles Normal
Weight of G 17
Started on Experiment
Age in days
Centimeters
Pounds
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
680
720
130
120
110
100
90
80
70

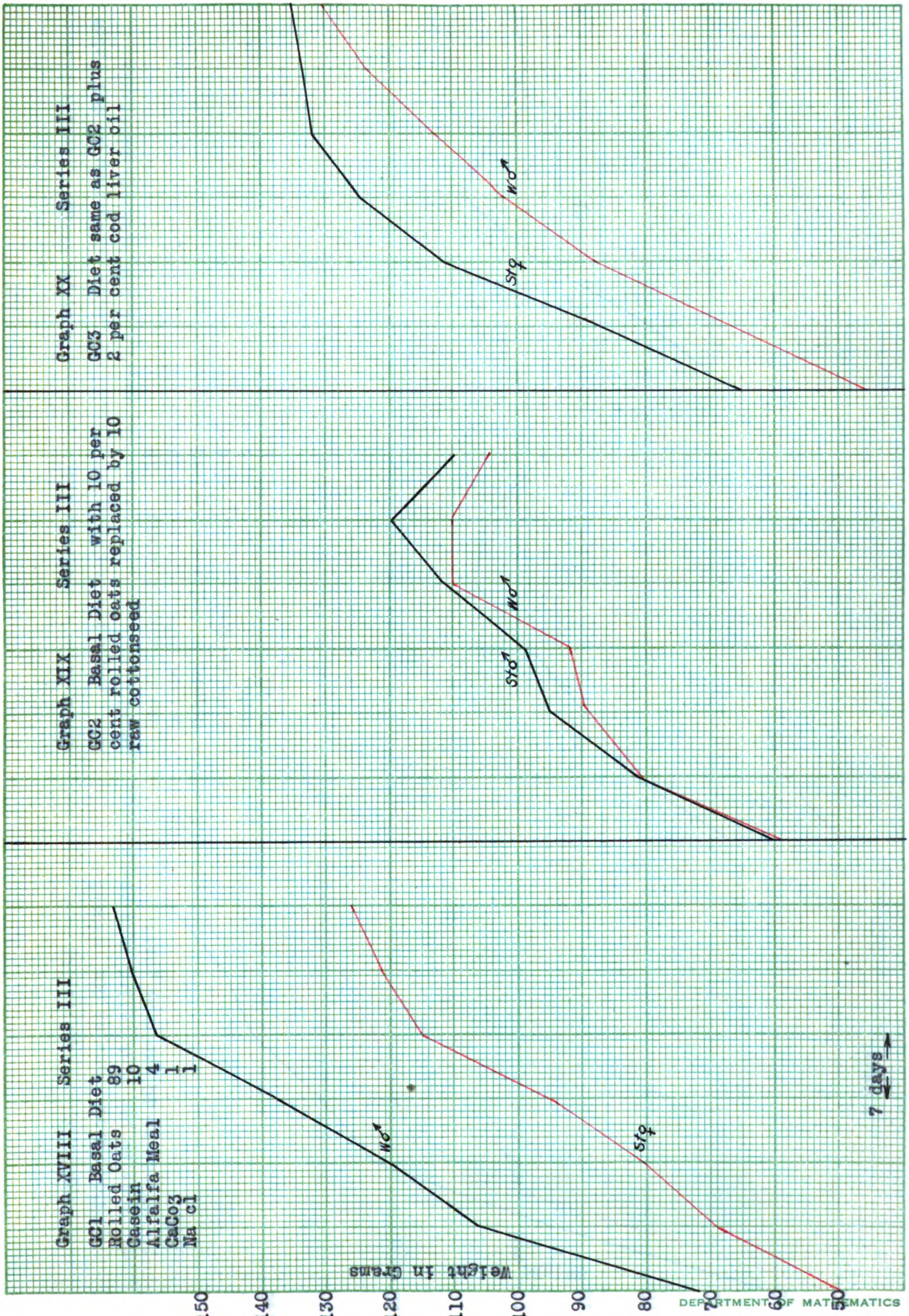
Graph V. Animal G 16 - Showing growth in weight and height at withers. Compared with Eckles Normal.

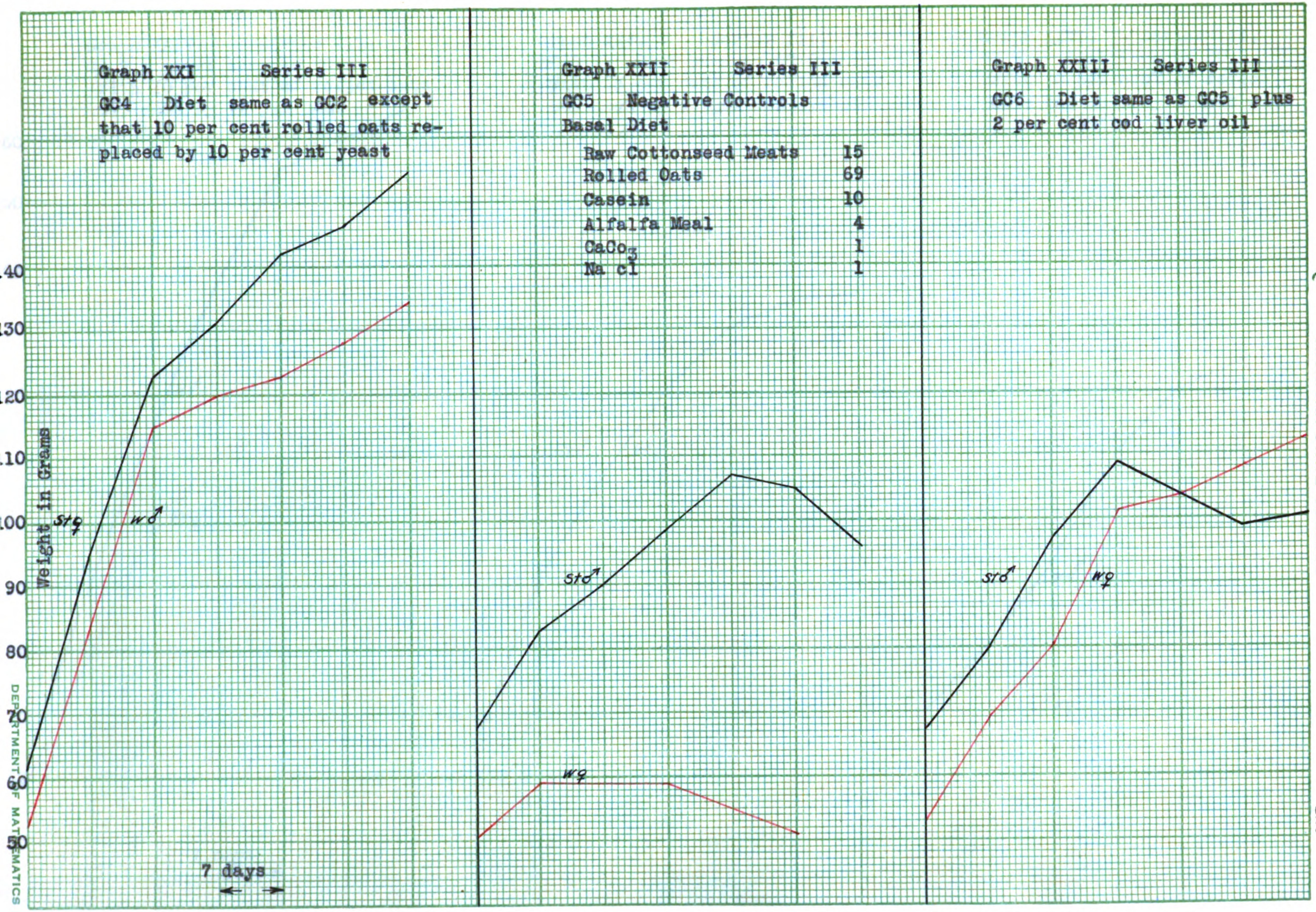

MICHIGAN STATE COLLEGE
Graph VI
Series I
G4 Diet
Raw Cottonseed 25
Alfalfa Meal 20
Yellow Corn 53
Bone Meal 1
Na cl 1
Graph VII
Series I
G5 Diet
Raw Cottonseed 25
Yellow Corn 73
Bone Meal 1
Na cl 1
Graph VIII
Series I
G6 Diet
Raw Cottonseed 25
Rolled Oats 73
Bone Meal 1
Na cl 1
Weight in Grams
140
130
120
110
100
90
80
70
killed
died
23 days
14 days
22 days
7 days
DEPARTMENT OF MATHEMATICS

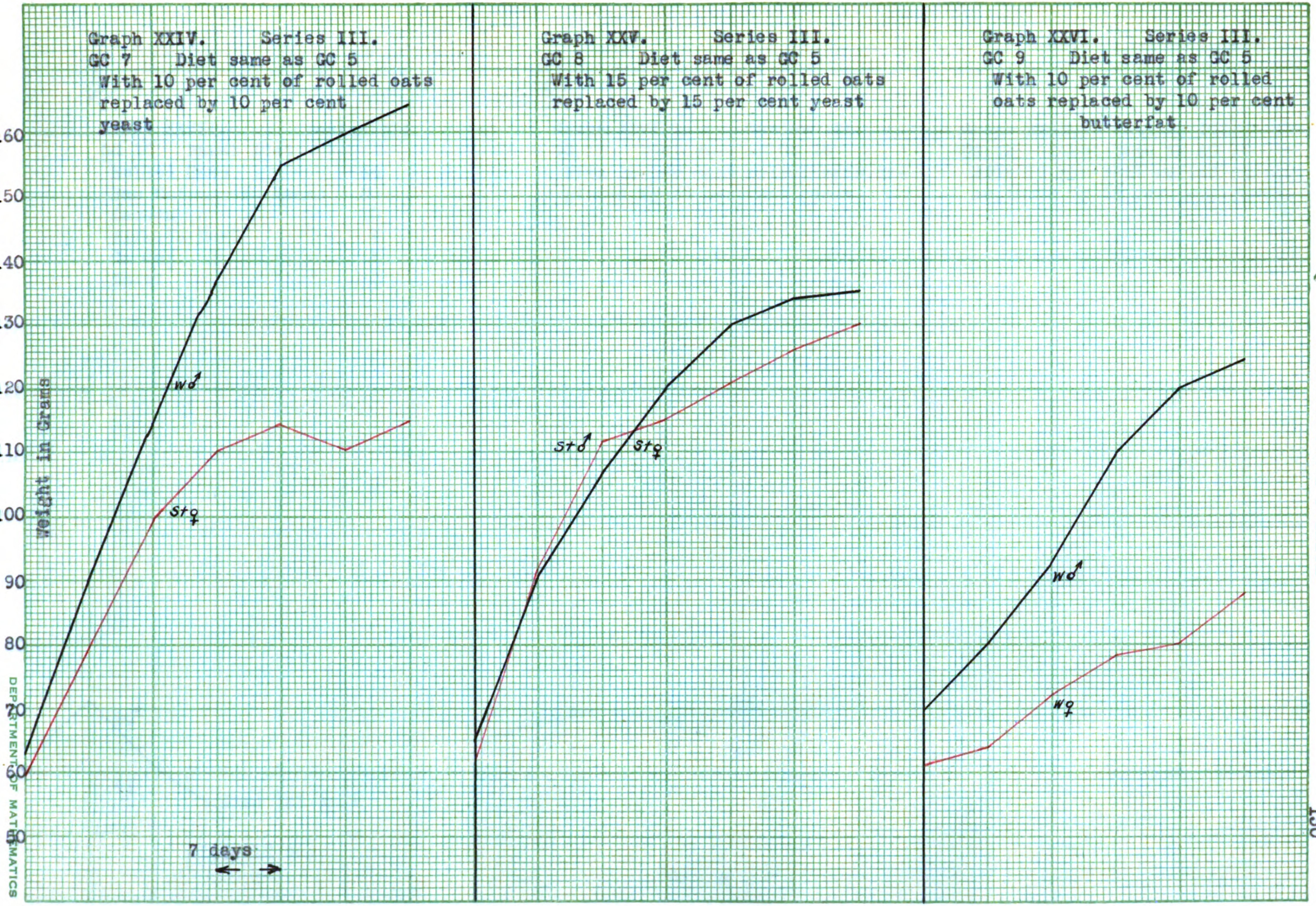

Graph IX
Series I
G7 Diet
Raw Cottonseed 25
Alfalfa Meal 20
Rolled Oats 53
Bone Meal 1
Na cl 1
Weight in Grams
140
130
120
110
100
90
80
70
W♂
St♂
died 26 days
died 11 days
Graph X
Series I
G8 Diet
Linseed Oil Meal 25
Alfalfa Meal 20
Rolled Oats 53
Bone Meal 1
Na cl 1
W♂
St♂
7 days

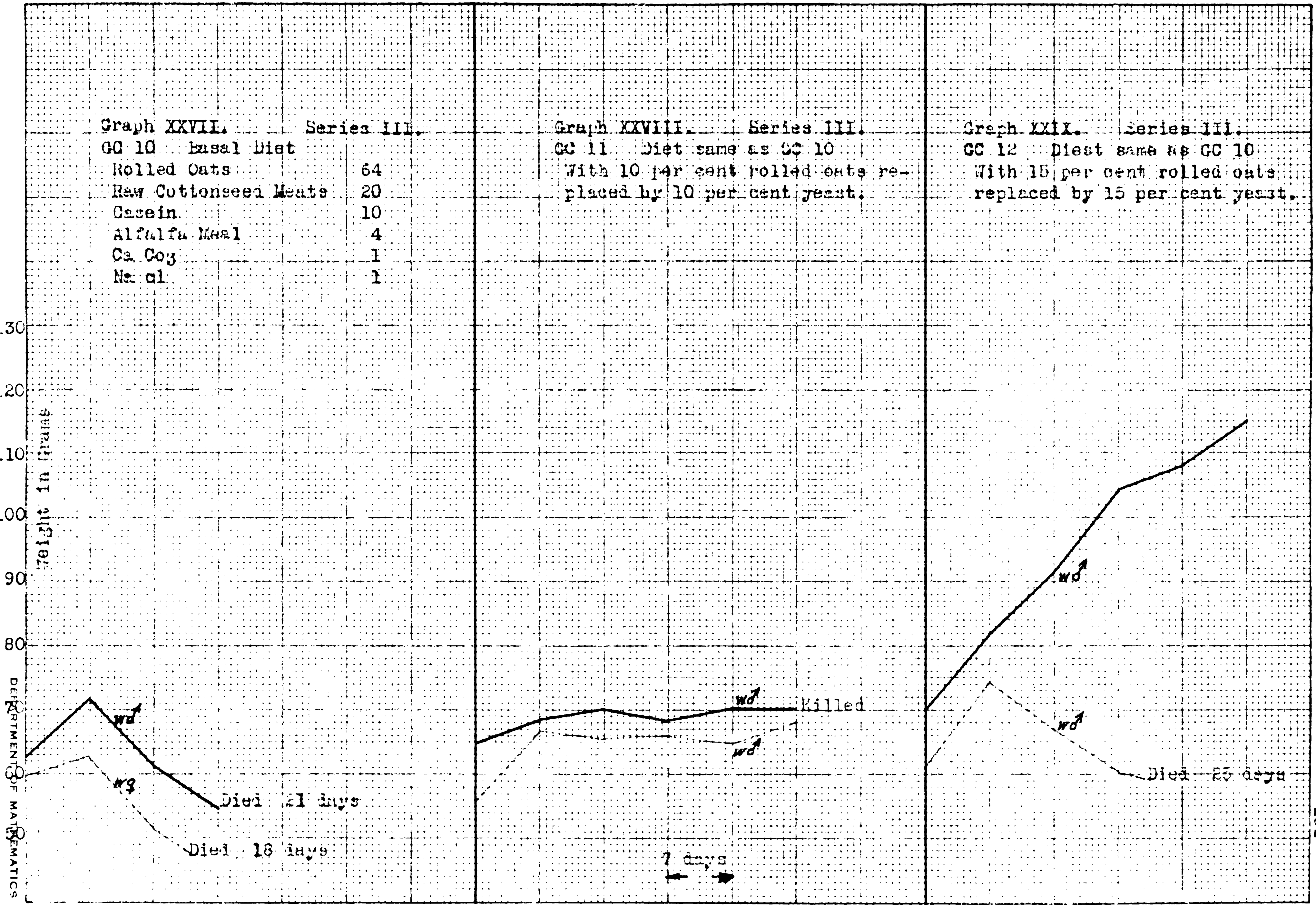

Graph XI
Series II
GW1 Basal Diet
Yellow Corn 44
Whole Wheat 40
Alfalfa Meal 4
Casein 10
Na cl 1
Ca Co3 1
110
100
90
80
70
60
50
Weight in Grams
W♂ #4
W♀ #3
7 days
Graph XII
Series II
GW2 Basal Diet plus
.2 per cent Gossypol
Acetate
W♂ #6
W♂ #7
Both died 11 days
Graph XIII
Series II
GW3 Basal Diet plus
.2 per cent Gossypol
Acetate
15 per cent wheat re-
placed by 15 per cent
yeast
W♂ #8
killed
W♂ #9
died 16 days
Graph XIV
Series II
GW6 Basal Diet plus
.2 per cent Gossypol
Acetate
plus 2 per cent cod
liver oil
W♂ #10
W♂ #11
Killed
died
14 days


Graph XV
Series II
Basal Diet plus .1 per cent gossypol
Further additions as indicated below -
Basal Diet
Cod liver oil added
one tenth cc. per day
gossypol
free diet
#13
#14
7 days
Graph XVI
Series II
Basal Diet plus .1 per cent
Gossypol Acetate, 15 per
cent whole wheat replaced
by 15 per cent yeast
Graph XVII
Series II
Basal Diet plus .1 per
cent Gossypol Acetate
plus 2 per cent cod liver
oil
140
130
120
110
100
90
80
70
60
50
Weight in Grams


Graph XVIII Series III
GC1 Basal Diet
Rolled Oats 89
Casein 10
Alfalfa Meal 4
CaCo3 1
Na cl 1
Graph XIX Series III
GC2 Basal Diet with 10 per cent rolled oats replaced by 10 raw cottonseed
Graph XX Series III
GC3 Diet same as GC2 plus 2 per cent cod liver oil
Weight in Grams
150
140
130
120
110
100
90
80
70
60
50
7 days


Graph XXI Series III
GC4 Diet same as GC2 except that 10 per cent rolled oats replaced by 10 per cent yeast
Graph XXII Series III
GC5 Negative Controls
Basal Diet
Raw Cottonseed Meats 15
Rolled Oats 69
Casein 10
Alfalfa Meal 4
CaCo3 1
Na cl 1
Graph XXIII Series III
GC6 Diet same as GC5 plus 2 per cent cod liver oil
Weight in Grams
140
130
120
110
100
90
80
70
60
50
7 days
St♀
W♂
St♂
W♀
St♂
W♀
DEPARTMENT OF MATHEMATICS
MICHIGAN STATE COLLEGE


Graph XXIV. Series III.
GC 7 Diet same as GC 5
With 10 per cent of rolled oats replaced by 10 per cent yeast
Graph XXV. Series III.
GC 8 Diet same as GC 5
With 15 per cent of rolled oats replaced by 15 per cent yeast
Graph XXVI. Series III.
GC 9 Diet same as GC 5
With 10 per cent of rolled oats replaced by 10 per cent butterfat
Weight in Grams
60
50
40
30
20
10
00
90
80
70
60
50
W♂
St♀
St♂
St♀
W♂
W♀
7 days


Graph XXVII. Series III.
GC 10 Basal Diet
Rolled Oats 64
Raw Cottonseed Meats 20
Casein 10
Alfalfa Meal 4
Ca Co3 1
Na cl 1
Graph XXVIII. Series III.
GC 11 Diet same as GC 10
With 10 per cent rolled oats re-
placed by 10 per cent yeast.
Graph XXIX. Series III.
GC 12 Diest same as GC 10
With 15 per cent rolled oats
replaced by 15 per cent yeast.
Weight in Grams
130
120
110
100
90
80
Died 21 days
Died 18 days
Killed
7 days
Died 25 days

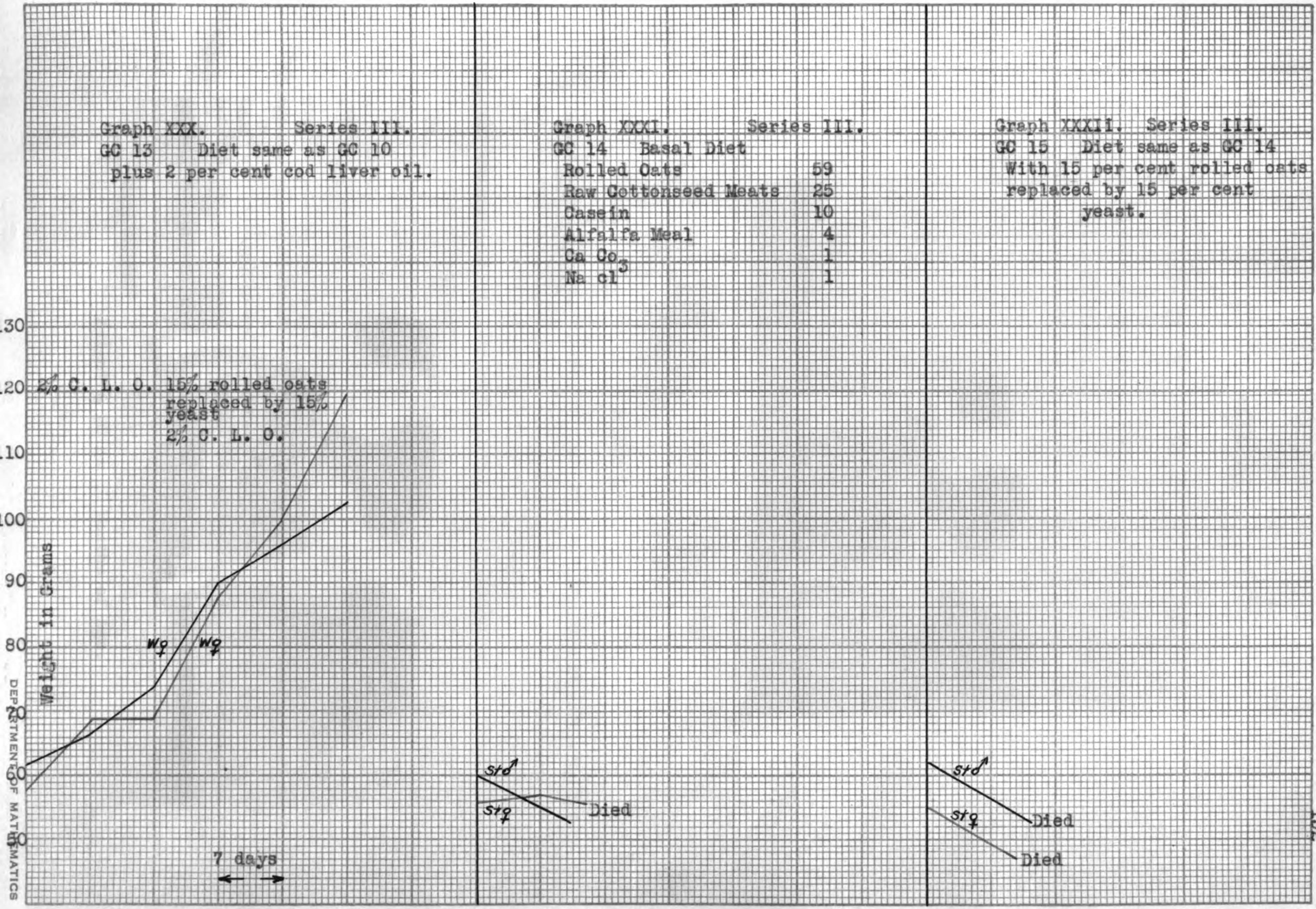
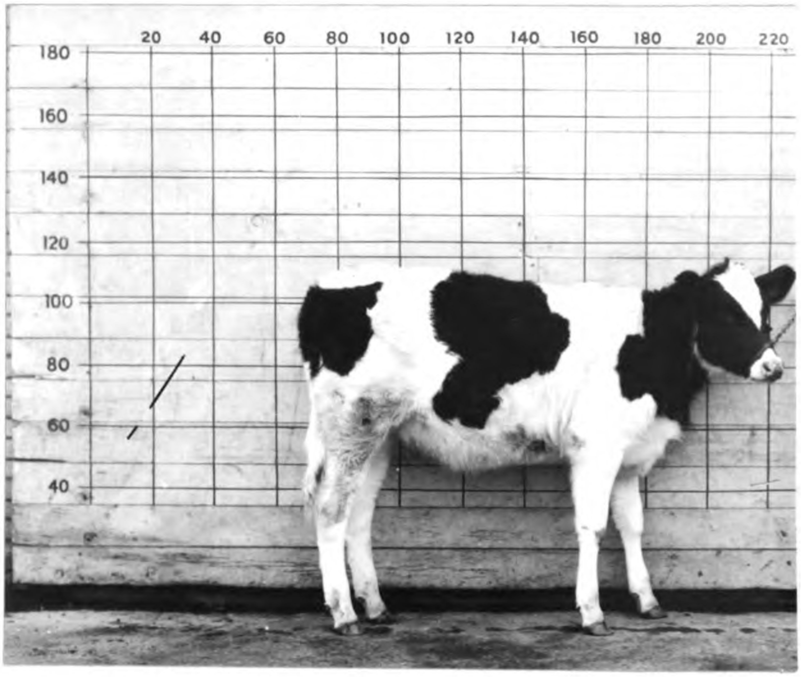
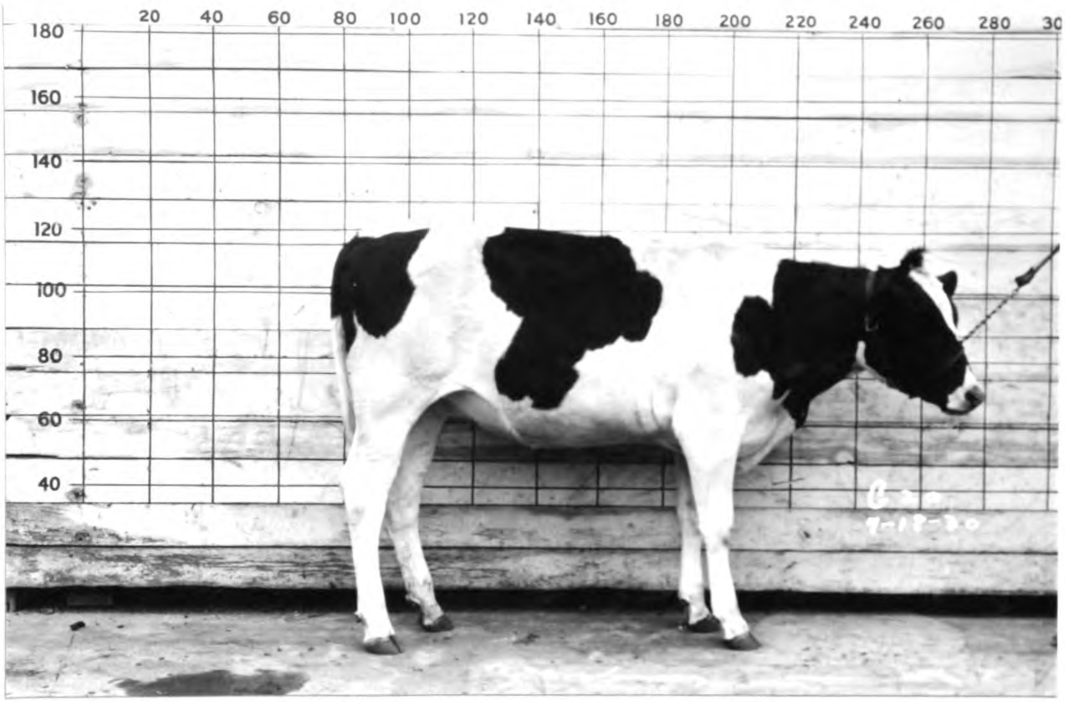

Graph XXX. Series III.
GC 13 Diet same as GC 10
plus 2 per cent cod liver oil.
Graph XXXI. Series III.
GC 14 Basal Diet
Rolled Oats 59
Raw Cottonseed Meats 25
Casein 10
Alfalfa Meal 4
Ca Co3 1
Na cl 1
Graph XXXII. Series III.
GC 15 Diet same as GC 14
With 15 per cent rolled oats
replaced by 15 per cent
yeast.
2% C. L. O.
15% rolled oats replaced by 15% yeast
2% C. L. O.
Weight in Grams
130
120
110
100
90
80
70
60
50
W♀
W♀
St♂
St♀
Died
Died
Died
7 days

Plate I.



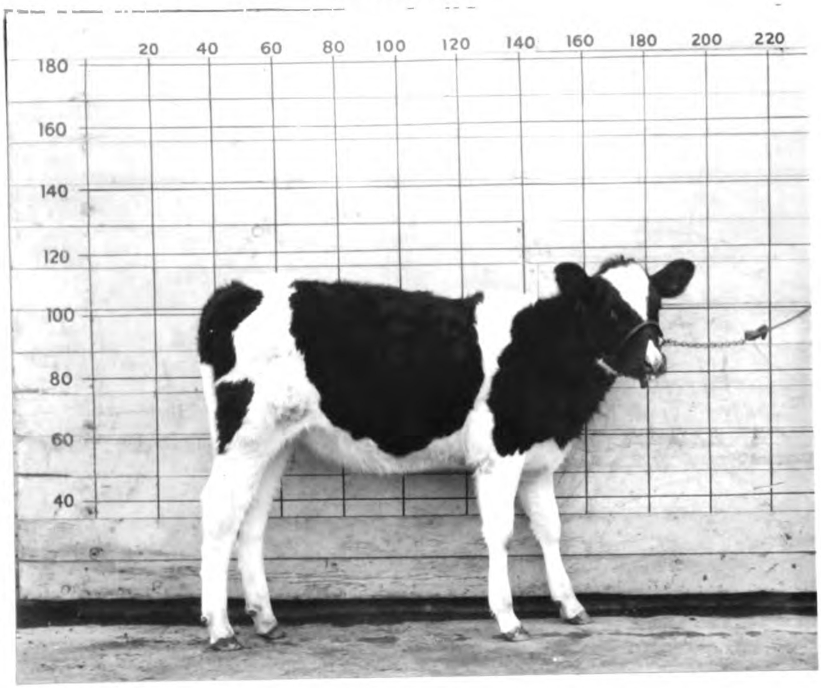
Animal G 20 at Start of Experiment
Ration - Raw Cottonseed Meats,
Timothy Hay, Silage and Corn.

Plate II.



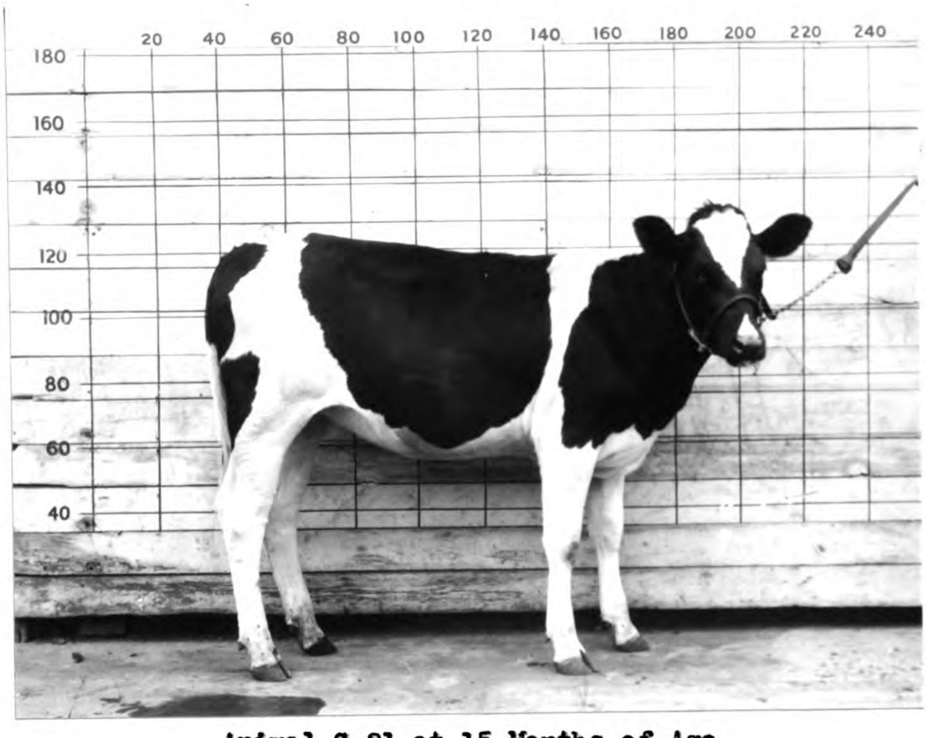
Animal G 20 at 15 Months of Age.

Plate III.



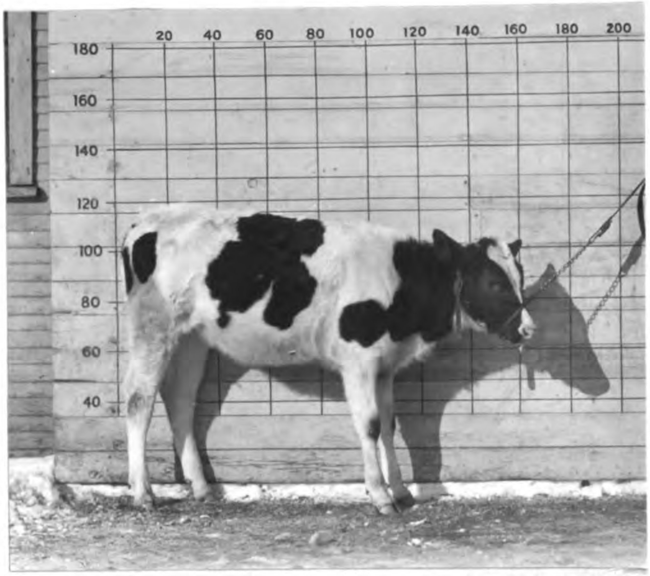
Animal G 21 at Start of Experiment
Ration - Raw Cottonseed Meats,
Timothy Hay, Silage and Corn

Plate IV.



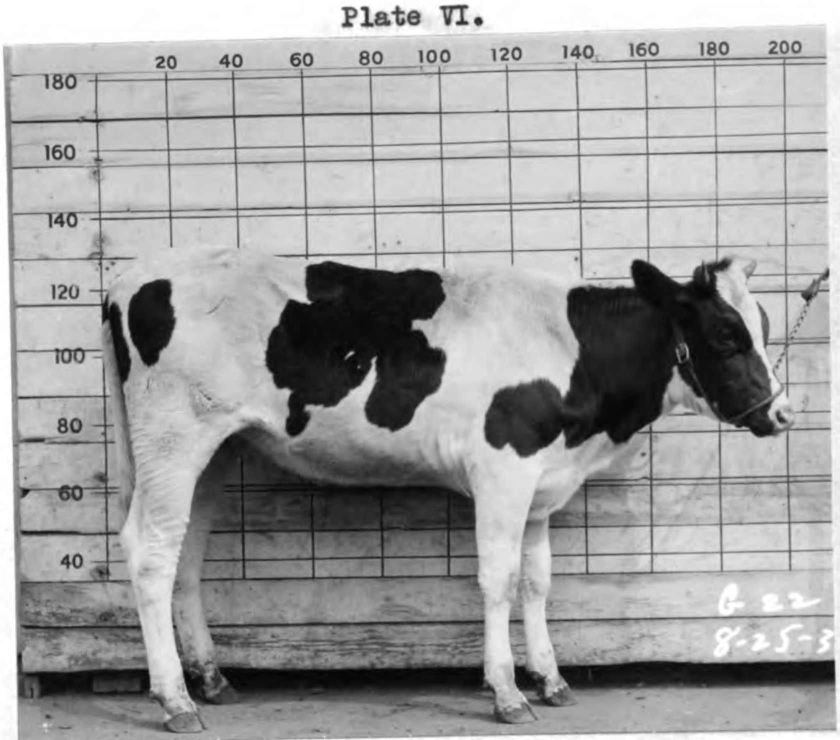
Animal G 21 at 15 Months of Age

Plate V.



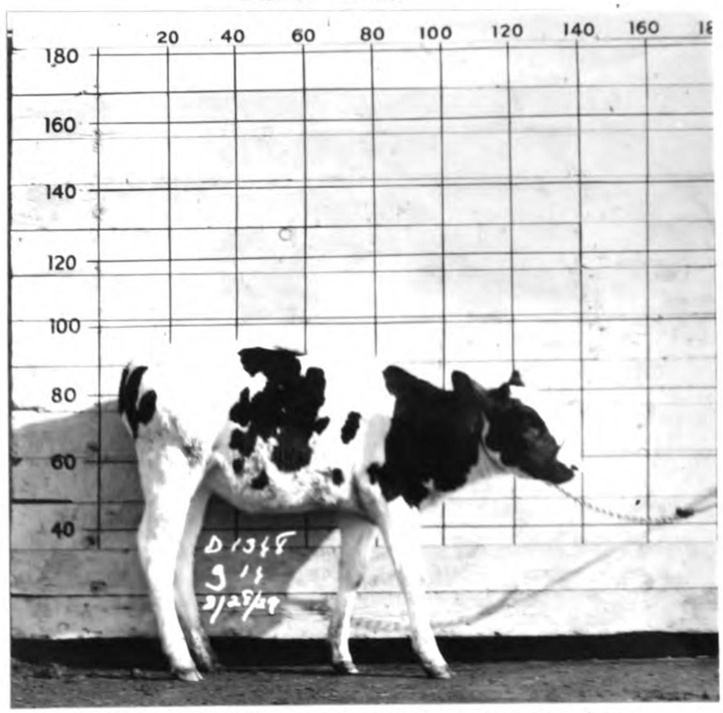
Animal G 22 at Start of Experiment
Ration - Raw Cottonseed Meats,
Timothy Hay, Silage and Corn.

Plate VI.



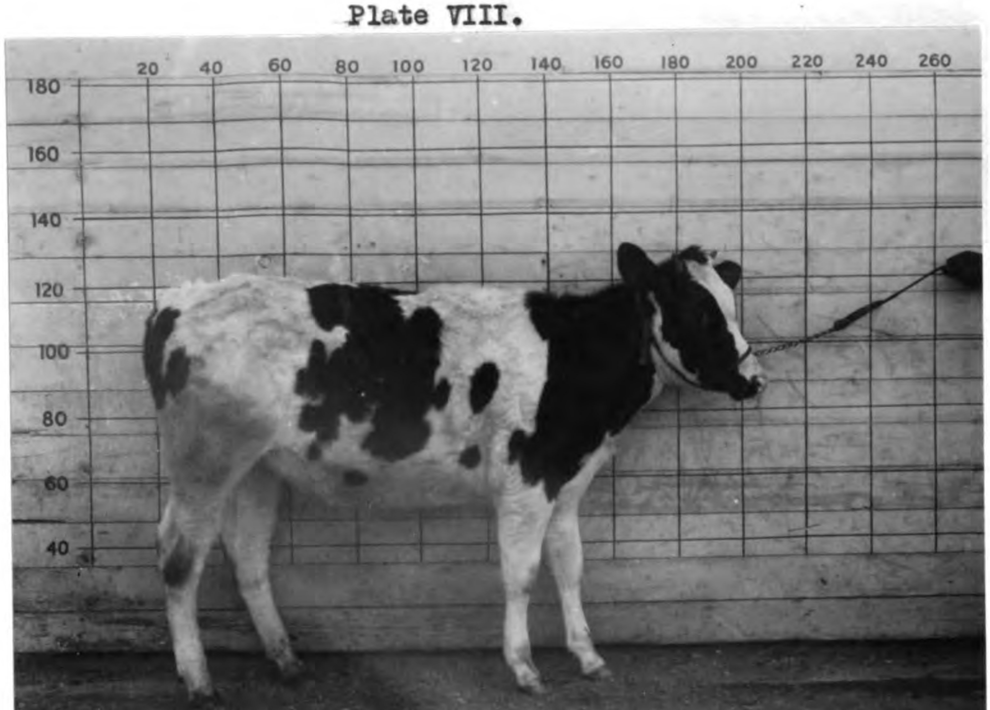
Animal G 22 at 15 Months of Age

Plate VII.



Animal G 16 at Three Months of Age

Plate VIII.



Animal G 16 at 15 Months of Age.
Ration - Linseed Oil Meal, Timothy
Hay and Silage