THE EFFECT OF GOSSYPOL ON THE PEPSIN ACTIVITY OF THE RAT

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DIANA E. CLOKEY, B.S., R.D.

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CHAPTER I

INTRODUCTION

The worldwide demand for protein to feed the population is continuously increasing (1). To meet this demand, investigators are seeking new protein sources and efforts are in progress to improve the quality of available sources (1,2).

In 1964, The Food and Drug Administration approved the use of whole kernel cottonseed and products derived from cottonseed intended for human consumption with the specification that the free gossypol content should not exceed 0.045 per cent by weight when in the diet at 20% or less (3). Glandless cottonseed flour has a protein efficiency ratio near that of casein (4), and has been used for supplementation of inadequate diets in tropical and sub-tropical countries (1). It is estimated that one-fourth of the cottonseed flour potentially available could satisfy the present serious shortage in protein in nations with inadequate food supply (1).

Cottonseed comes from a plant that is indigenous to protein-poor tropical areas of Asia, Africa and Latin America (2). Food techologists foresee cottonseed

becoming a more prominent source of food protein in the years ahead (5).

Although cottonseed has great potential as a protein source, the development of edible protein products from cottonseed has been limited because of the unfavorable physiological effects of free gossypol contained in cottonseed protein. Adverse effects attributed to gossypol have included depression of appetite and body weight (6), diarrhea (7,8), hemolysis (9), and hypothrombinemia (10). Fatalities from gossypol have been noted by several investigators (6,9,11). Although other gossypol-like pigments may be involved (12) gossypol appears to be the primary toxic agent.

Improvements in oilseed processing (4,5,13), increased knowledge of the inter-relationship between gossypol content and protein quality (15-17), and gossypol inactivation research (16,18) has promoted the use of cottonseed meal in non-ruminant rations in the past fifteen years. Nevertheless, gossypol remains a serious economic problem (19). Gossypol is responsible for the loss of revenue to the producer and the cottonseed processing industry because of restricted markets and increased processing costs necessary to remove gossypol-containing pigmented glands from the kernel (13,14).

Although the toxic level of free gossypol in the diet has been established in important species of monogastrics (16), little is known about the mechanism of toxicity. A decreased feed intake and lowered daily body weight gain are the first clinical signs observed in animals receiving gossypol in their diets (17). Diets supplemented with 10% raw cottonseed flour containing 0.083% free gossypol have been shown to have an inhibiting effect on growth performance in rats (17).

Gossypol has been shown to participate in reactions typical of amino compounds with carbonyl and aromatic hydroxyl groups (20). This suggests that gossypol may complex with many enzymes. If such complexes render enzymes inactive, normal metabolism of the body might be greatly altered, resulting in early characteristic symptoms observed in monogastric animals and poultry (21).

Tanksley reported that incubation of gossypol with pepsinogen (3:1 molar ratio of gossypol/pepsinogen) in the presence of 10% ethanol completely inhibited pepsinogen activation (22). No studies have been conducted to determine if pepsinogen inhibition by gossypol occurs in vivo. The purpose of this study is to investigate the possibility of inhibition of the conversion of pepsinogen to pepsin in rats fed cottonseed flour with different levels of gossypol.

CHAPTER II

REVIEW OF LITERATURE

Gossypol is a yellow pigment found in specific glands distributed throughout the cottonseed plant (genus Gossypium) and constitutes 20-40% of the weight of the pigmented glands in cottonseed (23). In the late nineteenth century, the Polish chemist Marchlewski first isolated the crystalline substance (gossypol-acetic acid) from cottonseed oil. He arrived at its name in recognition of its origin, Gossypium and chemical nature phenol (23).

Adams et al. (23) reviewed studies on gossypol toxicity in several monogastric animals including rats, poultry, dogs, and swine. Alsberg and Schwartze (11) stated that the most prominent effect of gossypol poisoning prior to death is generally caused by circulatory failure with post mortem lesions showing pulmonary edema and an enlarged flabby heart.

Characteristically, the first clinical sign observed in animals and poultry receiving dietary gossypol is decreased feed intake and daily weight loss which becomes more pronounced as the feeding period progresses (11, 24).

Even though gossypol is known to complex with several amino acids including lysine, glutamine, ornithine, glycine, arginine, and ∝-amino butyric acid, the binding site is thought to be mainly in the epsilon amino group of lysine in the protein (25). According to Finlay (26), formation of the Schiff's base between the carbonyls of gossypol and the amino group of amino acids and protein is a major factor in the toxicity of gossypol and its cumulative effects (Figure 1).

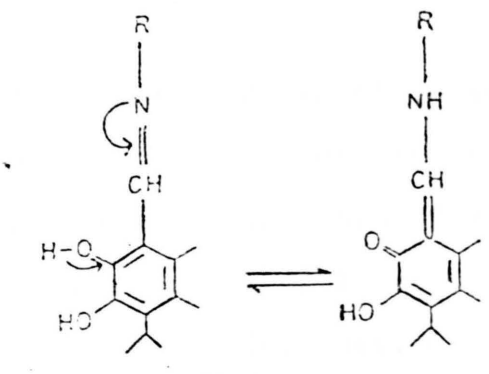

Figure 1. Reaction of Gossypol with lysine as described by Finlay (26).

Tanksley (21) suggested that the depression of weight gain and loss of appetite are perhaps initial signs of altered normal metabolism by the complexing of gossypol with proteolytic enzyme and lowering its enzymatic activity. He further stated that the chemical groups of gossypol which might be involved in reactions with digestive enzymes and result in enzyme inhibition include carbonyl groups, phenolic groups, aromatic ring systems, aliphatic side chains, and the carbon skeleton. The carbonyl and phenolic groups are most likely involved in such reactions.

The structural formula of gossypol was proposed by Adams et al. (27) and later verified by Edwards (28). The structure of gossypol is 1,1',6,6',7,7'-hexahydroxy, 3,3'-dimethyl, 5,5'-diisopropyl, 2,2'-binaphthyl, 8,8'-dicarboaldehyde. Synthesis and degradation of gossypol indicated that it exists in three tautomeric forms, the aldehyde form being the most prominent (Figure 2).

Gossypol is known to form complexes with protein. Clark (29) suspected the binding site to be the free amino groups in cottonseed meal. Baliga and Lyman (30) showed that a decrease of lysine availability from 82.9 to 48.7% was due to the formation of a complex between gossypol and purified cottonseed protein.

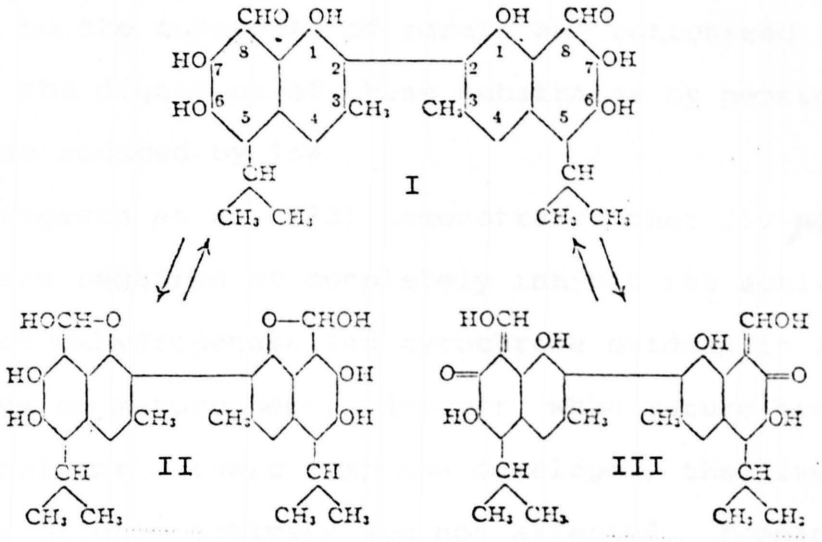

Figure 2. Tautomeric forms of gossypol. (I) aldehyde form, (II) hemiacetal form, (III) quinoid form (28).

Lyman et al. (31) showed that it is the free epsilon-amino group of lysine in the bovine serum albumin that combines with gossypol. Sedimentation velocity studies indicated the presence of 1 to 4 different gossypol protein complexes. Lyman suggested that one molecule of gossypol probably formed a cross-link between two or more protein molecules.

Jones and Waterman (32) found that when gossypol was added to the substrate of casein and cottonseed globulin, the digestion of these substrates by pepsin and trypsin was reduced by 15%.

Ferguson et al. (33) demonstrated that 250 µg of gossypol was required to completely inhibit the activities of succinic dehydrogenase and cytochrome oxidase in liver homogenates of mature hens. However, when mature hens were fed gossypol until toxic symptoms developed, the liver cytochrome oxidase activity was not affected. Feeding gossypol to 8-day old chicks for a period of six weeks would reduce the activity of the same enzyme in the liver homogenate. This indicates that feeding gossypol during the growing period can affect the activity of certain hepatic enzymes.

Meksongsee et al. (34) studied the effects of gossypol on cytochrome oxidase and succinoxidase of pigs, rabbits, and rats. Pigs were given a diet containing 15% protein and approximately 0.4% free gossypol until severe symptoms of toxicity appeared. Rabbits were given an adequate diet containing 15% protein and gossypol was administered in a saline solution intravenously in the ear vein until toxicity appeared. Weanling rats were fed a basal diet containing 10% protein and 0.14%

gossypol-acetic acid for three to six weeks. The data showed that cytochrome oxidase and succinoxidase activities in the livers of pigs, rabbits, and rats were not markedly affected by gossypol, even in animals exhibiting severe toxicity. These investigators (34) concluded that the level of gossypol found in the liver of the pigs (531 ppm) was not sufficiently high to affect the same enzymes studied by Ferguson (33). However, bound gossypol found in the liver tissue of these pigs indicated that interaction of gossypol and protein indeed took place.

Braham and co-workers (18) reported that the glutamic oxaloacetic transaminase activity in the serum of gossypol-fed pigs was increased, but lactic dehydrogenase, leucine amino peptidase and aldose activity were not affected significantly. The increase in glutamic oxaloacetic transaminase activity is an indication of liver necrosis which has been reported as a symptom of gossypol toxicity (35).

Among the proteolytic enzymes, pepsin and its precursor pepsinogen, is one of the most important endopeptidases in protein digestion of all domestic animals. The conversion of pepsinogen to the active form can be accomplised either by the presence of H^+ or by pepsin itself (36).

Gossypol has been shown to inhibit the transformation of pepsinogen to pepsin in in vitro studies (22,30, 37). According to these studies, gossypol, after conformational change, reacts with pepsinogen. This irreversible gossypol pepsinogen binding leaves pepsinogen no longer available for activation. Certain organic solvents such as ethanol and dimethylsulfoxide can cause conformational changes in pepsinogen.

Wong (37) reported that the inhibition of pepsinogen activation by gossypol was a specific reaction between gossypol and residues Lys 18 and Lys 358 from the two terminal regions of the pepsinogen either through an inter- or intramolecular cross linkage. The covalent linkage of these regions by gossypol prevented the transformation of pepsinogen to pepsin (Figure 3).

The nature of gossypol reacting with native pepsinogen has been studied by Perlmann (38-40) who has shown that lysl residues of pepsinogen can be acetylated, succinylated, and carbamylated extensively under conditions in which pepsinogen is in its native state, thus creating the possibility of a conformational change in vivo.

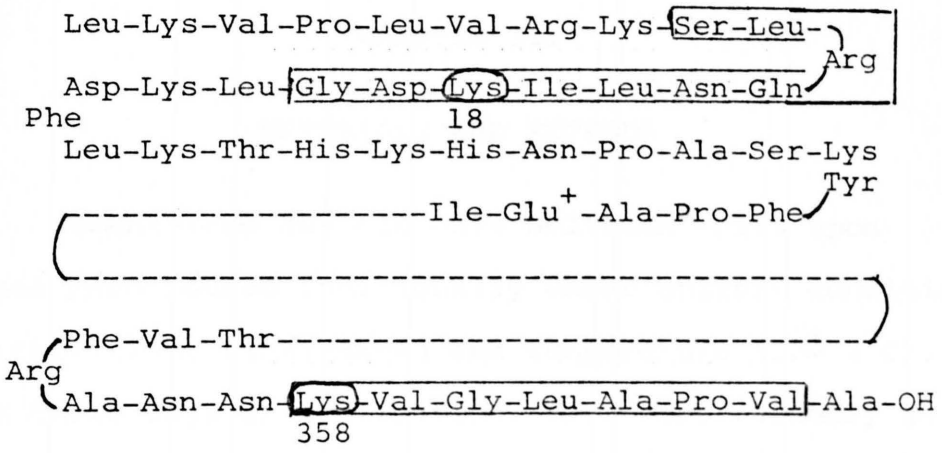

Figure 3. Schematic representation of the gossypol cross-linked regions of pepsinogen. The gossypol-containing peptides are enclosed in rectangular boxes and the two lysine residues involved in the cross-linkage are encircled. Glu^{+} denotes the amino acid residue which becomes the NH_2 terminal end of pepsin on activation of pepsinogen. The gossypol cross-linkage with lysine prevents this activation of pepsinogen to occur (37).

CHAPTER III

MATERIALS AND METHODS

Twenty-one day old male Holtzman[a] rats upon arrival were housed individually under uniform conditions of light (12-hr light/dark) and temperature ($22 \pm 2^{o}C$). After three days of acclimation, rats were randomly assigned to three groups. Two studies were carried out. Both studies consisted of three dietary groups.

In each study, Group 1 (control) was fed a casein diet containing 0 ppm of free gossypol. Group 2 was fed a diet with raw defatted glandless cottonseed flour as the protein source. Group 3 was fed the same diet as Group 2, in addition, gossypol-acetic acid was added to the diet.

The raw defatted glandless cottonseed flour used for the two studies was purchased from Texas A & M University at two different times. The proximate analyses of both lots are presented in Table 1. There were slight differences in the composition of the two lots of cottonseed flour. There was a 10-fold difference in the amount of free gossypol contained in the two lots of cottonseed

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[a]Holtzman Co., Madison, Wisconsin.

TABLE 1

PROXIMATE ANALYSIS OF RAW GLANDLESS DEFATTED COTTONSEED FLOUR[a]

Contents	Cottonseed Flour Used In	
	Study 1	Study 2
Moisture	9.00%	9.72%
Ash	7.43%	7.04%
Oil	3.24%	0.55%
Protein	55.41%	59.45%
Fiber	0.80%	0.81%
Free Gossypol	0.044%	0.0062%
Nitrogen Free Extract	24.12%	22.47%

[a]Analyses performed at the Food Protein Research and Development Center, Texas A & M University, and Pope Testing Laboratories, Inc., Dallas, Texas.

flour. The amount of gossypol-acetic acid added to the third dietary group of both studies was also different.

In study 1, the free gossypol content of the diet was 0 ppm for Group 1 (CA), 150 ppm for Group 2 (EA1), and 164 ppm for Group 3 (EA2). In study 2, Groups 1, 2, 3, contained 0 ppm (CB), 21 ppm (EB1), and 171 ppm (EB2) of free gossypol in their diets respectively. Since the content of protein and other nutrients of casein and cottonseed flour were different, the diets were formulated so that the composition of the three diets was similar. The dietary composition is presented in Table 2. All three groups of rats were provided with water and feed ad libitum. Feed intake was measured daily. Body weight was measured weekly.

After a 31-day period, the diets fed to each group of rats was replaced by granulated sucrose for 48 hours. Sucrose was then removed and the rats were fasted for 24 hours. The replacement of the diet by sucrose was to ensure that the stomach would contain minimum debris at the time of collection of gastric juice. Water was given ad libitum during the 72 hour fasting period.

The surgical procedure for pyloric ligation is the procedure of Shay (41). After 24 hours of feed deprivation, the rat was anesthetised with ether. The stomach

TABLE 2

COMPOSITION (%) OF DIET

INGREDIENT	Study 1			Study 2		
	CA	EA1	EA2	CB	EB1	EB2
			(g)			
CASEIN[a]	23	___	___	23	___	___
COTTONSEED FLOUR[b]	__	34.2	34.2	__	34.2	34.2
SUCROSE[c]	59.0	54.2	54.2	59.0	54.2	54.2
CORN OIL[d]	6.8	5.5	5.5	6.8	5.5	5.5
MINERALS[e]	4.9	2.2	2.2	4.9	2.2	2.2
VITAMINS[f]	2.2	2.1	2.1	2.2	2.1	2.1
FIBER[g]	4.1	1.8	1.8	4.1	1.8	1.8
GOSSYPOL-ACETIC ACID[h]			.0014			.015
Gossypol in the diet (ppm)	0	150	164	0	21	171

[a]High Protein Casein, Teklad, Madison, Wisconsin

[b] (85% Protein, 1.2% Fat, 10% Moisture, 0.43% Ash, 4.3% NFE) See Table 1 for proximate analysis

[c]Granulated sugar

[d]Mazola Corn Oil, Englewood Cliff, N.J.

[e]Hegsted Salt Mixture IV

[f]Vitamin Diet Fortification Mixture, ICN Pharmaceutical Inc.

[g]Alphacel Non-nutritive bulk, ICN Pharmaceutical Inc.

[h]Sigma Chemical Co., St. Louis, Missouri

was exposed by a mid-line incision, approximately 2.5 cm long below the xyphi-sternum (Figure 4). The body of the stomach was brought into view by gentle traction on the omentum. The pylorus was raised, exerting traction towards the right on the free border of the duodenal loop. A pyloric ligature was placed on the lower border of the pylorus. The incision was closed with sutures and wound clips. The rat was then placed back in the cage without feed and water for three hours at a constant temperature of 22°C.

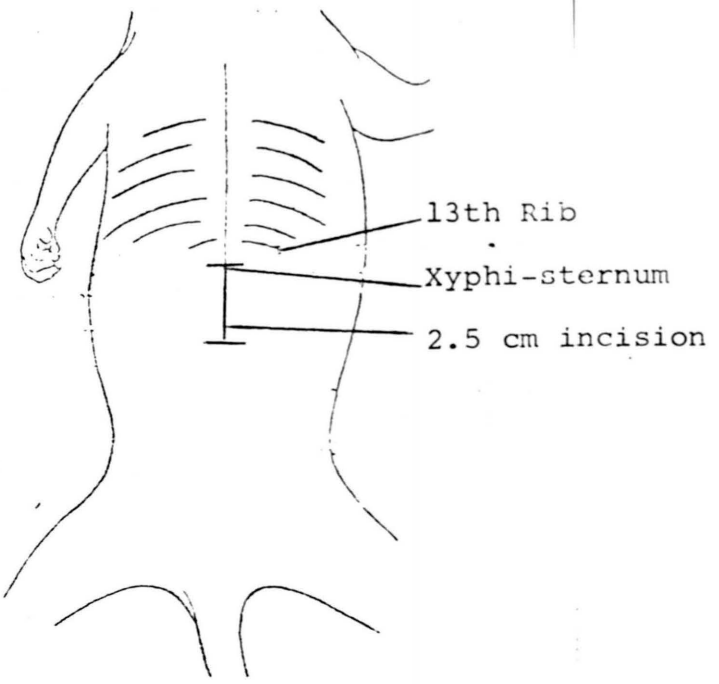

Figure 4. Diagram of Midline Incision of the Rat

At the end of the three-hour period, the animal was anesthetized with ether and a second ligation was placed at the esophageal-cardio juncture (Figure 5). Blood was drawn from the heart for determination of hematocrit and hemoglobin. The stomach was then excised, rinsed with physiological saline solution and blotted dry with surgical gauze. An opening was made in the greater curvature of the forestomach, and the gastric juice was collected into a centrifuge tube by the way of a funnel.

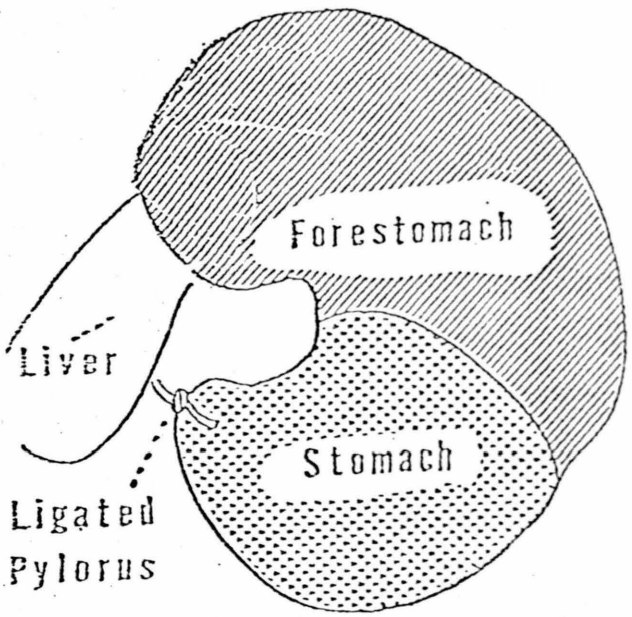

Figure 5. Diagram of Pyloric Ligation of the Rat (44)

Analytical Procedures

Hemoglobin was determined by the method of Drabkin (42). Activity of pepsin from the gastric juice was determined according to Anson (43). Gastric juice was centrifuged at 3000 rpm for ten minutes to separate the debris. Twenty µl of clear undiluted gastric juice, and 0.98 ml of 0.01 N. HCL were added to 5 ml of 2% acidified hemoglobin solution which had been warmed to 35.5°C in a constant temperature water bath. After ten minutes, 10 ml of 5% (w/v) tricholoracetic acid was added to terminate the reaction. The resulting solution was centrifuged at 4000 rpm for twenty minutes. To 5 ml of the supernatant, 10 ml of 0.05% NaOH and 3 ml of Folin's phenol reagent were added. Tyrosine was used as standard. The optical density of the digested hemoglobin was determined at 578 nm using a Perkin-Elmer 552 spectophotometer. A typical reading of the optical density from various concentrations of tyrosine standard solution is presented in Table 3.

The "Pepsin Unit" used in this study is the one defined by Anson (43). It is the amount of enzyme to hydrolyze 0.1 g hemoglobin at 35.5°C to form the amount of TCA-soluble hydrolysis products per minute which gives the same optical density with phenol reagent as 1 mmole tyrosine.

TABLE 3

OPTICAL DENSITY OF TYROSINE
STANDARD SOLUTION

TYROSINE (μM)	O.D. (at 578 nm)
0.2	0.056
0.3	0.099
0.4	0.142
0.5	0.198
0.6	0.250
0.7	0.294
0.8	0.351
0.9	0.405
1.0	0.459

Statistical Analysis

Data obtained from both studies on feed intake, growth, feed efficiency, hemoglobin, hematrocrit, and pepsin activity were analyzed statistically by analysis of variance to determine the effect of gossypol in the diet on these parameters. Pearson's correlation was performed on total free gossypol intake and pepsin activity of the rat.

CHAPTER IV

RESULTS AND DISCUSSION

The average feed intake and body weight of the rats from study 1 and study 2 are presented in Tables 4 and 5. All six groups of animals had similar feed intakes and body weights during the experimental period. Tone and Jenson (17) had shown an inhibition in growth performance when rats were fed a diet containing 830 ppm of free gossypol. In this study, the level of free gossypol containing in the four experimental diets ranged from 21-171 ppm. It is possible that at these levels, the growth of the rats is not affected.

Daily feed efficiency ratios expressed as gram of body weight change per gram of feed intake per day are shown in Table 6. In all cases, feed efficiency ratios decreased as the rats grew older which is a normal phenomenon. In study 1, the feed efficiency ratios of the two experimental groups are approximately the same or slightly less than the control group throughout the study. In study 2, both experimental groups exhibited a greater decrease in feed utilization during the third and

TABLE 4

AVERAGE FEED INTAKE OF RATS FED DIETS WITH DIFFERENT LEVELS OF FREE GOSSYPOL

	Group[a]	Free Gossypol in Diet	Average feed intake during			
			Week 1	Week 2	Week 3	Week 4½
		ppm	(g)			
	CA (10)[b]	0	105.0±14.91[c]	129.7±15.99	138.3±15.16	234.4±34.02
STUDY 1	EA_1 (10)	150	109.7±6.49	132.8±12.81	135.1±9.55	234.3±28.18
	EA_2 (10)	164	104.3±11.95	149.8±64.68	139.7±15.25	222.6±23.08
	CB (12)	0	113.4±14.20	134.5±13.81	149.5±18.06	247.1±12.01
STUDY 2	EB_1 (12)	21	111.6±17.01	142.9±18.28	154.9±14.45	234.1±21.25
	EB_2 (12)	171	116.8±11.86	151.1±11.37	163.0±14.59	243.1±29.1

[a] See p. 14 for diet designation

[b] Number of rats

[c] Mean ± S.D.

TABLE 5

AVERAGE BODY WEIGHT OF RATS FED DIETS WITH DIFFERENT LEVELS OF FREE GOSSYPOL

	Group[a]	Free gossypol in the diet	Initial Wt.	Final Wt.
		(ppm)	(g)	(g)
	CA (10)[b]	0	107.0±12.0[c]	328.9±31.8
STUDY 1	EA1 (10)	150	109.5±12.3	325.5±18.5
	EA2 (10)	164	108.6±14.9	313.4±30.4
	CB (12)	0	103.0±15.8	345.1±17.2
STUDY 2	EB1 (12)	21	99.4±16.2	323.1±26.8
	EB2 (12)	171	104.2±15.8	341.0±27.8

[a] See p. 14 for diet designation

[b] Number of rats

[c] Mean ± S.D.

TABLE 6

FEED EFFICIENCY RATIO OF RATS FED DIETS WITH DIFFERENT LEVELS OF FREE GOSSYPOL

	Group[a]	Free Gossypol in Diet	Feed Efficiency Ratio during			
			Week 1	Week 2	Week 3	Week 4½
		ppm	gm body wt. change/1gm feed intake/day			
	CA (10)[b]	0	.062±.01[c]	.055±.00	.053±.00	.030±.00
STUDY 1	EA_1(10)	150	.066±.00	.051±.00	.052±.00	.027±.00
	EA_2(10)	164	.062±.02	.053±.01	.044±.01	.029±.00
	CB (12)	0	.066±.00	.058±.01	.054±.00	.030±.00
STUDY 2	EB_1(12)	21	.066±.01	.057±.01	.046±.00	.025±.00
	EB_2(12)	171	.067±.00	.056±.00	.046±.00	.026±.00

[a] See p. 14 for diet designation

[b] Number of Rats

[c] Mean ± S. D.

and fourth weeks of the experiment when they were compared to the control group. However, no significant difference was found in either study.

Skutches et al. (45) reported that the primary pathway of gossypol excretion is via the biliary system as iron complexes. The iron-gossypol complex results in a reduced hematocrit thus decreasing the iron available for synthesis of hemoglobin. Hematocrit values were 51.0% (CA), 51.7% (CB), 49.9% (EA1), 50.1% (EB1), 50.6% (EA2), and 50.4% (EB2). Hemoglobin values,in g%,were 14.1 (CA), 13.5 (CB), 14.6 (EA1), 13.5 (EB1), 14.5 (EA2), and 14,3 (EB2) (Table 7). No significant difference was found between groups in both studies, suggesting that the levels of gossypol selected for each study did not have an adverse effect on the blood constituents of the rats.

The activity of pepsin in the gastric juice of the rats fed cottonseed flour with or without addition of gossypol is compared with that of the controls (Table 8). In study 1, the average pepsin units were: 4450 (CA), 3760 (EA1), and 3985 (EA2). In study 2, the mean pepsin units were: 6450 (CB), 5062 (EB1), and 4904 (EB2). There was no significant difference among the three groups in each study. Pearson's correlation between total gossypol intake of each rat during the entire period and the pepsin

TABLE 7

HEMATOCRIT AND HEMOGLOBIN OF RATS FED DIETS WITH DIFFERENT LEVELS OF FREE GOSSYPOL

Group[a]	Free Gossypol in Diet	HEMATOCRIT	HEMOGLOBIN
	ppm	%	g%
CA (10)[b]	0	51.0±4.96[c]	14.1±1.57
EA_1(10)	150	49.9±3.77	14.6±0.57
EA_2(10)	164	50.6-2.51	14.5-0.97
CB (12)	0	51.7-2.64	13.5-1.37
EB_1(12)	21	50.1-4.17	13.5-1.46
EB_2(12)	171	50.4-3.36	14.3-1.27

[a] See p. 14 for diet designation

[b] Number of rats

[c] Mean ± S.D.

TABLE 8

EFFECT OF GOSSYPOL ON THE PEPSIN ACTIVITY OF THE RAT

	Group[a]	Free Gossypol in Diet	Pepsin Unit
		ppm	
	CA (7)[b]	0	4450 ± 693.4[c]
STUDY 1	EA_1 (5)	150	3760 ± 549.3
	EA_2 (7)	164	3985 ± 889.1
	CB (11)	0	6450 ± 2342
STUDY 2	EB_1 (12)	21	5062 ± 1394
	EB_2 (12)	171	4904 ± 1094

[a] See p. 14 for diet designation

[b] Number of Rats

[c] Mean ± S.D.

activity of each rat is presented in Figure 6. The correlation is $r = -0.3173$ and it is significant at $p = 0.01$. This indicates that as total gossypol intake increased there was a decrease in pepsin activity in rats.

Skutches (45) reported in 1964 that the epithelial lining of the stomach was permeable to the lipid soluble gossypol. Pepsin is secreted by the chief cells of the stomach in the inactive form of pepsinogen. Gossypol is the only known naturally occurring inhibitor of pepsinogen found in food, specifically cottonseed flour products. It is conceivable to believe that the free gossypol present in cottonseed flour can react with pepsinogen in the stomach lining and inhibit the activation of pepsinogen to pepsin.

The level of 21 ppm of free gossypol in cottonseed flour diet is considered to be within the specified range of FDA's standard for human consumption (3). The levels of 150 ppm, 164 ppm, and 171 ppm of free gossypol used in this study are above the approved level of 90 ppm in the diet. All four levels of free gossypol in the cottonseed flour used in the two studies have proven to have no significant effect on body weight, feed intake, feed efficiency, blood constituents and pepsin activity in rats in comparison to rats fed diet with no free gossypol.

The negative correlation between total gossypol intake and pepsin activity demonstrated a slight decrease in

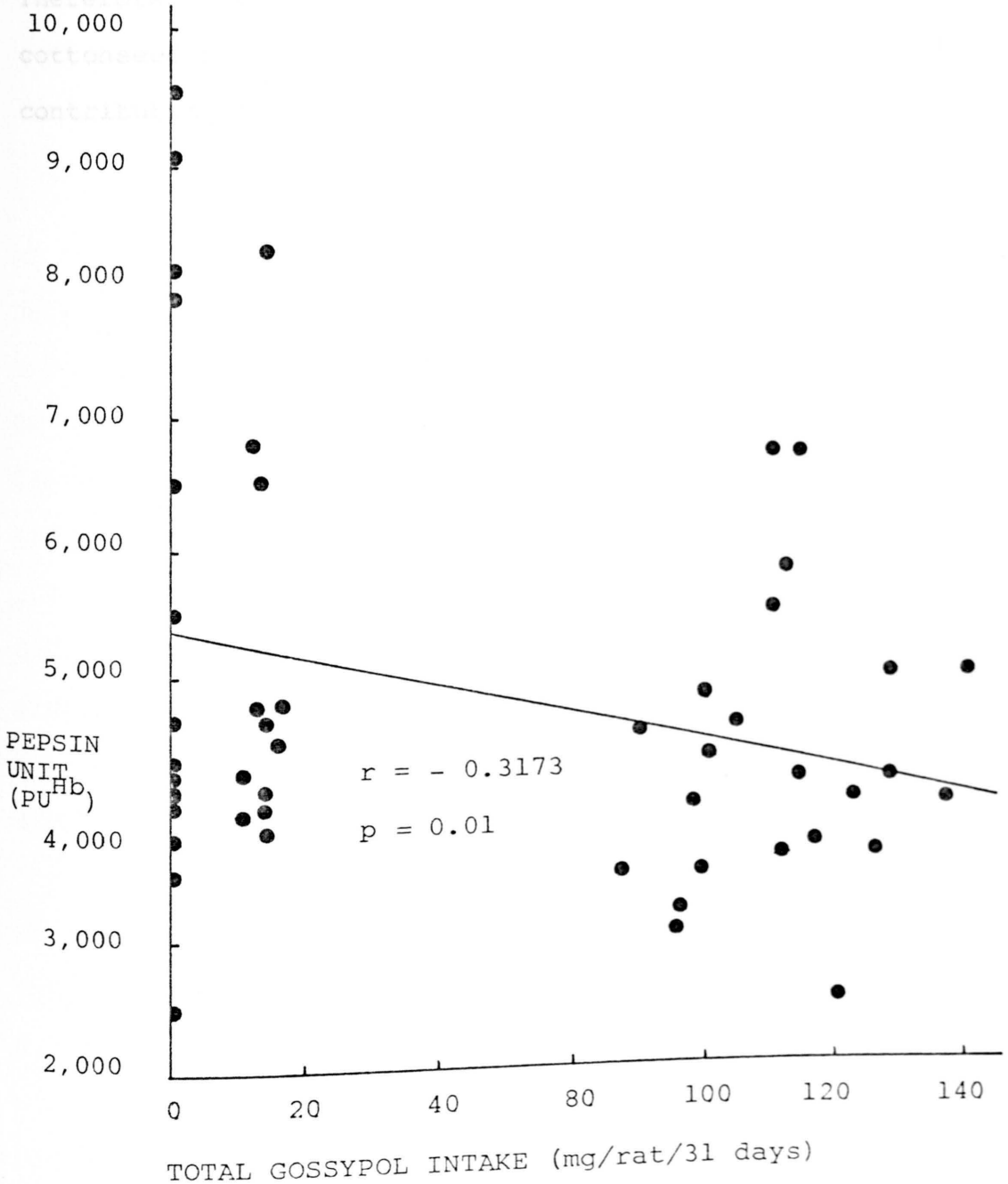

Figure 6. Pearson's correlation between the gossypol intake and pepsin activity of the rat

enzyme activity as the gossypol intake level increased. Therefore, the inhibition of pepsinogen by free gossypol in cottonseed flour, could be considered a possible factor in contributing to gossypol toxicity.

CHAPTER V

SUMMARY

The present study was designed to investigate the effect of free gossypol in diets on the pepsin activity of the rat. Two studies were conducted using Holtzman male rats. Each study contained three groups, Group 1 (control), Experimental group 1 (E1), and Experimental group 2 (E2). In study 1, the levels of free gossypol in the cottonseed flour diets were 150 ppm (EA1) and 164 ppm (EA2). In study 2, the levels of free gossypol in the diets were 21 ppm (EB1) and 171 ppm (EB2). The experimental period for both studies was a 31-day feeding period. The experimental groups in each study were compared to their study's control. All levels of free gossypol used in this study were either within or slightly above the FDA's regulation.

The parameters selected for investigation consisted of feed intake, body weight, hemoglobin and hematocrit values and pepsin activity. Statistical analysis performed on these parameters were analysis of variance, and Pearson's correlation was performed between total gossypol intake and pepsin activity.

In general, no statistically significant difference was found for all five parameters in both studies. Mean values for feed intake, body weight, and feed efficiency in the experimental groups were approximately the same as the control group. Although there was no statistically significant difference in the means of the parameters investigated, the trend for a decreasing pepsin activity with an increasing total free gossypol intake may be an indication of subtle change of enzyme inhibition.

This is the first study to investigate the possibility of the effect of feeding free gossypol to rats on the activity of pepsin. Data from this study can only provide groundwork for research in this area. Further studies are definitely indicated to elucidate the relationship between intake of gossypol and the activity of digestive enzyme, pepsin.

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