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Effect of pH on the free and total Gossypol and nutritive value of cottonseed and protein concentrate *

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SUMMARY

Studies were carried out to determine the effect of pH treatment on the free and total gossypol, epsilon amino lysine and nutritive value of cottonseed flour in chick and rats. Acid pH increased free gossypol and decreased total gossypol from the value found in cottonseed flour at pH 7. Alkaline pH, on the other hand, tended to decrease free and total gossypol in cottonseed flour and kernels. The increase in free gossypol at acid pH was not due to hydrolysis of bound gossypol or to pigments extracted by acetone-water extracts of the flour. The increase was only observed when flour or meal was treated. The decrease at basic pH value was due to destruction of gossypol. Biological tests with rats and chicks indicated lower weight gains which were due not to the increased gossypol detected but to a decrease in feed intake, conclusion reached from pair feeding studies and studies in which neutral flours were fed with added free gossypol. The above findings were discussed in terms of the pH changes in the gastro intestinal tract.

INTRODUCTION

The main factor limiting the more extensive use of cottonseed protein as flour or as a meal in human and animal feeding

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is gossypol (1-4). In spite of this, protein-rich foods containing 38% cottonseed flour have been developed and are being manufactured commercially to fight protein malnutrition (5-7). This mixture has been tested extensively (8, 9) and adverse physiological effects have never been observed in animals or humans. However, more knowledge of the behavior and fate of gossypol is needed in order that the utilization of this protein source may be increased to feed larger segments of undernourished populations.

Previous studies indicated that the addition of calcium ions as calcium hydroxide decreased free gossypol (10). Furthermore, iron addition as ferrous sulfate also decreased free gossypol (10). The simultaneous addition of both had a synergistically decreasing effect, shown both in *in vitro* (10) and *in vivo* studies with swine (11). With respect to the effect of calcium hydroxide addition on gossypol content, it was found that alkaline pH would also decrease free gossypol levels (10). In this study, therefore, the behavior of gossypol in cottonseed flour treated with solutions of different hydrogen ion concentration was investigated. Furthermore, the effect of this treatment on its nutritive value and toxicity was evaluated in biological trials with rats and baby chicks.

MATERIALS AND METHODS

Different samples of cottonseed protein, as kernel, flour and meal were used. Decorticated cottonseed kernels were obtained from local oil meals. The material was ground to pass 60 mesh in a micro Wiley mill before treatment. The flour and meal were obtained from a pre-press solvent extraction and press mill, respectively.

For the pH treatment studies, 20 grams of each sample were suspended and mixed in 100 cc of distilled water and the pH adjusted from 1 to 12 with standard hydrochloric acid or sodium hydroxide solutions. After adjusting the pH of the suspension to the desired value, the material was freeze-dried. The dried sample was again ground and analyzed for free and total gossypol by the AOCS Official Methods (12). Some of the extracts were placed in a DK4 Beckman recording spectrophotometric for the determination of maximum absorption curves. The samples were also analyzed for their free epsilon

amino lysine content by the method of Conkerton and Frampton (13).

For the biological tests in chicks and rats, 4 Kg batches of cottonseed flour (50% protein) were treated at pH 1, 5 and 7. After treatment the flours were freeze-dried and used to prepare diets made of 20% cottonseed flour, 4% mineral mixture (14), 1% cod liver oil, 5% vegetable oil and corn starch to adjust to 100%. All diets were supplemented with 5 ml of a complete vitamin mixture (15) per 100 g of diet, for the studies in weanling rats. In the studies with chicks, diets containing 40% cottonseed flour, 3% mineral mixture[5], 0.5% cod liver oil, corn starch to 100% and 1 ml of a complete vitamin mixture for 100 g of diet were used (16).

In the rat studies, 6 animals per treatment were used and placed in individual all-wire screen cages with raised screen bottoms. The changes in weight and food consumption were measured every week for 28 days. A pair-feeding experiment including similar diets, was also carried out. The food intake of the control cottonseed flour was adjusted to the food intake of the control cottonseed flour (50% protein pre-press solvent extracted) was adjusted to the food intake of the group eating the least diet.

In the chick experiments, eight one-day-old chicks were used per group and each treatment was replicated twice. They were placed in battery brooders with controled heat. The birds were fed *ad libitum* and changes in weight and feed consumed were recorded weekly for a total of 28 days.

RESULTS

Figure 1 shows the results for free gossypol in several samples treated at different pH values. It can be seen that, at pH 1, free gossypol content is significantly higher than the value at pH 6, which is close to the pH of the control suspension. As the pH shifts to the alkaline side, a minimum value is found at pH 5, which increases at pH 7, to decrease again at pH 12. The decrease at pH 12 is, however, not as pronounced as that found in previous experiments with calcium hydroxide. The affect of changing the pH was similar for all cottonseed

5 33% Calcium carbonate, 33% bone meal, 33% iodized salt, 1% micro element mixture.

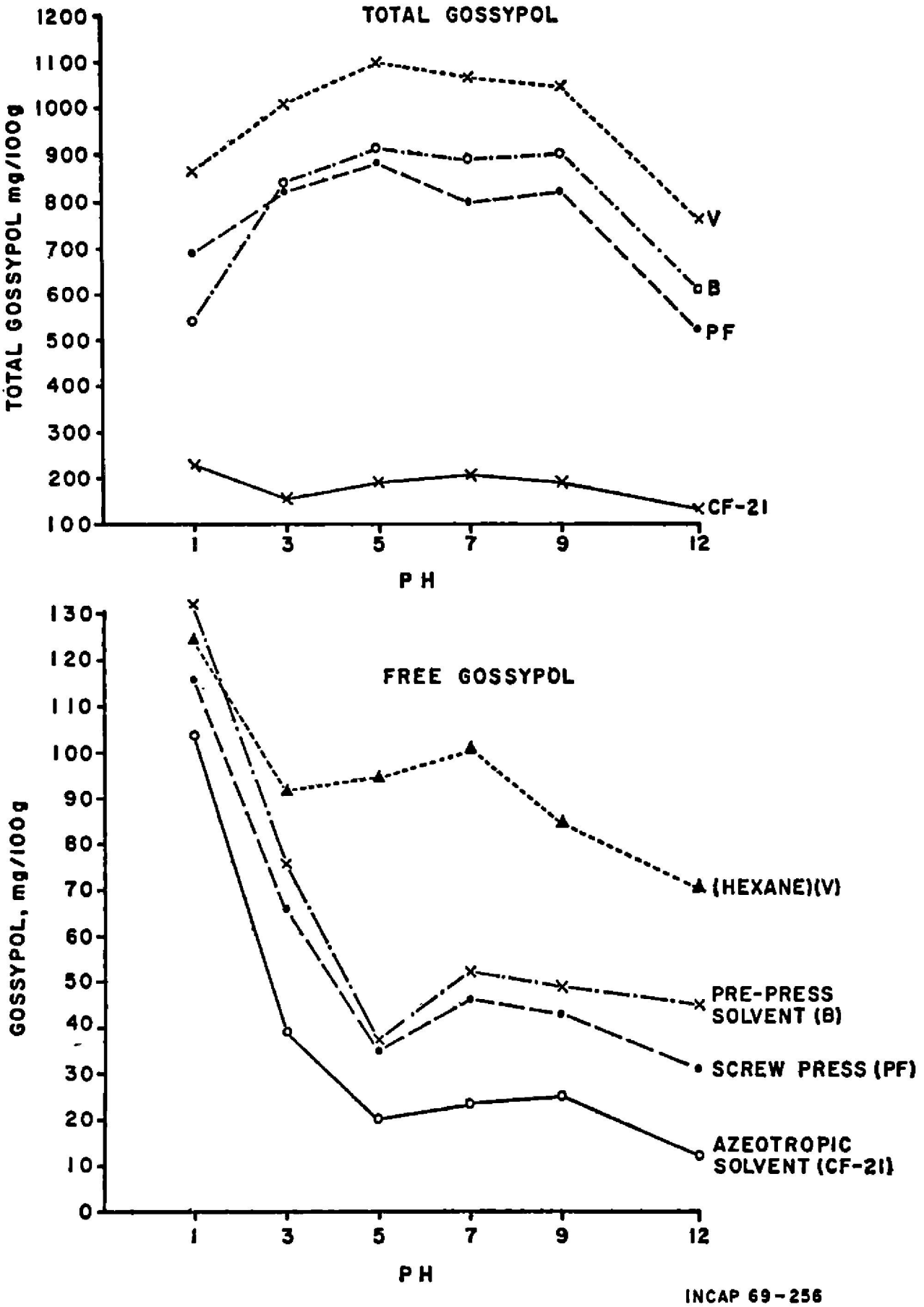

Fig. 1: Effect of pH treatment on free and total gossypol content of several cottonseed flours.

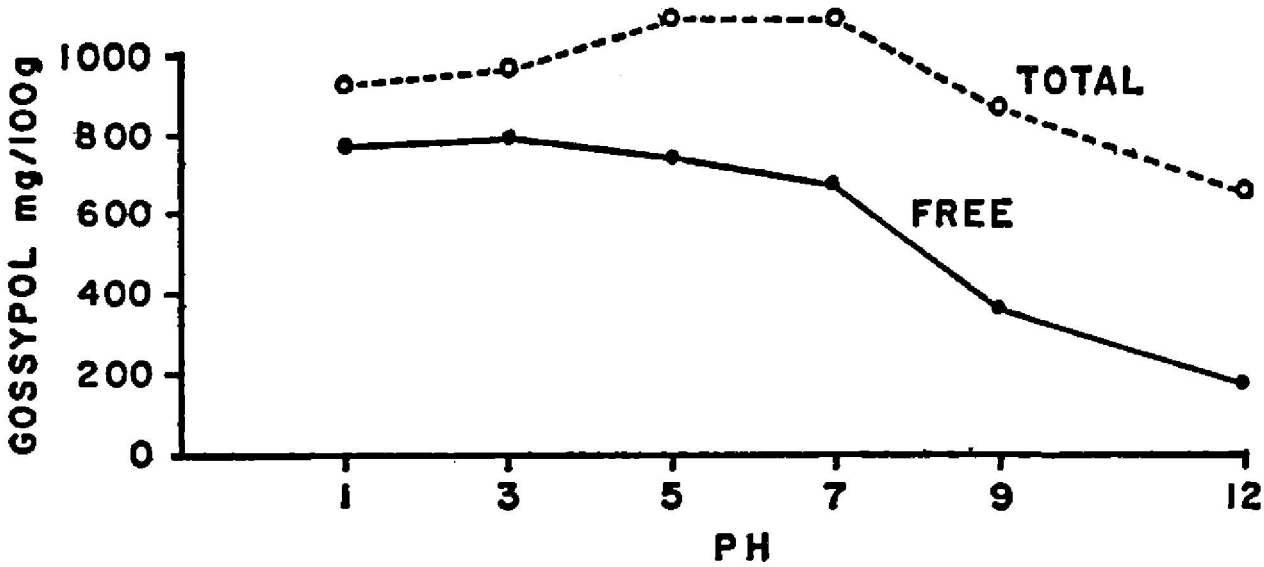

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Fig. 2: Effect of pH treatment on gossypol content of cottonseed kernels.

products and for cottonseed materials containing from 1.1 g to 0.021 g/100 g of free gossypol.

Figure 1 also shows the effect of the pH treatment on total gossypol in the same samples. In this case, acid pH gave lower total gossypol content than neutral pH, while the alkaline pH treatment yielded total gossypol concentrations slightly lower than those on the acid side of the pH scale. Maximum absorption curves of extracts of the samples treated at different pH values were identical, except with respect to concentration, which is to be expected in view of the increase in free gossypol. However, none of the extracts showed more than one maximum, which for free gossypol was 360 mμ and for the aniline derivative 440 mμ. If it is a precursor substance of gossypol, it does not absorb between 350 and 600 mμ. Figure 2 shows the changes in free and total gossypol with respect to pH in cottonseed kernels. In this case both free and total gossypol decreased as pH became alkaline.

Table I shows the results of experiments designed to test the possibility of hydrolysis on bound gossypol. Samples were treated at pH 1 and 12 and held for 1, 5, 10, 15, 20 and 30 minutes at room temperature. They were then freeze-dried and analyzed for free gossypol. As can be seen, acid pH increased free gossypol, but longer periods did not cause an increase in free and total gossypol. At pH 12, there is a slight decrease

TABLE I

EFFECT OF HOLDING TIME AT pH 1 OR 12 ON THE FREE AND TOTAL GOSSYPOL CONTENT OF COTTONSEED FLOUR

Treatment		Gossypol Free mg%	Gossypol Total %	Treatment		Gossypol Free mg%	Gossypol Total %
None		54	1.15	None		50	0.89
Moisted		40	1.07	Moisted		50	0.92
Held at pH 1 for minutes	1	97	1.12	Held at pH 12 for minutes	1	43	0.83
	5	95	0.77		5	48	0.84
	10	90	0.90		10	46	0.85
	15	99	0.77		15	53	0.82
	20	100	0.95		20	42	0.82
	30	94	1.17		30	45	0.79

TABLE II

EFFECT OF pH OF THE AQUEOUS ACETONE EXTRACTS OF COTTONSEED FLOUR ON FREE GOSSYPOL CONTENT

EXPERIMENT No. 1		EXPERIMENT No. 2	
pH of 70% aqueous acetone extract	Free Gossypol mg%	pH of 70% aqueous acetone extract	Free Gossypol mg%
1	75	1	72
3.2	74	3	73
5.3	74	4.8	78
7.4	73	7.0	74
8.8	72	9.0	70
9.5	71	10.0	55
12.5	44	12.3	42
Control	75	Control	75

TABLE III

CHANGES IN FREE AND TOTAL GOSSYPOL AND IN EPSILON-AMINO LYSINE WITH RESPECT TO pH OF COTTONSEED FLOUR

pH Treatment	GOSSYPOL		ε-Amino Lysine
	Free g%	Total g%	%
1.5	0.080	0.663	1.614
3.5	0.038	0.717	1.604
5.0	0.021	0.728	1.590
6.0	0.027	0.765	1.690
6.3 (Control)	0.030	0.770	1.687
7.0	0.033	0.747	1.650
8.0	0.031	0.787	1.557
10.0	0.029	0.684	1.595
11.5	0.024	0.435	1.562

in both free and total gossypol although longer periods of time did not diminish the values any further.

Table II summarizes the results of studies carried out to find out if the effect of pH was effective when the 70% aqueous acetone extracts, rather than the cottonseed flour, were treated at various pH. Except for low values in free gossypol between pH values 10.0 - 12.5, other pH treated extracts contained similar concentrations of free gossypol as the control extract. To learn if available lysine of the treated cottonseed flour was being affected by the pH treatment, a sample of cottonseed flour was treated at various pH. The results of the study are shown in Table III. Free and total gossypol varied as before although the changes were not as marked. The epsilon amino-lysine values seem to follow a similar trend, with respect to pH, to the one followed by free gossypol, with higher values occurring with high free gossypol and low values occurring with low levels of free gossypol.

TABLE IV

EFFECT OF pH TREATED COTTONSEED FLOUR ON ITS UTILIZATION BY RATS AND BABY CHICKS (AD LIBITUM)

TREATMENT	RATS			CHICKS	
	Avg. weight gain, g. [c]	F. E.	P.E.R.	Avg. weight gain, g [d]	F. E.
Cottonseed flour [a]	150	3.13	1.85	228	1.82
Cottonseed flour [b]	152	3.01	1.80	176	2.35
pH 1-2	126	3.22	1.94	88	2.68
3	140	3.16	1.88	182	2.08
5	153	3.01	2.04	233	1.93
7	144	3.23	1.83	199	2.42
11-12	133	3.55	1.62	176	2.36

[a] Cottonseed flour without any treatment.
[b] Cottonseed flour - wetted - dried.
[c] Average initial weight: 49 g.
% protein diets: 17.0.
[d] Average initial weight: 63 g.

Table IV presents the results of rat and chick experiments carried out to learn if the higher free gossypol values found in acid treated samples would have an effect on the growth of the animals. As can be seen, lower weight gains were obtained in animal consuming samples treated at pH 1 than in those consuming sample treated at pH 7. The effect was more marked in chicks than in rats, reaching statistical significance. PER values in rats were similar regardless of pH. F. E. followed the trend of the weight gain in the baby chicks. Feed intake decreased in the acid treatment samples. Paired feeding studies in rats, presented in Table V showed, however, that the lower weight gain in the rat fed acid samples was due to a lower food intake. Table VI shows the results of an additional experiment in which neutral flours containing gossypol added to give values similar to flours treated at pH 1 were tested.

The results were essentially the same as previously shown, with flour treated at pH 1.5 giving the lower weight gain and with lower food intake, although with a PER essentially the same as that in other groups. Apparently, the added gossypol did not affect the quality of the samples or the feed intake.

TABLE V

EFFECT OF TREATED COTTONSEED FLOUR ON ITS UTILIZATION BY RATS

pH Treatment	Protein in diet %	Avg. final utilization g	Avg. food intake g	P. E. R.
Control [a]	20.9	103	307	1.61
Control [b]	20.7	106	307	1.67
pH 1.5	21.4	100	296	1.58
pH 7	21.2	105	308	1.62
pH 12	19.5	102	309	1.69

[a] Cottonseed flour not treated.
[b] Cottonseed flour. Controlled feeding group.
[c] Controlled feeding.

TABLE VI

EFFECT OF ADDING GOSSYPOL TO NEUTRAL COTTONSEED FLOURS

Treatment	Protein in diet %	Avg. weight gain [c] g	Avg. food consumed g	P.E.R.
Cottonseed flour + gossypol [a]	22.7	159	448	1.56
Cottonseed flour + gossypol [b]	21.9	158	429	1.68
pH 1.5 CSF	21.4	118	355	1.55
pH 7.0 CSF + gossypol [d]	21.6	167	453	1.71
pH 12 CSF + gossypol [d]	20.4	154	437	1.73

[a] Cottonseed flour without any treatment.
[b] Cottonseed flour - wetted - dried.
[c] Average initial weight: 48 g.
[d] Gossypol added in the form of unproessed kernels in levels varying etween 1.9 - 2.3% of the diet. It had a free gossypol control of 700 mg/100 g of flour.

DISCUSSION

The results presented, clearly indicated that acid treatment of cottonseed flours causes an increase of free gossypol, while alkaline treatment causes a decrease in the concentration of the pigment.

The increase in free gossypol could be explained on the basis of the hydrolisis of the gossypol, which has reacted with sugars (4) but not with lysine during the processing of cottonseed. This is suggested by the experiments in which the pH was maintained constant up to 30 minutes without further increases in free gossypol beyond that observed at 0 time. That the increase was not due to other pigments reacting as gossypol is indicated by the results obtained when an aqueous

acetone extract of cottonseed flour was adjusted to pH 1 and the results compared to same extracts but not treated. Furthermore, whatever the effects of pH are, they are effective only when the cottonseed meal or flour are treated.

On the other hand, the effect of the alkaline pH treatment which cause a decrease in free gossypol is probably due to destruction of the pigment. It has been shown that alkaline pH destroys gossypol (10, 17). Furthermore, these results corroborate previous findings in which a cottonseed flour containing diet upon treatment with calcium hydroxide or alkaline pH showed lower levels of free gossypol (10).

This procedure could, therefore, be used advantageously to produce a cottonseed flour with lower levels of free gossypol. It would involve suspending the cottonseed flour at an alkaline pH and passing air through it. This treatment will destroy the free gossypol. The next step would require neutralization, followed by washing and drying of the product. Likewise, the increase in free gossypol could also be used as a means of producing cottonseed flours with less gossypol. In this case, after treating the material to pH 1, the sample can be washed with acetone added to give a 70% solution. This treatment would be followed by washing and drying. This procedure could be better, than the alkaline treatment, because of the cottonseed suspension at acid pH is held for at least 30 minutes, total gossypol would also decrease, as indicated by the results presented in this paper. It would be necessary to remove excess H+ or OH-ions since the tests with animals indicated that flours treated at pH 1 or pH 12 were not well consumed by either chicks or rats.

The results presented have some practical implications for the utilization of cottonseed flour as food. During protein digestion at the stomach level, the reaction is acid as a result of hydrochloric acid excretion. This implies that free gossypol in the stomach is higher than that which was ingested with the flour. This acid-formed gossypol, together with the gossypol ingested, could raise the total to toxic levels or a least to levels sufficiently high to cause some alteration in the utilization of the food by the animal. As food moves along the intestine, the pH will shift to the alkaline side, which might destroy some of the gossypol ingested. These possibilities are being studied *in vivo* and will be reported in the near future.

In studies carried out with dogs fed cottonseed flour (18), and under which gossypol balances were computed, it was found that more free gossypol appeared in the feces than that ingested. Total gossypol in feces, however, balanced total gossypol intake. The findings of the present study could be used to explain the results with dogs (18), and also to explain the toxic effects of gossypol. Since acid pH increases free gossypol part is excreted, explaining the higher free gossypol levels in feces, and part is absorbed explaining the toxic effects of the pigment.

RESUMEN

Efecto del pH sobre el contenido de gosipol libre y total y sobre el valor nutritivo de la harina de semillas de algodón

Se realizaron estudios en pollos y ratas, encaminados a determinar el efecto del tratamiento del pH sobre el contenido de gosipol libre y total, grupos epsilon amino libres de la lisina, y valor nutritivo de la harina de semilla de algodón.

El pH ácido incrementó el gosipol libre e hizo disminuir el gosipol total de los valores encontrados en la harina de semilla de algodón al usarse un pH de 7. En cambio, el pH alcalino tendió a reducir el gosipol libre y total de la harina y de la almendra de la semilla de algodón. El aumento en gosipol libre observado con el pH ácido no se debió a hidrólisis del gosipol ligado a los pigmentos extraídos de la harina con extractos de acetona y agua. El incremento únicamente se observó cuando la harina o torta habían sido sometidas a tratamiento. El descenso al valor básico del pH se debió a la destrucción del gosipol.

Las pruebas biológicas efectuadas en ratas y pollos señalaron menores incrementos ponderales que no se debían al mayor contenido de gosipol determinado, sino a una menor ingesta de alimento. Se llegó a esta conclusión con base en estudios pareados de alimentación y a partir de ensayos en que los animales consumieron harinas neutras con el agregado de gosipol libre.

Se comentan los hallazgos citados en términos de los cambios de pH en el tracto gastrointestinal.

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