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Raymond Borchers

W. E. Ham

R. M. Sandstedt

C. W. Ackerson

R. H. Thayer

See next page for additional authors

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The Authors

Raymond Borchers is assistant agricultural chemist; W. E. Ham,[1] formerly assistant agricultural chemist; R. M. Sandstedt, agricultural chemist; C. W. Ackerson, chairman of the department of agricultural chemistry; R. H. Thayer,[2] formerly assistant poultry husbandman; and F. E. Mussehl, chairman of the department of poultry husbandry, at the Nebraska Experiment Station.

[1] Present address, State College of Washington, Pullman.
[2] Present address, Oklahoma Experiment Station, Stillwater.

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THE MARKED improvement in the apparent nutritive value of soybean protein after heating has long been recognized. The fundamental cause of this improvement has never been fully understood. Furthermore, commercial soybean oil meal varies considerably in nutritive value although heat treatment is common in most processing plants. Since heating is generally regarded as deleterious to the nutritive quality of protein, the following investigations of various treatments of soybean oil meal are of particular importance in developing a soybean or soybean oil meal of maximum and uniform nutritive value.

Review of Literature

OSBORNE AND MENDEL (23) in 1917, investigating the nutritive value of soybeans for rats, were the first to report the improvement of soybeans after heating. Later investigations by Wilgus, Norris, and Heuser confirmed these findings for the chicken (27), and they were confirmed by Robison for the pig (24), by Scharrer and Nebelsiek for sheep (25), and by Lewis and Taylor for humans (19).

Since unheated soybeans have a raw, "greenish" taste while heated soybeans have a more agreeable nutty flavor, the suggestion was made that the former were unpalatable and as a consequence had an apparently lower nutritive value. A number of investigations (13, 21, 26) as well as data presented in this bulletin from the paired feeding tcchnic, have disproved this hypothesis. The second suggestion made was that unheated soybeans were less digestible than heated soybeans. Soybean digestibility studies of Johnson, Parsons, and Steinbock (16) showed that the difference in digestibility between raw and heated soybeans was neligible, an observation confirmed in other laboratories (13, 20) as well as by data reported in this bulletin. Johnson *et al.* (16) also observed that rats receiving raw soybeans retained less nitrogen and sulfur than those receiving heated soybeans. From this, they concluded that raw soybean protein was digested and absorbed as fully as heated soybean protein but that part of the raw soybean protein was absorbed in a form which was not utilized for growth but was instead directly catabolized and excreted as urinary nitrogen and sulfur end-products.

Mitchell and Smuts (21) observed that the addition of cystine to a raw soybean ration improved growth. Almquist *et al.* (2) extended this investigation using methionine additions to both raw and heated soybean rations. They found that methionine supplements improved growth with the raw soybean ration more than with the heated. Therefore, they concluded that the availability of the methionine of raw soybeans was enhanced by heating. However, the studies of cystine or methionine supplementation of both raw and heated soybeans reported by Mitchell (22), Hayward and Hafner (15), Evans and McGinnis (10) and in this bulletin show that cystine or methionine improved growth on either raw or heated soybean rations.

Shrewsbury, Vestal and Hauge (26) concluded from their studies on the improvement of soybeans by heating that, ". . . . there is some reason for believing that certain materials of a toxic nature are removed or destroyed during the heating process." Ham, Sandstedt, and Mussehl (12) have reported that a dilute acid (pH 4.2) extract of raw soybean meal, but not heated soybean meal, contains a factor which when incorporated into a ration, depresses the rate of growth, a finding confirmed by Klose, Hill and Fevold (17). This tends to confirm the hypothesis of Shrewsbury *et al.* (26) that raw soybeans contain a toxic, though not lethal, substance which inhibits or depresses the rate of growth and which is removed by heating.

Nutritive Value of Treated Soybean Oil Meal

THE SOYBEAN OIL meal used in this study was a commercial solvent process meal prepared with a minimum of heat treatment.

Soybean oil meal extracted with NaCl solution. Raw soybean oil meal was suspended in a minimum of 0.4 per cent NaCl solution and allowed to stand at 4°C. for 24 hours. The liquid portion was then removed by filtering through cheese cloth. This procedure was repeated on the residue and the second filtrate obtained added to the first. Nitrogen originally present in the raw meal was about equally distributed between the final residue and the combined filtrates. The residue was dried at room temperature and ground before inclusion in rations. The filtrate was mixed with the other ingredients in the ration, dried at room temperature and the whole ration ground before feeding.

Day-old, single-comb White Leghorn chicks were placed in electrically-heated battery brooders. Food and water were available *ad libitum*. All chicks consumed the ration of Lot 2, Table 2, during the first week, after which they were selected for uniform weight and distributed in the several experimental lots. Feed consumption during the experimental feeding period was recorded. The rations and growth data are presented in Tables 1 and 2.

TABLE 1.—*Base ingredients.*

Ingredient	Parts by weight
Yellow corn meal	28
Wheat shorts	10
Oats, pulverized	10
Wheat bran	10
Alfalfa leaf meal	5
Meat scraps	3
Fish meal	3
Dried whey	4
Limestone	1
Bone meal	1
NaCl	1
Vitamin D carrier (200 AOAC units per g.)	1
Total	77

That soybean meal is improved by heating is confirmed by comparison of Lot 1 which gained 105 g. and Lot 2 which gained only 55 g. Lot 3, which received the residue from NaCl extraction, gained 92 g. indicating that at least a part of the growth inhibitor was removed from the raw meal. A gain of only 50 g. by Lot 4 receiving the filtrate from NaCl extraction indicates that the growth inhibitor was concentrated in this fraction while Lot 5 receiving heated filtrate gained 84 g., showing that the growth inhibitor can be partially destroyed in this filtrate by heating.

TABLE 2.—*Gains in weight of chicks fed soybean oil meal extracted with NaCl solution.*

Ingredient parts by weight	Lot number 1	2	3	4	5
Base ingredients	87.8	87.8	87.8	87.8	87.8
Heated soybean meal (15 lbs. for 30 min.)	12.2				
Raw soybean meal		12.2			
Residue			6.5		
Raw filtrate				7.2	
Heated filtrate (15 lbs. for 15 min.)					7.2
Starch			5.7	5.0	5.0
Number of chicks	20	20	20	20	20
Deaths	0	2	0	0	0
Av. weight 1 wk. (g.)	69	69	70	68	70
Av. weight 3 wks. (g.)	174	124	162	118	154
Average gain (g.)	105	55	92	50	84
G. gain per g. feed consumed	0.33	0.24	0.29	0.18	0.31
Protein (N x 6.25) (Pct.)	20.4	20.9	18.3	20.3	19.6

Soybean oil meal supplemented with cystine and methionine. In a second feeding experiment, the effect of adding cystine and methionine to rations containing either raw or heated soybean meal was investigated. Day-old, single-comb White Leghorn chicks were fed the ration of Lot 7, Table 3, for one week, after which they were selected for uniform weight and distributed in the experimental lots. The chicks were housed in individual electrically-heated batteries. Water was available *ad libitum*. Feed consumption was equalized during the two-week experimental period among all chicks, the lot consuming the least amount of feed governing the amount fed to each of the other lots according to the method of Ackerson *et al.* (1). Cystine and methionine were added at a level calculated to furnish an amount equal to that present in the soybean oil meal at the level fed (4). The rations and growth data are presented in Tables 1 and 3. The results in Lots 6 and 7 confirm the findings of other workers (13, 21, 26) that palatability is not a factor in the nutritional difference observed between raw and heated soybeans since Lot 6 with heated soybean oil meal gained 100 g. while Lot 7 with raw meal gained only 76 g., although both lots consumed the same amount of feed. The addition of cystine and methionine improved growth on heated soybean oil meal from 100 to 111 g. and on raw meal from 76 to 91 g.

Soybean oil meal treated with ethyl alcohol. In the third feeding experiment, a study was made of the effect of ethyl alcohol upon the nutritive value of the soybean meal. Raw soybean meal was extracted for 45 minutes either with 45 per cent ethyl alcohol at 70° or at 95° C., or with 95 per cent ethyl alcohol at room temperature. The composition of the rations and growth data are presented in Tables 1 and 4. These data indicate that treatment of raw soybeans with ethyl alcohol improved the nutritive value of the soybean oil meal somewhat although not all treatments appear to be as effective as autoclaving.

Soybean oil meal extracted with dilute acid. Because extraction of soybean oil meal with NaCl solution or distilled water removes such a large portion of the soybean protein, it was important to find a suitable solvent which might remove the growth inhibitor with little other material. The use of alcohol is undesirable since it is known to denature proteins and hence make recovery of a possible factor difficult. Circle (8), in studies on soybean protein, found that the isoelectric point of most of the soybean protein was at pH 4.2, that is, the minimum amount of protein would be extracted by a dilute acid solution at pH 4.2. The extraction of raw soybean meal was then carried out by mixing in the proportion of 200 g. meal with 1 liter of water plus sufficient concentrated HCl (7.6 ml.) to bring the final pH to 4.2. The suspension was stored at 4°C. overnight and then filtered through

TABLE 3.—*Gains in weight of chicks fed soybean oil meal supplemented with cystine and methionine.*

Ingredient parts by weight	Lot number 6	7	8	9
Base ingredients	87.8	87.8	87.8	87.8
Heated soybean meal (15 lbs. for 30 min.)	12.2			
Raw soybean meal		12.2		12.2
l-cystine mg./100 g.			56.9	56.9
dl-methionine mg./100 g.			92.5	92.5
Number of chicks	8	7	8	8
Deaths	0	1	0	0
Av. weight 1 wk. (g.)	62	57	61	59
Av. weight 3 wks. (g.)	162	133	172	150
Average gain (g.)	100	76	111	91
G. gain per g. feed consumed	0.42	0.31	0.46	0.38

cloth. The residue was dried at room temperature and ground. The filtrate, which contained about 10 per cent of the original soybean protein, was mixed with the other ingredients of the ration, dried at room temperature, and ground.

Day-old, single-comb White Leghorn chicks were maintained on the ration of Lot 16, Table 5, for 6 days, after which they were housed in individual electrically-heated brooders during a 10-day experimental period. Food and water were available *ad libitum*. The rations and growth data are presented in Tables 1 and 5. Dilute acid extraction was found to be as efficient as heat in removing the growth inhibitor since Lot 15 and Lot 17 consuming heated and acid-extracted

TABLE 4.—*Gains in weight of chicks fed soybean oil meal treated with ethyl alcohol.*

Ingredient parts by weight	Lot number 10	11	12	13	14
Base ingredients	87.8	87.8	87.8	87.8	87.8
Heated soybean meal (15 lbs. for 30 min.)	12.2				
Raw soybean meal		12.2			
Soybean meal extr. 45 min. 45% EtOH at 95° C.			12.2		
Soybean meal extr. 45 min. 45% EtOH at 70° C.				12.2	
Soybean meal extr. 45 min 95% EtOH at room temp.					12.2
Number of chicks	16	16	16	16	16
Average gain (g.)	80	66	84	76	72
G. gain per g. feed consumed	0.39	0.31	0.41	0.40	0.38

TABLE 5.—*Gains in weight of chicks fed soybean oil meal extracted with dilute acid.*

Ingredient parts by weight	Lot number 15	16	17	18	19
Base ingredients	87.8	87.8	87.8	87.8	87.8
Heated soybean meal (15 lbs. for 30 min.)	12.2				
Raw soybean meal		12.2			
Residue			12.2	11.0	11.0
Heated filtrate (15 lbs. for 30 min.)				1.2	
Raw filtrate					1.2
Number of chicks	8	8	8	8	7
Average gain 10 days (g.)	37	27	37	40	24
G. gain per g. feed consumed	0.34	0.24	0.34	0.31	0.20

meal, respectively, made the same growth of 37 g. When the acid-extracted meal and the filtrate were combined and fed, the growth was reduced to 24 g. in Lot 19, slightly less than on the original raw meal of 27 g. in Lot 16. Lot 18, fed the acid-extracted meal plus the heated filtrate, made a better gain, 40 g., than did Lot 15 on heated meal.

In order to study the nutritive value of acid-extracted soybean meal under practical conditions, batches of both heated and acid-extracted soybean meal were prepared in sufficient amounts to feed 150 chicks on each diet for 8 weeks. The soybean meal was extracted by mixing with 0.05 N HCl in the proportions of 1 g. meal plus 10 ml. acid which gave a final pH of 4.2. The suspension was allowed to stand at room temperature for 3 hours and then filtered. The residue was dried with forced air at 35–40° C. and ground. One lot of 150 day-old White Rock chicks was placed on each diet under practical brooder house conditions with food and water available *ad libitum*

TABLE 6.—*Gains in weight to 8 weeks of chicks fed soybean oil meal extracted with dilute acid.*

Ingredient parts by weight		Lot number 794	795
Base ingredients		77	77
Heated soybean meal (15 lbs. for 30 min.)		23	
Acid-extracted soybean meal			23
Number of chicks		150	150
Deaths		7	13
Av. weight 4 wks. (g.)		223	251
Av. weight 8 wks. (g.)	All	678	650
	Cockerels	733	695
	Pullets	623	607

and electrically heated brooders. The rations and growth data are presented in Tables 1 and 6. Although the average weight at 4 weeks favored the acid-extracted soybean oil meal, the heated soybean meal ration and the acid-extracted soybean meal ration gave similar average 8-week weights.

Some Characteristics of a Trypsin Inhibitor

IN 1944, Ham and Sandstedt (11) reported the existence of a factor in unheated soybean oil meal which inhibited the digestion of casein by commercial trypsin as measured by formol titration, a finding confirmed by Bowman (6) who has since reported (7) the existence of two distinct trypsin inhibitors in soybeans. Kunitz (18) has succeeded in obtaining purified crystalline trypsin inhibitor from soybeans and has characterized the substance as a protein.

Relation of the trypsin inhibitor to other proteolytic enzymes. Extracts of raw soybean oil meal were prepared by mixing 1 g. of the meal with 10 ml. of 0.05 N HCl, allowing the mixture to stand overnight at 4° C. and centrifuging it to obtain an extract as a clear supernatant liquid. The possible inhibition of pepsin by soybean extract was first investigated. One ml. of soybean extract was mixed with 1 ml. of 0.1 per cent pepsin (Merck and Co., U. S. P. granular) in 0.1 N HCl and diluted to 10 ml. Pepsin activity units were then determined by the method of Anson (3) on this solution as well as on a control solution without the soybean extract. The pepsin activity units found in the two solutions were identical. Hence, it was concluded that soybean extracts do not inhibit pepsin, a conclusion confirmed by the data of Evans (9). A similar procedure was applied to a 0.1 per cent papain (Merck and Co.) solution, using the method of Anson (3). Again no differences were found, indicating no inhibition of papain by a soybean extract. To study the effect of the soybean extract on "erepsin," 1 ml. of a 4 per cent solution of desiccated duodenal powder (Armour and Co.) as a source of "erepsin" was mixed with 0.5 ml. of soybean extract and diluted to 10 ml. The proteolytic power of this solution was determined by the procedure of Anson (3) as given for trypsin, as well as of a similar solution without soybean extract. The apparent digestion of the solution with soybean extract was only 25 per cent of that given for the same amount of "erepsin" without soybean extract. Hence, the soybean contains a factor which inhibits *in vitro* proteolysis by "ereptic" enzymes as well as proteolysis by trypsin.

Borchers *et al.* (5) reported that incubation of soybean extracts with trypsin does not reduce the subsequent inhibition of trypsin by the soybean extract. Similar procedures were applied to find the effect of other proteolytic enzymes on the trypsin inhibitor. To four 10 ml.

portions of soybean extract were added respectively 100 mg. pepsin, 100 mg. desiccated duodenal powder, 25 mg. papain, and 10 mg. ficin. The pH was adjusted by addition of 6 N HCl or 10 per cent NaOH as required to pH 2 for pepsin, pH 8 for "erepsin," pH 4.5 for papain, and pH 5 for ficin. The extract plus enzyme after proper adjusting of the pH was covered with toluene and incubated at 37° C. for 48 hours, after which the trypsin inhibitor was determined (5) on each and the results compared with the untreated extract. No diminution in trypsin inhibitor was noted after incubation with pepsin, "erepsin" or papain, but the ficin-treated preparation had lost all trypsin inhibiting power. It was therefore concluded that of the five proteolytic enzymes investigated (pepsin, trypsin, "erepsin," papain, and ficin), only ficin was capable of digesting the inhibitor and that therefore, the inhibitor is resistant to the proteolytic enzymes of the digestive tract.

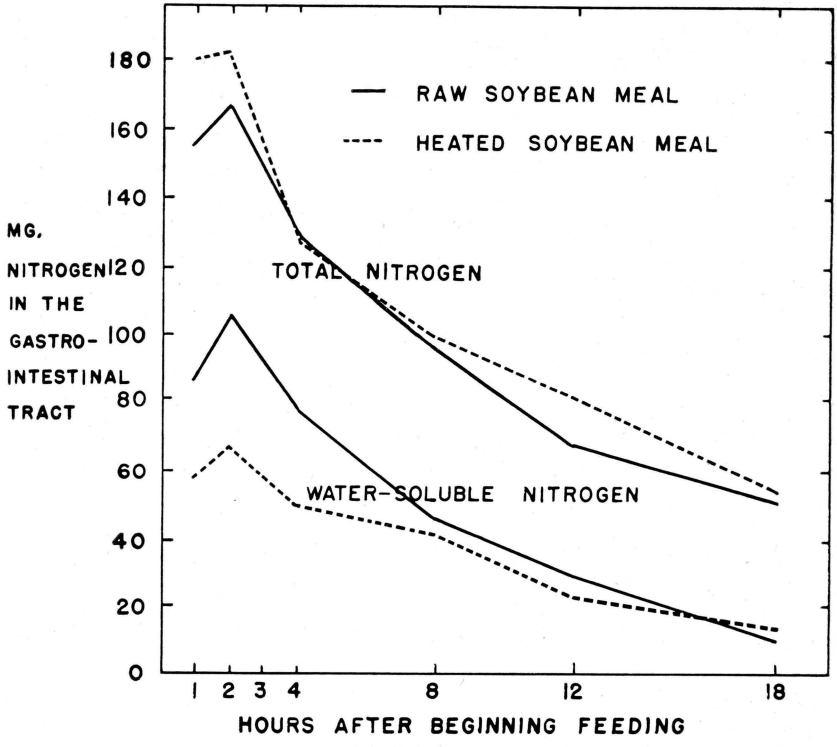

FIGURE 1.—Digestion of raw and heated soybean meal *in vivo*.

Effect of the trypsin inhibitor on digestion in vivo. Since all the investigations which have been reported concerning the trypsin inhibitor have been done *in vitro,* it seemed of interest to study digestion *in vivo* in the presence of the trypsin inhibitor as compared with normal digestion. This seemed of particular importance since Evans (9) has reported that raw soybeans when digested *in vitro* with pepsin, trypsin, and "erepsin," successively, simulating the natural order, were digested equally as well as heated soybeans. In this study, 24 albino rats weighing from 97 to 129 g. were fasted overnight. At 12 o'clock noon, 12 were fed 2 g. of raw soybean meal and 12 the same amount of soybean meal heated at 15 lbs. pressure for 30 minutes. All of the rats consumed the food within 30 minutes. At one hour after the beginning of the feeding two rats, one female and one male, from each group were killed and the contents of the gastro-intestinal tract were washed out, diluted to 50 ml. volume and stored at 4° C. At 2, 4, 8, 12, and 18 hours after the beginning of feeding, this procedure was repeated. The contents of the gastro-intestinal tract were then analyzed for total nitrogen and for water-soluble nitrogen after filtration through filter paper (E. H. Sargent & Co., No. 501). The results are given in Figure 1 in graphical form. From these results, the trypsin inhibitor seems to have no effect on the course of gross digestion *in vivo,* which conforms with the results of the digestibility studies of Johnson, Parsons, and Steenbock (16), and others (13, 20).

Effect of sulfaguanidine on digestibility of raw soybean meal. Although Johnson, Parsons, and Steenbock (16) have reported that raw soybean meal has a digestibility value but little different from heated soybeans, it appeared possible that the completion of the digestion of raw soybean may have been accomplished by bacterial action. The diet of Johnson *et al.* (16) was prepared and fed to albino rats, duplicating as nearly as possible their procedure. Sulfaguanidine at a level of 0.5 per cent was included in the ration to suppress intestinal bacteria. The results are presented in Table 7. Although the digestibility value of heated soybean oil meal was slightly better than that of raw soybean oil meal either with or without sulfaguanidine, the

TABLE 7.—*Effect of sulfaguanidine on digestibility of soybean oil meal.*

Ration containing:	No. of animals	Digestibility g. N absorbed/g. N ingested X 100
Raw soybean meal	6	77
" plus 0.5% sulfaguanidine	6	78
Heated soybean meal (15 lbs. for 30 min.)	6	84
" plus 0.5% sulfaguanidine	6	81

difference is without significance. Sulfaguanidine, therefore, appears to have no effect on the digestibility value of either raw or heated soybeans.

Discussion

FROM THE REVIEW of the literature, five hypotheses may be drawn which have been presented to explain the improvement of soybeans after heating. First, unheated soybeans are less palatable than heated soybeans. Second, unheated soybeans are not as digestible as heated soybeans. Third, part of the protein of raw soybeans is absorbed in a form which is not utilized for growth. Fourth, the cystine and methionine are less available in unheated soybeans than in heated soybeans. And fifth, raw soybeans contain a toxic substance which is destroyed by heating.

Data cited in the literature review have disproved the first, second, and fourth of these hypotheses. These reports have been confirmed by the additional experimental results reported herein. The data presented in Table 3, Lots 6 and 7, in which chicks were maintained on equalized food intake, showed that those consuming a diet containing heated soybeans grew more rapidly and efficiently than those consuming an equal amount of ration containing raw soybeans. In this experiment food consumption was not permitted to be a variable and, hence, confirms the conclusion that palatability is not the cause of the lower nutritive value of raw soybeans compared with heated soybeans. Digestibility data shown in Figure 1 and Table 7 likewise confirm the previous findings that raw soybean protein is digested and absorbed as readily as heated soybean protein. Concerning the fourth hypothesis, if cystine and methionine are less available in raw soybeans than in heated soybeans, addition of these amino acids in adequate amounts to both types of rations should equalize the rate of growth. The growth data in Table 3 show this is not the case. The only valid conclusion to be drawn from feedings with supplements of cystine and methionine is that the nutritive plane of either a raw or heated soybean ration has been raised but that animals consuming a raw soybean ration plus cystine and methionine do not attain the growth rate of the heated soybean ration plus cystine and methionine. Therefore, the factor operating to prevent maximum growth with raw soybeans is still operating in the presence of cystine and methionine.

The experimental results reported here support the fifth of these hypotheses, that is, that raw soybeans contain a "toxic" factor which has been termed a growth inhibitor. Extraction of raw soybean oil meal with either NaCl or dilute acid solution removes this growth inhibitor, leaving an insoluble portion or residue which generally equals heated soybean oil meal. That the growth inhibitor is present

in the soluble portion or filtrate is indicated from the results of adding the filtrate to rations containing either heated or properly extracted meal.

The identity of the growth inhibitor has not been established, but it has been assumed that the growth inhibitor is identical with the trypsin inhibitor. Although the trypsin inhibitor has no appreciable effect on gross digestibility as shown by *in vivo* digestion studies reported in Figure 1 and Table 7, it is assumed that the inhibitor, due to its action on trypsin and "erepsin," prevents the complete digestion of a part of the soybean protein which is, however, absorbed but in a form not capable of being utilized for growth by the animal body. This is essentially in agreement with the third hypothesis presented and as concluded by Johnson *et al.* (16). That the trypsin inhibitor is available to exert its action throughout the course of digestion is indicated by the fact that it is not affected by incubation with any of the commonly recognized proteolytic enzymes of the gastro-intestinal tract.

Summary

RAW OR UNHEATED soybean oil meal when extracted with 0.4 per cent NaCl solution, hot 45 per cent ethyl alcohol or 95 per cent ethyl alcohol at room temperature, or dilute acid leaves an insoluble residue which compares favorably with heated soybean oil meal in nutritive value. The soluble portion of the filtrate from NaCl or acid extraction contains a growth inhibitor which, when added to rations containing heated soybean meal or the residue, reduces the nutritive value to that of raw soybean meal rations. It is concluded that raw soybeans contain a growth inhibitor soluble in 0.4 per cent NaCl or in dilute acid at pH 4.2.

A trypsin inhibitor is present in the same fractions that contain the growth inhibitor and it is assumed that the two are identical. The trypsin inhibitor acts, not by depressing the digestibility value of soybean protein or decreasing its rate of digestion, but by preventing the complete digestion of a part of the soybean protein which is, however, absorbed but not utilized for growth.

Extracts of unheated soybeans inhibit the enzymatic action of trypsin and "erepsin" but not that of pepsin or papain. The trypsin inhibitor present in extracts of unheated soybean is not affected by incubation with pepsin, trypsin, "erepsin," or papain, but is inactivated by incubation with ficin.

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