Effect of Moisture Content and Cooking Time on Soybean Meal Urease Index, Trypsin Inhibitor Content, and Broiler Growth

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(Received for publication November 2, 1979)

ABSTRACT Soybean meal (SBM) was solvent-extracted at a commercial processing plant and subjected to added moisture and cooking time treatments. Results in Experiments 1 and 2 indicated that regardless of cooking time, the addition of either 10 or 25% moisture to raw SBM prior to either autoclaving or dry heating decreased urease index. Results in Experiments 3 and 4 indicated that when raw SBM contained either 0, 4, 8, 12, or 16% added moisture and autoclaved at 120 C in sealed containers from 0 to 135 min with 15-min intervals, urease and trypsin inhibitor contents decreased with either increasing added moisture or cooking time.

With 16% moisture, either 30 or 45 min of cooking time was required to decrease urease index and trypsin inhibitor contents, respectively, to an acceptable level. Raw SBM autoclaved with less than 12% moisture contained urease and trypsin inhibitor contents considered excessive, regardless of cooking time. Raw SBM autoclaved for either 60 min at 12% or 30 min at 16% moisture showed lysine degradation when compared to SBM autoclaved for shorter periods of time.

Increasing moisture and cooking resulted in decreased trypsin inhibitor and urease contents, increased broiler growth, improved feed efficiency, and decreased pancreas hypertrophy. Trypsin inhibitor was found in Experiment 5 to be a more reliable criterion for determining processing adequacy than was urease index. These data indicate that both SBM moisture and cooking time affected urease, trypsin inhibitor contents, and broiler growth, and that urease and trypsin inhibitor destruction during heat treatment immediately precedes lysine degradation.

(*Key words:* soybean meal, trypsin inhibitor, urease, broilers, growth)

1980 Poultry Science 59:2300–2306

INTRODUCTION

The largest single source of supplementary protein used for livestock feeding is soybean meal (SBM). Raw soybeans have proteolytic inhibiting substances that may be destroyed with heat (Hayward *et al.*, 1936). The proteolytic inhibiting substances present in unheated SBM have a retarding effect upon the growth of chicks when given with either a ration containing autoclaved SBM or one containing a supplement composed of nutritionally adequate protein from animal sources (Caskey and Knapp, 1944; Ham *et al.*, 1945; Westfall and Hauge, 1947; Saxena *et al.*, 1963). The most important proteolytic inhibiting substance in animal nutrition is regarded by many to be trypsin inhibitor. In addition to trypsin inhibitor, urease is an important constituent since it is used by commercial processing plants as an index to determine adequacy of processing. Caskey and Knapp (1944) noted that the heat treatment required to destroy the urease parallels the treatment required to destroy the trypsin inhibitor; therefore, urease index has been used to determine adequacy of SBM processing.

Heat is applied to soybeans to destroy the proteolytic inhibiting substances (Borchers *et al.*, 1948); however, overheating will either destroy or render unavailable several essential amino acids, particularly lysine and arginine (Hayward *et al.*, 1936; Renner *et al.*, 1953). Hayward *et al.* (1936) found that the most effective process for both preserving the nutritive value of the soybean protein and destroying proteolytic inhibitors was to autoclave ground soybeans for 1.5 hr at 1.16 atmospheres of pressure. Borchers *et al.* (1948) found also that raw SBM autoclaved at 1.02 atmospheres of pressure for 4, 10, 15, and 30 min contained 82, 39, 15, and 0% trypsin inhibitor activity, respectively, as compared to that of raw SBM trypsin inhibitor activity (100%). Trypsin inhibitor therefore, may be used along with urease as a criterion for determining adequacy of SBM processing.

Contrasting results have been reported as to the effect of moisture. Graham *et al.* (1949) concluded that moisture content of SBM partly determines the quality of soybean protein resulting from the heating process. However, Renner *et al.* (1953) concluded that the deleterious effects of overheating SBM can be partly overcome by the addition of water before overheating in open containers. Furthermore, Hayward *et al.* (1936) found that the effects of overheating were greater when a material was sealed than not sealed when both meals were subjected to autoclaving at 1.16 atmospheres of pressure for 1.5 hr. These results imply that moisture may affect the destruction of amino acids.

In a literature review, Sunde (1973) concluded that moisture generally tends to increase the orderly protein structure and makes digestion more effective. Also, Clandinin *et al.* (1951) showed that less lysine was liberated from a lysine-glucose mixture heated in an autoclave oven in the absence of water than when heated in the presence of water. These results imply that the exposure of amino acids during heat treatments is greater in the presence of moisture than the absence of moisture and, thus, may result more readily in overheating.

Moisture content of SBM influences protein quality; however, the extent to which moisture affects proteolytic inhibitors, total amino acid content, and broiler growth has not fully been explored. Therefore, research was conducted to determine the effect of moisture content and cooking time on urease, trypsin inhibitor, amino acid, and total protein (N × 6.25) contents in SBM and on broiler growth.

MATERIALS AND METHODS

Five experiments were conducted to determine the effect of moisture content and cooking time on soybean meal (SBM) quality. SBM samples with either 0 or 25% added moisture levels were dry heated for 0 to 6 hours with 1-hour intervals (Experiment 1). The SBM samples with either 0 or 10% added moisture levels were autoclaved in sealed containers for 0, 30, 60, or 120 min (Experiment 2). Only urease activity was determined in Experiments 1 and 2. Experiments 3 and 4 were conducted to determine the minimum cooking time required to destroy either urease or trypsin inhibitor without sacrificing either protein or amino acid quality. Raw SBM in Experiments 3 and 4 containing either 0, 4, 8, 12, or 16% added moisture was autoclaved at 120 C in sealed containers from 0 to 90 min in Experiment 3 and 0 to 135 min in Experiment 4, with 15-min intervals. Experiment 5 was conducted to determine the effect of feeding soybean meals to broiler chicks containing either 12 or 16% added moisture prior to processing. The SBM containing either 12 or 16% moisture was sealed in pint jars and processed for either 15, 30, 45, or 60 min.

Raw, solvent-extracted, flaked SBM was obtained from a commercial soybean processing plant. The soybeans had previously undergone all processing techniques at the plant site with the exception of toasting and grinding. All soybeans used in this study were of the same source and stored in plastic bags at 20 C.

Heat treatments were applied by using either a dry heat oven at 50 C in Experiment 1 or an autoclave oven at 1.02 atmospheres of pressure in Experiments 2 to 5. Cooking time began when maximum pressure was reached in the autoclave oven.

In Experiments 1 to 4, raw SBM weighing 32 g was placed in 50-ml screw-top Erlenmeyer flasks; an extra stopper was fitted to create a complete moisture seal. In Experiment 5, raw SBM weighing approximately 200 g was placed in pint jars and sealed prior to processing. After processing for various lengths of time, all samples were dried at 38 C in a dry-heat oven and ground to 40 mesh before urease, trypsin inhibitor, amino acids, and N-equivalent protein were determined.

Three replicates of each sample were used to determine urease index, and total protein contents were determined by the procedures of AOAC (1965). The total N-equivalent protein was determined by the macro-Kjeldahl procedure. The difference between the pH of the test and the pH of the blank was used as an index of urease activity. The urease index is currently used by the commercial soybean processing plants as a test for SBM processing adequacy. Three replicates of each sample were used to determine trypsin inhibitor by the procedure of Kunitz (1947) as modified by Kakade *et al.* (1969). Trypsin inhibitor contents are reported as micrograms trypsin inhibited per milligram protein in crude soybean extract (Kakade *et al.*, 1969). Samples were prepared for amino acid analysis by the procedure of Roach (1966). Amino acid compositions were determined on a Beckman 119 BL.[1,2]

[1] Beckman Instruments, Inc., Fullerton, CA.

[2] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

Broiler strain cockerels obtained from a commercial hatchery were used in Experiment 5. At one day of age, the chicks were wing-banded and randomly assigned to decks in electrically heated battery brooders. Test diets (Table 4) and water were furnished *ad libitum.* The SBM sources were added as 35.96% of the diet. Calculated analyses are shown based on composite commercial SBM analyses tables. All nutrients were added to either meet or exceed the NRC (1977) recommendations for poultry. Four replicates of 10 chicks each were reared in Experiment 5 to 21 days of age, at which time each bird was weighed and feed consumption determined. Each bird was sacrificed for pancreas weight determinations.

A factorially-arranged, randomized, complete block design was used in Experiment 5. Statistical examination was performed using the analysis of variance (Steel and Torrie, 1960). Significant differences among means were separated by using Duncan's new multiple range test (1955). All statements of significant differences refer to the 5% level of probability.

RESULTS AND DISCUSSION

Results of Experiment 1 (Table 1) indicated that urease index was influenced by the addition of moisture before dry-heating raw SBM for 0 to 6 hr with 1-hr intervals. Increased cooking times decreased urease activity; however, urease index was decreased to a larger extent with the addition of 25% moisture before heating than with no moisture. These results show that moisture is necessary in destroying urease. A urease index of <.15 Δ pH is used by the commercial soybean processors and, thus, used in these studies to indicate processing adequacy. At least 3 hr cooking time was necessary to obtain the required minimum urease index (<.15 Δ pH), with the addition of 25% moisture to raw SBM before processing.

TABLE 1. *Effect of added moisture content and cooking time on soybean meal[a] urease activity, Experiment 1*

Cooking time, hr[b]	Urease activity (ΔpH): 0% added moisture	Urease activity (ΔpH): 25% added moisture
0	2.11	2.13
1	1.66	.54
2	1.01	.31
3	.68	.14
4	.54	.06
5	.45	.02
6	.46	0

[a]Raw soybean meal was dehydrated in a vaccum oven at 50 C. In an effort to obtain the desired 25% added moisture level by weight, moisture was added to one-half of the meal before dry heating.

[b]SBM was dry heated for various lengths of time. Cooking time began when the samples were placed in a preheated dry-heat oven.

TABLE 2. *Effect of moisture content and cooking time on soybean meal[a] urease activity, Experiment 2*

Cooking time, min[b]	Urease activity (ΔpH): 0% added moisture	Urease activity (ΔpH): 10% added moisture
0	2.28	2.21
30	1.98	1.17
60	1.65	.27
120	1.14	0

[a]Raw soybean meal was dehydrated in a vacuum oven at 50 C. In an effort to obtain the desired level of added 10% moisture by weight, moisture was added to one-half of the meal before autoclaving.

[b]SBM was autoclaved at 120 C for various lengths of time. Cooking time began when maximum pressure was reached in an autoclave.

In Experiment 2 the raw SBM was autoclaved rather than dry-heated. Both moisture and cooking time affected destruction of urease (Table 2). Results indicate that less time was required to sufficiently decrease urease index to <.15 Δ pH when moisture was added than when moisture was not added. With a 10% added moisture level, approximately 120 min are required to adequately autoclave SBM (Experiment 2, Table 2); with a 25% added moisture level, 3 hr are required to adequately dry-heat SBM (Experiment 1, Table 1). With no added moisture, the urease index did not sufficiently decrease to an acceptable processing level with the cooking times used in Experiment 1 and 2.

Experiments 3 and 4 were conducted to determine whether the addition of moisture and cooking time is linear in its effect on urease index and trypsin inhibitor contents. The same moisture and cooking times were used in both experiments, but in Experiment 4, the maximum cooking times were increased from 90 to 135 min. Within each moisture level, both urease (Figure 1) and trypsin inhibitor contents (Figure 2) decreased with increased cooking

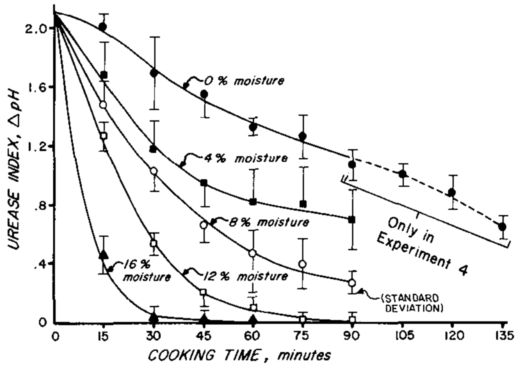

FIG. 1. Composite data of Experiments 3 and 4 showing the effect of moisture and cooking time on soybean meal urease index.

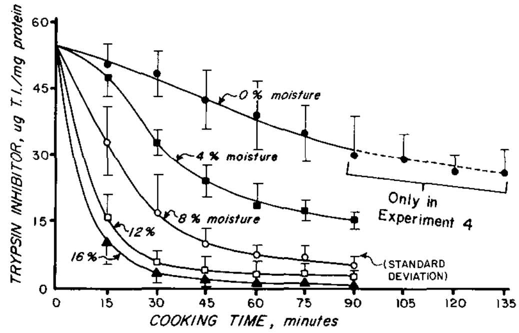

FIG. 2. Composite data of Experiments 3 and 4 showing the effect of moisture and cooking time on soybean meal trypsin inhibitor contents.

times. However, both soybean constituents decreased at a faster rate with increasing moisture levels. We hypothesized that increasing the maximum cooking time from 90 to 135 min (Experiment 4) with no added moisture might allow for complete degradation of proteolytic inhibitors and urease; however, this did not occur.

Urease index was essentially equivalent when SBM was processed for either 135 min with no added moisture or 90 min with 4% moisture. Trypsin inhibitor contents did not show this trend. With 30 min cooking time and added moisture levels of 0, 4, 8, 12, and 16%, urease index decreased by 22, 46, 52, 75, and 97%, respectively, when compared to raw SBM. Within 30 min cooking time and added moisture levels of 0, 4, 8, 12, and 16%, trypsin inhibitor activity decreased by 11, 42, 69, 87, and 94%, respectively. With 8 and 12% moisture and 30 min cooking time, percentages for trypsin inhibitor destroyed are greater than those for urease destroyed, indicating that urease is destroyed more efficienctly with heat than is trypsin inhibitor.

When raw SBM was cooked with either 0, 4, or 8% moisture added, urease and trypsin inhibitor contents were not adequately decreased to assure proper processing of SBM, regardless of cooking time. Urease index and trypsin inhibitor contents were sufficiently decreased by processing raw SBM for either 45 min at 12% added moisture or 30 min at 16% added moisture.

N-Equivalent protein contents (Table 3) indicate that protein was unaffected when raw SMB was processed with 0, 4, or 8% moisture added. Amino acid determinations indicate that only lysine was affected by heat treatments. Lysine determinations are shown in Figure 3. Lysine contents remained unchanged when either 0, 4, or 8% moisture was added to SBM before heat treatment. However, when raw SBM was autoclaved for either 60 min with 12% added moisture or 30 min with 16% added moisture, lysine contents began to decrease and proceded to decrease with increasing moisture levels.

Table 5 shows results of Experiment 5. Body weight increased, feed/gain decreased, and pancreas weight decreased with either increasing moisture level added prior to cooking or increasing cooking time. Diets containing soybeans processed with 16% added moisture for 60 min obtained essentially equivalent body weights as diets containing commercial soybean meal.

Figure 4 shows the comparison of soybean meal urease index and trypsin inhibitor contents as an indicator of protein processing adequacy. Body weight increased and trypsin inhibitor contents decreased with increasing cooking times. With 16% added moisture, urease index increased up to 45 min of cooking time. Additional cooking time did not change the urease index.

Results of this study agree with those reported by Hayward *et al.* (1936), Graham *et al.* (1949), Clandinin *et al.* (1951), Sunde (1973), and Sandholm *et al.* (1976) that moisture increases the effectiveness of heating SBM. However, Renner *et al.* (1953) concluded that the deleterious effects of overheating SBM can be partly overcome by the addition of water before heating in open con-

TABLE 3. *Effect of added moisture and cooking time on soybean meal[a] protein content, Experiment 3*

	N-Equivalent protein (N × 6.25), %					
Cooking time, min[b]	0% Added moisture	4% Added moisture	8% Added moisture	12% Added moisture	16% Added moisture	Mean
0	50.66	50.03	50.24	50.59	50.35	50.37
15	50.96	50.45	50.53	50.69	50.44	50.61
30	50.85	50.38	50.46	50.83	50.56	50.62
45	50.92	50.34	50.41	50.89	50.70	50.65
60	50.86	50.45	50.40	50.53	50.37	50.52
75	50.38	50.62	50.82	50.13	50.06	50.40
90	50.89	50.30	50.23	50.08	49.97	50.29
Mean	50.79	50.37	50.44	50.53	50.35	

[a]Raw soybean meal was dehydrated in a vacuum oven at 50 C. Moisture was added by weight to each group before autoclaving.

[b]SBM was autoclaved at 120 C for various lengths of time. Cooking time began when maximum pressure was reached in an autoclave.

tainers. When sealed containers were used in Experiments 1 to 4, the findings of Renner *et al.* (1953) were not substantiated. Moisture not only increases the destruction of proteolytic inhibitors, but it also causes greater exposure of lysine and thus increases lysine degradation. Other amino acids tested were unchanged. Lysine is degraded when more than 8% moisture is added to the raw SBM before processing. Sealing the containers, which allows for moisture retention, decreases the cooking time required to obtain an adequately heated SBM. Autoclaving was more effective than dry heat in decreasing the urease index. Raw SBM, therefore, should contain approximately equal moisture contents when cooking times are established in a soybean meal processing plant.

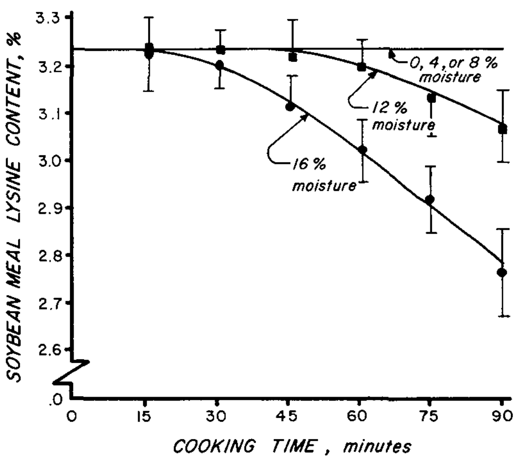

FIG. 3. Effect of moisture and cooking time on total lysine content of soybean meal.

TABLE 4. *Composition of basal diet*

Ingredient	(%)
Yellow corn	54.25
Soybean meal source (variable)	35.96
Animal fat	5.94
Limestone	1.29
Dicalcium phosphate (18.5% P, 22% Ca)	1.83
Salt	.30
Methionine hydroxy analogue-Ca, 93%	.18
Vitamin and trace mineral premix[a]	.25
Total	100.00
Calculated analysis:	
Crude protein, %	22.4
Metabolizable energy, kcal/kg	3190
Total calcium, %	1.00
Available phosphorus, %	.45
Total lysine, %	1.26
Total methionine plus cystine	.87

[a]The broiler premix furnished the following amounts of other ingredients per kilogram of feed: vitamin A, palmitate, gelatin coated, 6614 IU; vitamin D_3, 1654 ICU; vitamin E, 2.2 IU; riboflavin, 4.4 mg; niacin, 27.6 mg; d-pantothenic acid, 8.8 mg; folic acid, 275.6 μg; vitamin B_{12}, 8.8 μg; choline choride, 551 mg; ethoxyquin, 55 mg menadione sodium bisulfite complex, 2.8 mg or menadione sodium bisulfite, 1.7 mg; pyridoxine, .55 mg; manganese, 66.25 mg; zinc, 44 ppm; iodine, 1.25 mg; iron (in sulfate form), 20 mg; copper (in sulfate form), 2 mg.

TABLE 5. *Effect of moisture content and cooking time on subsequent broiler performance, Experiment 5*

Soybean meal source[1]	Added moisture content, %	Cooking time	21 day performance		
			Chick weight	Feed/gain	Pancreas weight
	(%)	(min)			(mg/dg BW)
Raw	12	15	352 ± 3[f]	1.84 ± .02[f]	921 ± 29[f]
		30	372 ± 10[f]	1.74 ± .02[e]	863 ± 27[e]
		45	400 ± 11[d]	1.55 ± .05[c]	759 ± 23[d]
		60	448 ± 18[b]	1.46 ± .03[b]	594 ± 14[c]
Mean			388	1.65	784
Raw	16	15	379 ± 7[e]	1.68 ± .03[d]	783 ± 17[d]
		30	430 ± 8[c]	1.50 ± .03[b]	622 ± 22[c]
		45	459 ± 12[b]	1.46 ± .01[b]	551 ± 19[b]
		60	496 ± 17[a]	1.35 ± .02[a]	482 ± 14[a]
Mean			441	1.50	610
Commercial soybean meal		. . .	480 ± 12[a]	1.34 ± .02[a]	474 ± 15[a]

[1]Raw soybean meal was dried at 38° and either 12 or 16% moisture (w/o) was added prior to autoclaving for either 15, 30, 45, or 60 min after a maximum 1.02 atmospheres of pressure was reached.

[a,b,c,d,e,f]Values within a column with different superscripts are significantly different (P<.05).

Results from chemical analyses collected in this study indicated that urease index and trypsin inhibitor content are highly correlated (+.91) and confirms results of Caskey and Knapp (1944). Furthermore, the correlation coefficient between mean 21-day body weight and both urease index and trypsin inhibitor contents were −.89. Urease was unchanged, trypsin inhibitor contents declined, and 21-day body weights increased with soybean meals processed longer than 45 min.

Urease and trypsin inhibitor contents of raw soybean meal varied between experiments due to different cooking conditions. Raw soybean meal was cooked in Erlenmeyer flasks in Experiments 3 and 4 and in pint jars in Experiment 5; thus, heat distribution was different in each experiment.

These data indicate that both SBM moisture and cooking time affected urease, trypsin inhibitor contents, and broiler growth. Furthermore, urease and trypsin inhibitor destruction during heat treatment immediately precedes lysine degradation.

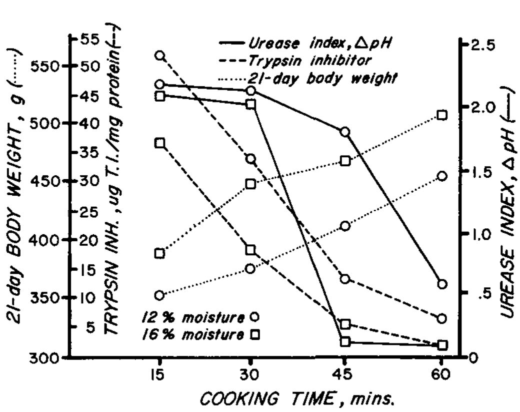

FIG. 4. Comparison of urease and trypsin inhibitor as indicators of soybean meal processing adequacy.

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