Color, Trypsin Inhibitor and Urease Activity As It Affects Growth of Broilers

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ABSTRACT

In five experiments, the relationships among trypsin inhibitor contents, urease index and color in soybean meal (SBM), and the effects of those relationships on broiler chick performance were determined. Raw, solvent-extracted SBM was dehydrated to 2% moisture; then 0, 4, 8, 12, or 16% moisture was added 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 moisture content or cooking time. With 16% added moisture, 30 min of cooking time was required to decrease urease index to an acceptable level, and 45 min was required to decrease trypsin inhibitor contents. Raw SBM autoclaved with less than 12% added moisture contained urease and trypsin-inhibitor contents that were not considered undesirable, regardless of cooking time used. Increased moisture and cooking time resulted in decreased trypsin inhibitor and urease contents, increased broiler growth, improved feed efficiency and decreased pancreas hypertrophy. Commercial SBM, containing 12 ug trypsin inhibited per mg of protein (μg/mg), a tristimulus *+a* (redness) color value of +3.21, and a urease index of 0.19 ΔpH, was autoclaved for 0–25 min with 5-min intervals to determine the effect of additional heat on broiler performance and SBM color. The commercial SBM, heated an additional 10 min, contained 1.77 μg trypgin inhibitor per mg of protein, a *+a* color value of +4.76, and urease index of 0.02 ΔpH. Broiler growth and feed efficiency were improved when the commercial SBM was heated an additional 10 min. Results of this study show that color is a good predictor of trypsin-inhibitor content and broiler chick performance. Color may be used to predict underprocessing, adequate, and overprocessing of SBM; however, trypsin inhibitor and urease index do not show the effect of overprocessing.

INTRODUCTION

Soybean meal (SBM) is the major protein ingredient in practical poultry diets; approximately 25% of the poultry diet is SBM.

Raw soybeans are heated to destroy proteolytic-inhibiting substances and urease (1–3). The proteolytic-inhibiting substances present in unheated SBM have a retarding effect upon the growth of chicks when they are given with either a ration containing autoclaved SBM or one containing a supplement of nutritionally adequate protein from animal sources (4,5).

The most important proteolytic-inhibiting substance in animal nutrition is trypsin inhibitor. Urease is also important because it is used as an index to determine processing adequacy. Caskey and Knapp (6) noted that the heat treatment required to destroy urease in SBM approximately paralleled the treatment required to destroy the trypsin inhibitor. On the basis of this work (6), urease index is used to determine SBM processing adequacy.

Steam heat will destroy proteolytic-inhibiting substances; however, overheating will either destroy or render unavailable several essential amino acids, particularly lysine and arginine (1,3,7). Borchers et al. (2) found that raw SBM autoclaved at one atmosphere pressure for 4, 10, 15, and 30 min contained 82, 39, 15, and 0%, respectively, of the original trypsin inhibitor activity. Under experimental conditions, trypsin inhibitor may, therefore, be used along with urease as a criterion for determining adequacy of SBM processing.

Moisture also affects destruction of proteolytic-inhibiting substances. Sunde (8) concluded that SBM moisture tends to increase the effectiveness of the heating process, and that the heating interrupts the orderly protein structure, and, thus makes digestion more effective. Clandinin et al. (9) found that the exposure of amino acids during heat treatments is greater in the presence of moisture than in the absence of moisture; therefore, exposure in the presence of moisture may result more readily in overheating.

Several chemical methods have been used to determine SBM quality; these include urease index, trypsin inhibitor and lysine availability. Although these methods indicate some SBM quality, none shows the effect of both under- and overprocessing. Furthermore, these methods are cumbersome and time consuming. Establishing a quick and reliable method to determine SBM quality is essential if the SBM processing industry is to provide a consistently high-quality product.

In the present studies, the relationships between color, trypsin inhibitor content and urease index of SBM and the effect of those relationships on broiler growth were determined.

MATERIALS AND METHODS

In experiments 1 and 2, the minimum cooking time required to destroy urease and trypsin inhibitor without sacrificing either protein or amino acid quality was determined. Raw, solvent-extracted, flaked SBM was obtained from a commercial SBM processing plant. Either 0, 4, 8, 12, or 16% moisture was added after SBM had been dehydrated in a vacuum oven to 2% moisture. The SBM was then autoclaved at 120 C in sealed containers from 0–135 min, with 15-min intervals. The soybeans had previously undergone all processing techniques at the plant site with the exception of toasting and grinding.

In experiment 3, the effect of feeding SBM to broiler chicks when the SBM contained either 12 or 16% total moisture before processing was determined. SBM flakes were placed in sealed containers, autoclaved for 15, 30, 45, or 60 min, dried in a vacuum oven to standardize moisture levels, and fed to broiler cockerels from 1 to 21 days of age.

A commercial sample of soybean meal contained 12 μg trypsin inhibited per mg protein. In experiment 4, the effect of additional cooking time on commercial SBM quality and broiler chick performance was determined. SBM color was also determined. In experiment 5, the relationship between SBM trypsin inhibitor contents and color was determined. Raw, solvent-extracted SBM was dehydrated to 2% moisture, and 0, 2, 4, 6, 8, 10, or 12% moisture was added before the new SBM was autoclaved for 0–135 min with 15-min intervals.

In experiment 5, three replicates of each sample were used to determine urease index (10) and trypsin inhibitor

content (11,12). Trypsin inhibitor contents are reported as μg trypsin inhibited per mg protein in crude soybean extract. Samples were prepared for amino acid analysis by the procedure of Roach (13). Amino acid compositions were determined on a single-column amino acid analyzer.

Broiler strain cockerels obtained from a commercial hatchery were used in experiments 3 and 4. When they were 1 day old, the chicks were wingbanded and randomly assigned to decks in electrically heated battery brooders. Test diets and water were furnished ad libitum. SBM sources were added as 35.96% of the diet. The basal diet in experiment 3 contained 22% protein, 1.26% lysine and 0.87% methionine plus cystine. In an effort to show the influence of low dietary levels of trypsin inhibitor, the basal diet in experiment 4 contained 19% protein, 1.04% lysine and 0.75% methionine plus cystine. All other nutrients were added to either meet or exceed the NRC (14) recommendations for poultry. Four replicates of 10 chicks each were fed each experimental diet in experiments 3 and 4. Bird weights, feed consumption and pancreatic weights were determined at 21 days of age.

A factorially arranged, randomized, complete block design was used in experiments 3 and 4. Statistical examination was performed using the analysis of variance (15). Significant differences among means were separated by using Duncan's New Multiple Range Test (16). All statements of significant differences refer to the 5% level of probability.

RESULTS AND DISCUSSION

Experiments 1 and 2

Results of experiments 1 and 2 are shown in Figures 1-3. At each moisture level, both urease (Fig. 1) and trypsin inhibitor contents (Fig. 2) decreased with increased cooking times. However, both soybean constituents decreased at a faster rate with increasing moisture levels. Trypsin inhibitor and urease were not completely destroyed when the maximum cooking time was increased from 90 to 135 min (experiment 2), with no moisture added.

With 30 min cooking time and added moisture levels of 0, 4, 8, 12 and 16%, urease index was 22, 46, 52, 75 and 97%, respectively—less than that of raw SBM. Trypsin inhibitor contents were 11, 42, 69, 87 and 94%, respectively—less with the same moisture levels. These results show the influence of moisture in processing SBM.

Lysine contents (Fig. 3) remained unchanged when either 0, 4, or 8% moisture was added to SBM before heat treatment. However, lysine contents began to decrease and continued to decrease when 12% moisture was added for 60 min or 16% was added for 30 min.

Experiment 3

Body weight increased (Table 1), feed/gain decreased and pancreatic weight decreased with either an increasing moisture level (12 or 16%) added prior to cooking or increasing cooking time. Diets containing soybeans, which were processed for 60 min with 16% moisture, obtained essentially the same trypsin inhibitor content and body weights as diets containing commercially processed SBM.

Experiment 4

A commercial SBM was found to contain 12.12 μg trypsin inhibited per mg protein (μg/mg) and 0.19 ΔpH urease index. Results of this experiment to determine the effect of additional cooking time on trypsin inhibitor content, urease index and broiler performance are shown in Table 2. Both trypsin inhibitor contents and urease index decreased with increased cooking times. When commercial SBM, containing 12.12 μg/mg of trypsin inhibitor and a urease index of 0.19

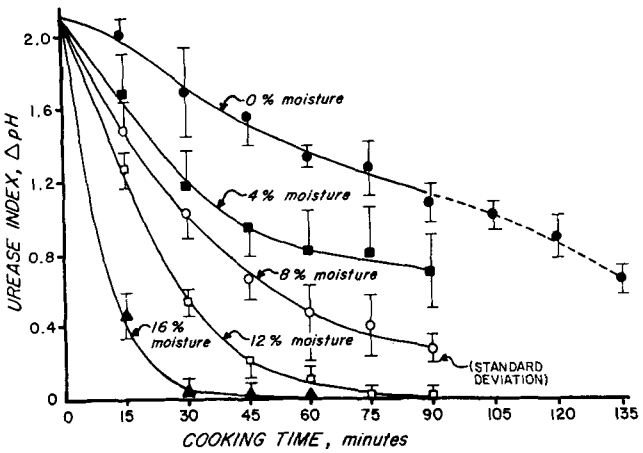

FIG. 1. Composite data of experiments 1 and 2 showing the effect of moisture and cooking time on soybean meal urease index.

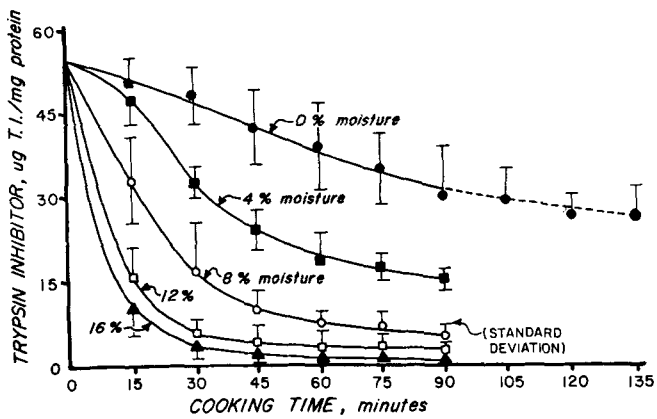

FIG. 2. Composite data of experiments 1 and 2 showing the effect of moisture and cooking time on soybean meal trypsin inhibitor contents.

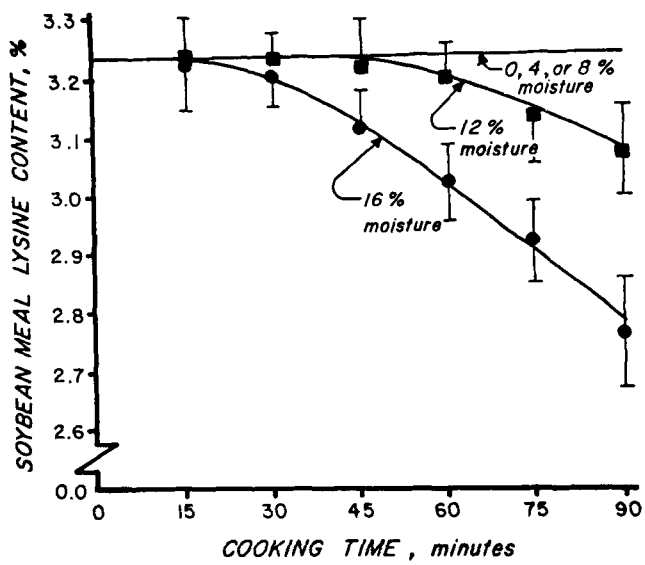

FIG. 3. Effect of moisture and cooking time on total lysine content of soybean meal.

TABLE I

Effect of Cooking Moisture Content and Time on Subsequent Broiler Performance, Experiment 3

Soybean meal source	Total moisture content, %	Cooking time (min)	Trypsin inhibitor, μg trypsin inhibited per mg protein	21-day performance[2] Chick wt,g	Feed/Gain	Pancreatic wt, wt/dg body wt
Raw[1]	12	15	52.16	352±3^{f}	1.84±.02^{f}	921±29^{f}
		30	34.35	352±10^{f}	1.74±.02^{e}	863±27^{e}
		45	13.30	400±11^{d}	1.55±.05^{c}	759±23^{d}
		60	6.93	448±18^{b}	1.46±.03^{b}	594±14^{c}
	Mean[2]			388	1.65	784
Raw	16	15	36.59	379±7^{e}	1.68±.03^{d}	783±17^{d}
		30	18.13	430±8^{c}	1.50±.03^{b}	622±22^{c}
		45	6.85	459±12^{b}	1.46±.01^{b}	551±19^{b}
		60	1.66	496±17^{a}	1.35±.02^{a}	482±14^{a}
	Mean[2]			441	1.50	610
Commercial source[3]		0	3.44	480±12^{a}	1.34±.02^{a}	474±15^{a}

[1] Raw soybean meal was dried at 38 C with either 12 or 16% moisture (weight-weight) before it was autoclaved for either 15, 30, 45, or 60 min after a maximum .545 kg/cm^2 (15 p.s.i.) of gauge steam pressure was reached.

[2] Means within a column or row and without a common superscript are significantly different ($P<0.05$).

[3] Commercial soybean meal containing 3.44 μg/mg trypsin inhibitor was fed to broiler chicks without additional autoclaving.

TABLE II

Effect of Additional Cooking of Commercial Soybean Meal[1] High in Trypsin Inhibitor on Broiler Performance and Color (Experiment 4)

Cooking time (min)	Hunterlab color value[2] L	+a	b	Urease index, ΔpH	Trypsin inhibitor, μg trypsin inhibited per mg protein	21-day test results[3] Mean body wt,g	Feed/Gain
0	70.05	+3.21	+18.15	.19	12.12	605^{bc}	1.61^{b}
5	67.19	+3.96	+17.90	.11	7.84	625^{ab}	1.53^{a}
10	60.78	+4.76	+17.40	.02	1.77	643^{a}	1.51^{a}
15	58.50	+5.81	+16.90	0	0	626^{ab}	1.54^{a}
20	55.72	+6.79	+16.76	0	0	596^{c}	1.59^{b}
25	50.33	+7.09	+16.32	0	0	565^{d}	1.68^{c}

[1] Soybean meal processed commercially was purchased on the open market and autoclaved for 0–25 min with 5-min intervals in open flat pans with 2.54 cm of material in each pan. Immediately after autoclaving, the soybean meal was tumbled to release the heat quickly.

[2] SBM was ground with a 20-mesh screen and tristimulus color values were determined by a color reflectance meter.

[3] Means within each column and without a common superscript are significantly different ($P<0.05$).

ΔpH, was fed, growth and feed efficiency were less than those when SBM was fed in a 19% protein diet to broiler chicks. However, an additional 10 min of autoclaving decreased trypsin inhibitor content, increased body weight and improved feed efficiency. With 15 min or longer cooking time, broiler performance was depressed (Table 2). These results indicate the effect of both under- and overprocessing. The commercial SBM used in this study was underprocessed, as evidenced by its failure to support optimum broiler performance when it was fed with a low-protein, lysine, and methionine plus cystine diet.

The SBM processing plants are now using a urease index of <0.15 ΔpH to show processing adequacy. The urease index of the commercial SBM used in this experiment showed that the SBM was properly cooked with 5 min of additional cooking; however, growth was slightly better with additional heat. Furthermore, neither trypsin inhibitor level nor urease index could adequately determine overprocessing. SBM color was a good indicator of both under and overprocessing.

Experiment 5

The relationships among SBM trypsin inhibitor and urease contents and tristimulus color values *L* (lightness) and *+a* (redness) were determined. These data show that both *L* (Fig. 4) and *+a* (Fig. 5) tristimulus color values can predict trypsin inhibitor content of SBM. Furthermore, SBM color can predict overprocessing of SBM because the Maillard reaction (browning effect) continued, even though all trypsin inhibitor had been destroyed. The data from experiment 5 show that trypsin inhibitor was completely destroyed when either the *L* color value was 63 or the *+a* color value was +5.2. Both trypsin inhibitor contents and urease index (Table 3) were affected by both cooking time and added moisture level.

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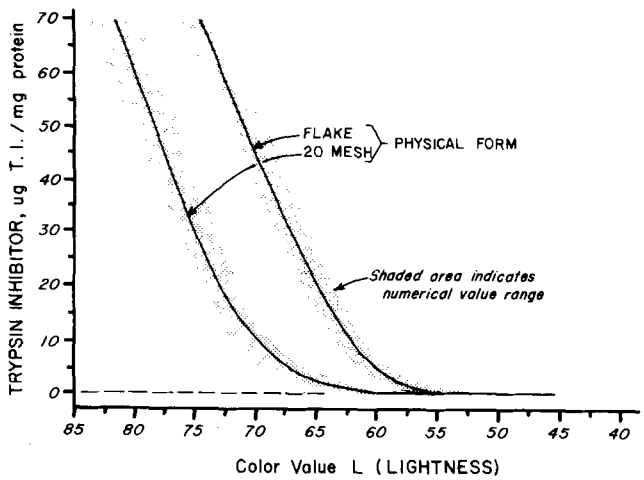

FIG. 4. Relationship between soybean meal trypsin inhibitor content and tristimulus color value *L* (lightness).

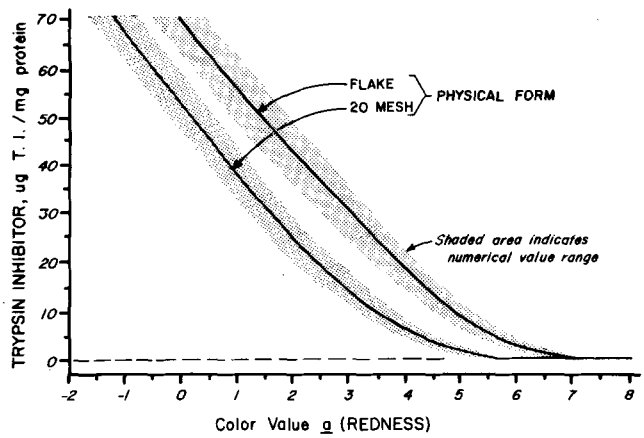

FIG. 5. Relationship between soybean meal trypsin inhibitor content and tristimulus color value *+a* (redness).

TABLE III

Effect of Added Moisture and Cooking Time on Trypsin Inhibitor and Urease Contents of Soybean Meal[1] (Experiment 5)

Cooking time (min)	Trypsin inhibitor, μg trypsin inhibited/mg protein							Urease index, ΔpH						
	Added moisture level							Added moisture level						
	2%	4%	6%	8%	10%	12%	Mean	2%	4%	6%	8%	10%	12%	Mean
0	70.00	69.68	67.82	62.50	62.16	59.38	65.26	2.19	2.16	2.16	2.14	2.13	2.10	2.15
15	65.47	59.30	52.50	44.53	31.10	27.94	46.81	2.22	2.16	2.18	2.16	2.10	2.02	2.14
30	47.97	44.84	27.97	23.75	10.62	7.18	27.06	2.18	2.16	2.13	2.16	2.02	1.99	2.11
45	28.28	21.56	11.88	10.00	2.81	1.48	12.67	2.12	2.11	2.10	1.88	.80	.08	1.52
60	21.95	10.54	5.78	5.01	.92	0	7.37	2.06	1.92	1.53	1.49	.07	0	1.18
75	16.78	9.54	3.44	2.20	0	0	5.33	1.88	1.68	.56	.41	0	0	.76
90	13.28	7.50	3.12	1.88	0	0	4.30	1.76	.80	.28	.08	0	0	.49
105	9.37	4.38	2.81	1.24	0	0	2.97	1.14	.36	.11	.02	0	0	.27
120	7.81	3.12	1.25	.94	0	0	2.19	.46	.17	0	0	0	0	.10
135	7.34	1.56	1.20	0	0	0	1.68	.46	.05	0	0	0	0	.08
MEAN	28.82	23.20	17.78	15.20	10.76	9.60		1.65	1.36	1.10	1.03	.71	.62	

[1] A total of 250 g of raw, flaked, solvent-extracted soybeans was placed in sealed glass containers and autoclaved for 0–135 min with 15-min intervals. Timing began when the maximum 0.545 kg/cm^2(15 psi) gauge steam pressure was reached. Each sample was dried at 37 C before determining trypsin inhibitor and urease contents.

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