Relationships Between Color, Trypsin Inhibitor Contents, and Urease Index of Soybean Meal and Effects on Broiler Performance

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ABSTRACT Three experiments were conducted to determine the relationships between trypsin inhibitor contents, urease index, and color of soybean meal, and the effect on broiler performance. Either 0, 8, 10, 12, 14, or 16% moisture (Experiment 1) or 0, 2, 4, 6, 8, or 10% moisture (Experiment 2) was added after dehydrating raw, flaked, solvent-extracted soybeans to 2% moisture. Each sample was then sealed in .473-liter jars, autoclaved for 0 to 135 min, with 15-min intervals, and dried at 37 C in a vacuum oven to remove all remaining moisture. In Experiment 3 commercial soybean meal with 12.12 μg trypsin inhibited/mg protein (μg/mg) was autoclaved for 0 to 25 min with 5-min intervals to determine the effect of additional heat on broiler performance and SBM color.

Commercial soybean meal with 12.12 μg/mg trypsin inhibitor, tristimulus +a color value of 3.21, and a urease index of .19 Δ pH supported poorer growth and feed efficiency in broilers than the same soybean meal heated an additional 10 min and, thus, 1.77 μg/mg trypsin inhibitor, a + a color value of +4.76, and urease index of .02 Δ pH. Results indicated that color is a good predictor of trypsin inhibitor content and broiler performance. Additional heating resulted in higher +a color values and poorer broiler performance, indicating that color may also be used to determine overprocessing.

Particle size of the soybean meal influenced the color. The soybean meal ground to pass a 20 mesh screen supported the best broiler performance when tristimulus color values ranged from +4.5–5.5 ±a color values or 58–65 *L* color values. These results confirm that color is a quick and reliable method of determining soybean meal quality.

(*Key words:* trypsin inhibitor, urease, color, soybean meal, cooking, processing, broilers)

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INTRODUCTION

Soybean meal (SBM) is the major protein supplement in practical poultry diets. It makes up 25% or more of a complete practical poultry ration. Compared to other plant-source proteins SBM is particularly high in lysine.

Raw soybeans are heated to destroy proetolytic inhibiting substances and urease (McNaughton and Reece, 1980; Borchers *et al.*, 1948; Hayward *et al.*, 1936). Trypsin inhibitor is regarded by many to be the most important proteolytic inhibitor in poultry diets. Excessive heat will either destroy or render unavailable several essential amino acids, particularly lysine and arginine (Renner *et al.*, 1953; Hayward *et al.*, 1936).

Chemical availability of lysine has been used to determine SBM quality under experimental conditions. Reported lysine availability values have ranged from 76 to 110% (Robel and Frobish, 1977; Netke and Scott, 1970; Hill *et al.*, 1966; Kelly and Scott, 1966; Combs *et al.*, 1965; Ousterhout *et al.*, 1959; Gupta *et al.*, 1958). Baker (1978) attributes the differences to protein level, amino acid balance, delayed release of amino acids from intact protein as opposed to ready availability of crystalline amino acids, caloric density of the diet, area of the growth curve used, and assay methodology used. Although all of these factors may contribute to variation between assays, other possible reasons for the differences in reported results include an excessive trypsin inhibitor content of SBM and the effects of overheating SBM used in these studies.

Evaluation of the quality of processed SBM has been a major concern in poultry nutrition. Although urease itself is not important in poultry production, urease index is currently used by the soybean meal processing plants to evaluate processing time and technique. However, trypsin inhibitor activity in SBM is important in monogastric nutrition. Trypsin inhibitor determinations have been used under experimental conditions to further test SBM processing adequacy (McNaughton and Reece, 1980; Sandholm *et al.*, 1976; Borchers *et al.*,

1948). McNaughton and Reece (1980) indicated that both the moisture and cooking time of SBM affected its contents of trypsin inhibitor and urease and affected broiler growth. Also, the destruction of trypsin inhibitor and urease during heat treatment was found to immediately precede lysine degradation. Borchers *et al.* (1948) found that raw SBM autoclaved at 1.02 atmospheres of pressure for 4, 10, 15, and 30 min contained 82, 39, 15, and 0% of the original trypsin inhibitor activity, respectively. Trypsin inhibitor may, therefore, be used along with urease as a criterion for distinguishing adequate SBM processing from underprocessing. However, techniques other than growth trials to determine overprocessing have not been devised.

The food industry has used food color in determining food quality and acceptance. Color has a tremendous impact upon consumer acceptance of orange juice (CMCR, Inc., 1965), tomatoes (MacGillivray, 1928), sugar (Brice *et al.*, 1958), and egg yolks (Kahlenberg, 1949; Pohle and Mehlenbacher, 1955).

Maillard (1912a,b) demonstrated the browning of SBM and attributed it partly to changes in carbohydrates and moisture. Bird growth, chemical lysine availability, trypsin inhibitor content, and urease index have been used under both practical and experimental conditions to determine SBM quality. However, determination of these criteria is cumbersome and time consuming. Establishing a quick and reliable method of determining SBM quality is essential to the SBM processing industry to provide a consistently high-quality product. Therefore, the present study was conducted to determine the relationships of color, trypsin inhibitor content, and urease index of SBM from various heat treatments and their effects on broiler growth.

MATERIALS AND METHODS

Three experiments were conducted to determine the efficacy of using color to evaluate SBM quality. SBM processing plants are seeking a method of determining quality that is quick, reliable, repeatable, and accurate for all SBM moisture levels and cooking conditions. Raw, flaked, solvent-extracted soybeans were collected from a commercial SBM processing plant and stored in plastic bags until experimentation began. The soybeans had previously undergone all processing techniques except toasting and grinding at a processing plant. Before experimentation, raw soybeans were dried to 2% moisture at 37 C in a vacuum oven.

Experiments 1 and 2 were conducted to determine the relationship between trypsin inhibitor content and color when SBM was cooked under various conditions. Either 0, 8, 10, 12, 14, or 16% moisture (Experiment 1) or 0, 2, 4, 6, 8, or 10% moisture (Experiment 2) was added after dehydrating raw soybeans to 2% moisture. Each sample was then sealed in .473-liter jars, autoclaved for 0 to 135 min with 15-min intervals at .545 kg/cm^2 (15 psi) gauge steam pressure, and dried at 50 C in Experiment 1 and 37 C in Experiment 2 in a vacuum oven to remove excessive moisture. After drying, a portion of each sample was ground in a Wiley Mill[1] to pass either a 20- or 40-mesh screen. A portion of each sample in Experiment 2 was left as processed flakes for determination of the effect of particle size on SBM color.

Three replicates of each sample were used to determine urease index by the procedure of the AOAC (1965). The difference between the pH of the test sample and the pH of the blank (Δ pH) was used as an index of urease activity. Three replicates of each sample were used to determine trypsin inhibitor by the procedure of Kunitz (1947) as modified by Kakade *et al.* (1969). Samples were ground to pass a 40-mesh screen before their trypsin inhibitor content was determined. Trypsin inhibitor contents are reported as micrograms of trypsin inhibited per milligram of protein (or trypsin units) in crude soybean extract (Kakade *et al.*, 1969). One trypsin unit is arbitrarily defined as an increase of .01 absorbance units at 280 mμ in 20 min/10 ml of the reaction mixture under the conditions set forth herein. For simplicity, trypsin inhibitor contents are referred to as micrograms per milligram.

Experiment 3 was conducted to determine the effect of heating commercial SBM on its trypsin inhibitor content and color and on broiler performance. Commercial SBM was autoclaved in open 2.5 × 25.4 × 35.6 cm flat pans for 0 to 25 min with 5 min intervals. After autoclaving, each meal was tumbled to

[1] Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA. 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.

coolness and dried before it was fed to 1- to 21-day-old broiler chicks.

Broiler strain cockerels (Arbor Acres × Peterson) obtained from a commercial hatchery were used in Experiment 3. At 1 day of age, the chicks were wingbanded and randomly assigned to decks in electrically heated battery brooders. Test diets (Table 1) and tap water were furnished *ad libitum*. SBM sources were added as 31.60% of the diet. The calculated analyses (Table 1) are based on commercial SBM analysis tables. All nutrients other than crude protein, lysine, and sulfur-containing amino acids were added to either meet or exceed the NRC (1977) recommendations for poultry. Six replicates of 10 chicks each were reared in Experiment 3 to 21 days of age, at which time individual bird and feed intake weights were determined.

Hunter (1941) proposed an α, β uniform-chromaticity scale representing surface colors measured with the Hunter multipurpose reflectometer with tristimulus filters. The L (lightness or darkness), $+a$ (redness), and +b (yellowness) tristimulus values were determined by the Hunterlab Color and Color Difference meter (Model D25-2). Samples were placed in a petri dish for testing color using SBM ground to pass a 20-mesh screen in Experiment 1 and both to pass a 20-mesh screen and flaked material in Experiment 2.

A factorially arranged, randomized, complete block design was used in Experiment 3 to determine broiler performance. The data were statistically examined by the analysis of variance (Steel and Torrie, 1960). Significant differences among means were separated with Duncan's New Multiple Range Test (1955). All statements of significant differences refer to the 5% level of probability.

RESULTS AND DISCUSSION

Experiment 1. Both trypsin inhibitor content and urease index (Table 2) were influenced by both the addition of moisture and cooking time. Increasing the cooking times and moisture levels decreased both trypsin inhibitor content and urease index.

Generally, urease index decreased with less increase in cooking time than did trypsin

TABLE 1. *Composition of basal diet (Experiment 3)*

Ingredient	Content (%)
Yellow corn	59.25
Soybean meal (test material)[a]	31.60
Animal fat	5.45
Limestone	1.00
Dicalcium phosphate (22% Ca, 18.5 P)	1.82
Salt	.48
Methionine hydroxy analogue – Ca, 93%	.15
Vitamin and trace mineral premix[b]	.25
Total	100.00
Calculated analysis:	
Crude protein, %	19.00
Metabolizable energy, kcal/kg.	3135
Total lysine, %	1.04
Total methionine + cystine, %	.75
Total calcium, %	.90
Available phosphorus, %	.45
Total sodium, %	.20

[a]Commercial 44% crude protein soybean meal was placed in 2.5 cm layers in open, flat cooking pans and autoclaved at .545 kg/cm^2 (15 psi) gauge steam pressure for 0 to 25 min with 5 min intervals. Timing began when maximum pressure was reached.

[b]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, 2766 mg; d-pantothenic acid, 8.8 mg; folic acid, 275.6 mg; vitamin B_{12}, 8.8 mg; choline chloride, 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 mg; iodine, 1.25 mg; iron, (in sulfate form), 20 mg; copper (in sulfate form), 2 mg.

TABLE 2. *Effect of added moisture and cooking time on trypsin inhibitor and urease contents of soybean meal,*[a] *Experiment 1*

Cooking time (min)	Trypsin inhibitor, Trypsin inhibited/protein (μg/mg)							Urease index, Δ pH						
	Total moisture level							Total moisture level						
	2%	10%	12%	14%	16%	18%	Mean	2%	10%	12%	14%	16%	18%	Mean
0	62.50	59.20	57.50	56.12	41.51	38.30	52.52	2.01	2.08	1.96	1.91	1.86	1.73	1.92
15	41.88	38.75	8.12	3.25	1.25	....	15.54	2.00	1.97	1.87	1.58	.29	....	1.28
30	30.62	16.88	6.25	2.50	....	....	9.38	1.96	1.88	.35	.12	....	....	.71
45	20.62	3.75	3.28	....	....	....	4.61	1.91	1.06	.02	....	....	....	.33
60	9.53	1.20	....	....	....	....	1.79	1.89	....	....	....	....	....	.32
75	7.52	....	....	....	....	....	1.25	1.72	....	....	....	....	....	.29
90	6.31	....	....	....	....	....	1.05	1.68	....	....	....	....	....	.28
105	4.69	....	....	....	....	....	.78	1.04	....	....	....	....	....	.17
120	3.25	....	....	....	....	....	.54	.35	....	....	....	....	....	.06
135	3.12	....	....	....	....	....	.52	.28	....	....	....	....	....	.05
Mean	19.00	11.98	7.52	6.19	4.28	3.83		1.48	.60	.42	.36	.22	.17	

[a]A total of 250 g of raw, flaked, solvent-extracted soybeans was placed in sealed glass containers and autoclaved for 0 to 135 min with 15 min intervals. Timing began when the maximum .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.

TABLE 3. *Effect of added moisture and cooking time on trypsin inhibitor and urease contents of soybean meal*[a], *Experiment 2*

Cooking time (min)	Trypsin inhibitor, Trypsin inhibited/protein (μg/mg)							Urease index, Δ pH						
	Total moisture level							Total 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	....	7.37	2.06	1.92	1.53	1.49	.07	....	1.18
75	16.78	9.54	3.44	2.20	....	....	5.33	1.88	1.68	.56	.41	....	....	.76
90	13.28	7.50	3.12	1.88	....	....	4.30	1.76	1.30	.28	.08	....	....	.49
105	9.37	4.38	2.81	1.24	....	....	2.97	1.14	.36	.11	.02	....	....	.27
120	7.81	3.12	1.25	.94	....	....	2.19	.46	.17	....	....	....	....	.10
135	7.34	1.56	1.20	....	....	....	1.68	.46	.05	....	....	....	....	.08
Mean	28.82	23.20	17.78	15.20	10.76	9.60		1.65	1.36	1.10	1.03	.71	.62	

[a]A total of 250 g of raw, flaked, solvent-extracted soybeans was placed in sealed glass containers and autoclaved for 0 to 135 min with 15 min intervals. Timing began when the maximum .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.

inhibitor content. This finding indicates that more heat is required to completely suppress trypsin inhibitor content than urease index. For example, with an 8% added moisture level the maximum required cooking time (Table 2) was 75 min for destruction of trypsin inhibitor and 45 min for nearly complete destruction of urease. Furthermore, the maximum required cooking time with a 10% added moisture level was 60 min for destruction of trypsin inhibitor and 45 min for nearly complete destruction of urease. A urease index of <.15 Δ pH is used by the commercial soybean processors to indicate processing adequacy and, thus, was used in these studies. These results are similar to those obtained by McNaughton and Reece (1980).

Figure 1 shows the relationship of trypsin inhibitor content and Hunter tristimulus color values *L* (lightness) and +*a* (redness). In SBM ground to pass a 20-mesh screen, both *L* and +*a* color values were related very closely to the SBM trypsin inhibitor content. As the SBM trypsin inhibitor contents approached zero, the ranges in both *L* and +*a* color values, indicated by the shaded area in Figure 1, became narrower.

These data indicate that both *L* and +*a* 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 indicate that trypsin inhibitor was completely destroyed when either *L* color value of 63 or +*a* color value of +5.2 is obtained.

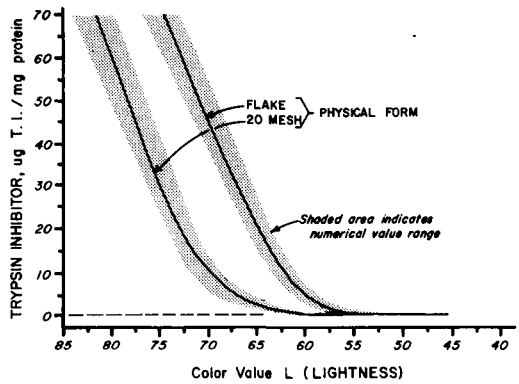

FIG. 2. Relationship between soybean meal and trypsin inhibitor content and Hunterlab color value *L* (Experiment 2).

Experiment 2. Experiment 2 was conducted to determine the effect of particle size on the subsequent color values in addition to checking results obtained in Experiment 1. As in Experiment 1, trypsin inhibitor content and urease (Table 3) decreased with increasing moisture levels and cooking times. These results agree with those reported by McNaughton and Reece (1980), Graham *et al.* (1949), Clandinin *et al.* (1951), Sunde (1973), Sandholm *et al.* (1976), and Hayward *et al.* (1936).

At the soybean meal processing plant, rolled flakes travel on-line from the solvent-extractor to the toaster. Therefore, it seemed logical to test the color of the flakes, as well as a 20-mesh material, in relation to the trypsin inhibitor contents. Grinding flakes to 20 mesh allowed for greater exposure of the inner or white parts

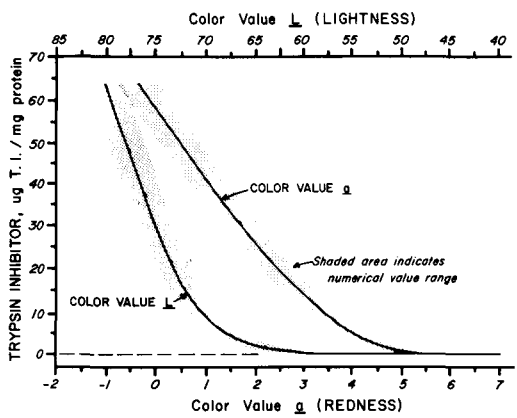

FIG. 1. Relationship between soybean meal trypsin inhibitor content and Hunterlab color values *a* and *L* (Experiment 1).

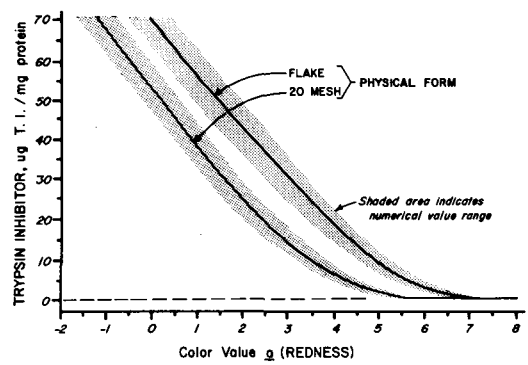

FIG. 3. Relationship between soybean meal trypsin inhibitor content and Hunterlab color value *a* (Experiment 2).

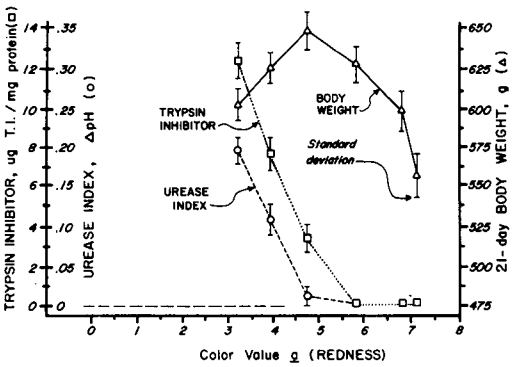

FIG. 4. Relationship of trypsin inhibitor content and urease index of soybean meal and broiler chick body weight (Experiment 3).

of the SBM flakes; therefore, the 20-mesh material was lighter in color than the flaked material (Figs. 2 and 3).

Trypsin inhibitor was destroyed with an *L* tristimulus color value of either 62 (20 mesh) or 56 (flake) and *+a* color value of either +5.5 (20 mesh) or +6.8 (flake). Although color differed significantly with particle size as was expected, the curves paralleled for both *L* and *+a* tristimulus color values. Grinding the sample to pass a 20-mesh screen has the advantage in that various similar sized materials, such as cooked soybean meal or flakes, can be tested on a comparable basis.

Experiment 3. Both trypsin inhibitor content and urease index (Table 4; Fig. 4) decreased with increasing cooking times. Commercial SBM was found to contain 12.12 μg/mg trypsin inhibitor. Growth was less than optimum when this SBM was fed in a 19% protein diet to broiler chicks. However, an additional 10 min of cooking decreased trypsin inhibitor content, increased body weight, and improved feed efficiency. With 15 min or more of additional cooking, broiler performance was depressed. These results indicate the effect of underprocessing 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 in a protein-deficient diet.

The SBM processing plants are now using a urease index of <.15 Δ pH to indicate processing adequacy. The urease index 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.

Conclusions. In Experiments 1 and 2, tristimulus *+a* color values of +5.2 and +5.5, respectively, coincided with the minimum concentration of trypsin inhibitor. The *+a* color value should be between 4.5 and 5.5 to assure optimum cooking conditions when processed under experimental conditions. Broiler weights were suboptimum with a *+a* color value of less

TABLE 4. *Effect of additional cooking of commercial soybean meal*[1] *high in trypsin inhibitor on broiler performance and color, (Experiment 3)*

Cooking time (min)	Hunterlab color value[2]			Urease index, Δ pH	Trypsin inhibitor, trypsin inhibited /protein (μg/mg)	21-day test results[3]	
	<	a	b			Mean body weight gain	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 to 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 Hunterlab Color and Color Difference Meter.

[3] Means within each column and without a common superscript are significantly different (P<.05).

than +3.96 or more than +5.81. Color may be used as an indicator of SBM processing adequacy. It can determine both underprocessing and overprocessing.

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