EVALUATION OF HEATED SOYBEAN MEAL USING CRESOL RED TECHNIQUE

by

OPES K. MATANMI
Department of Animal Science University of Ife, Ile-Ife

SUMMARY

Occasional problems with improperly heated soybean meal persist even though methods and rather good evaluation procedures are available. The technique of Olomucki and Bornstein (1960) involves absorption of cresol red during a shaking process of a specific grind on soybean meal and will evaluate overheated as well as underheated soybean meal. Attempts have been made to determine if the 3.8 (mg cresol red absorbed per g meal) reported was the lowest safe level. Unheated soybean meal samples averaged 2.55.

Genetically uniform day old chicks fed heated soybean meal sample testing 3.3 averaged 61.9 g at one week compared with 68.3g for soybean meal testing 3.6. Poults fed the same meals averaged 129g and 138g respectively at one week. In both tests at two weeks these differences had decresed to only 126g vs 133 and 266 vs 271. Effects on pancreas weights were not significant except with raw soybean meals.

INTRODUCTION

Adequately heat-treated soybean meal is gaining increased acceptance as a protein source in poultry rations in the U.S.A., Latin America and in East Africa.

In Nigeria, soybean is grown mainly for export purposes with a production of 33,000 tons on 38,000 hectares in 1971. These is evidence that hectarage is increasing and with the development of preheating techniques soybean meal will become an important source of protein in poultry diets. It is important therefore that techniques for evaluating heated soybean meal to destroy the trypsin inhibitor without denaturing the protein need to be investigated.

Several workers have reported that raw or improperly heated soybean meal when fed as the sole source of protein and amino acid depresses body weight of pigs, chicks, growth rate of poults and induces pancreatic hypertrophy Saxena, Jensen and McGinnis (1961); Bornstein and Lipstein (1963); Bray (1964); Shen, Bird and Sunde (1970); and Morgan, Somers and Larmand (1973). There are several methods of evaluating proper heating of soybean meals for optimum growth of chicks and poults. Ingram, Riesen, Cravens and Elvehjem (1949) described an *in vitro* method for evaluating overheated soybean meals by enzymatic release of amino acids. The urease activity test involving a pH change also evaluates underheated soybean meal. All these tests are very tedious and time consuming.

The purpose of this study was to use the cresol red technique as described by Olomucki and Bornstein (1960) with slight modification to evaluate the soybean meals and correlate these results with the growth of chicks and poults fed the corresponding samples.

MATERIALS AND METHODS

The soybean samples used in this study were either collected from feed mills near the laboratory of the University of Wisconsin (U.S.A) by the author or were obtained by feed mill inspectors during routine checks by them throughout the state of Wisconsin. The method of Olomucki and Bornstein (1960) was modified as follows. The soybean samples

are ground in a Wiley mill fitted with a 1mm sieve. Four hundred mg of the ground meal is weighed into a 15ml plastic centrifuge tube and 10ml of one part 0.2% alcoholic solution of cresol red to nine parts (volume by volume) of 0. 1N HCL (prepared and left over night) is added. The tubes are closed with rubber stoppers and shaken horizontally for one hour in a mechanical shaker (180 strokes/min). Care is taken so that no material is in the conical end of the tube when shaking is begun. The samples are then centrifuged for 10 minutes at 3000 rpm and 1 ml of the supernatant is added to 10 ml of 0.2 N NaOH in a test tube. The intensity of the colour is measured on the Beckman spectronic 20 at 570 nm. A standard curve is prepared using 0.05 ml to 0.25 ml of the acidic solution of cresol red with 0.02N NaOH solution making a final volume of 11 ml and the amounts of cresol red absorbed is expressed in mg/11ml using the equation:

$$x = (2.00 - (10 \times A)\ 2.5)$$

x = mg dye absorbed by 1 g meal

A = mg cresol red obtained by spectronic reading.

Soybean samples testing 2.55 to 4.25mg cresol red were fed to genetically uniform 10 day-old NHXWL chicks and 10 day-old Large White turkey poults for 3 weeks in duplicate groups and 7 experiments were conducted. At the end of experiment, 4 chicks or 4 poults were randomly selected, killed and their pancreas weights recorded.

In the first 2 experiments, raw soybean flakes were autoclaved in the laboratory at different pressures and for various length of time. The apparent degree of heating of these soybean meals was determined using the modified cresol red test.

Experiments 3 to 7 were performed with raw and commercial 44% protein soybean meals in addition to the earlier ones on laboratory meals. These were also fed to day-old poults and chicks for 3 weeks. The composition of the diets fed is shown in Table 1. These diets were calculated to meet the NRC (1971) nutrient requirements but the methionine supplementation was decreased because previous work by Shen, Bird and Sunde (1979) has shown that adding methionine to the diet above the NRC (1971) nutrient requirements could partially correct the growth depressions caused by raw soybean meals.

A survey of the ratings of commercially available soybean meal in Wisconsin (U.S.A) was carried out using the modified cresol red score test. Samples were furnished by state department of Agriculture inspectors from various parts of the state of Wisconsin for 4 years during the fall and winter months.

RESULTS

Samples of raw and autoclaved soybean flakes were ground and subjected to the modified cresol red test. Results showed that both the raw and those beans held at temperature of 105°C for five minutes at 51b presure gave absorption values of 2.55 and 3.50 and are therefore underheated. Heating at 112°C for 5 minutes up to 40 minutes at 151b pressure produced beans with absorption values ranging from 3.95 to 4.25 (Table 2). This is within the range of properly heated soybean as reported by Olomucki and Bornstein (1960). In this work the author reported that meals prepared from raw soybean had a value of 2.0 to 3.0 mg cresol red absorbed/g while values for underheated were in the range of 3.3 – 3.4; 3.5 — 3.7; 3.8 — 4.3 and over 4.3 respectively.

Samples of soybean processed by commercial feed meals used in experiments 3 to 7 gave absorption values of 3.30 to 3.8

TABLE 1

Gross Composition of diets from prepared soybean meal fed (g/kg)

	Experiments 1,& 2		*Experiments 3 & 7*	
Ingredients	Chicken	Turkey	Chicken	Turkey
Yellow corn	565	469	544	354
Soybean meal	310[a]	342[a]	335[b]	500[b]
Animal fat	50	50	50	50
Meat scrap	0	50	0	40
Fish meal	0	50	—	—
Fish solubles	10	0	10	10
Alfalfa meal	20	20	20	20
Iodized salt	5	5	5	3
Calcium carbonate	16	5	12	10
Dicalcium phosphate	18	5	18	8
Vitamin Mix[C]	5	5	5	5
Mg/kg				
D-L methionine	1000	500	330	330
MnS04	330	330	100	100
ZnCo3	100	100	500	—

C This mixture was composed by the author and supplied the following in mg/kg of diet.

Vitamin A activity	(IU)	1,55C
Vitamin D	(ICU)	220
Vitamin E	(IU)	15
Riboflavin	(mg)	3.6
Niacin	(mg)	28
Biotin	(mg)	(mg) 0.10
Choline	(mg)	1,300
Folacin	(mg)	0.56
Vitamin B	(mg)	0.01
Manganese[12]	(mg)	55
Iodine	(mg)	0.35
Iron	(mg)	80
Zinc	(mg)	50

a – Laboratory Prepared soybean meal

b – Commercially Prepared soybean meal

(Table 3). In accordance with the results of Olomucki and Bornstein (1960), these will be graded as underheated to slightly underheated. The results however indicated that all commercial samples of soybean were similarly utilised by both chicks and poults (Table 3).

EXPERIMENTS 1 AND 2

The chick response to the laboratory prepared meals is shown in Table 2. The raw soybean meal reduced growth rate and increased pancreas weights. Only slight weight differences existed between the heated soybean samples for the entire period and similarly pancreas weights were not affected by the heated meals. Table 2 also shows the poult response to the same soybean meals. The raw soybean meal again reduced growth rate and increased pancreas weight. Only small weight differences were observed with the heated soybean meals except that with the meals 4.25 cresol red score appear to be overheated for poults at 2 and 3 weeks. Effects on pancreas weights were consistent except that the weights were increased with raw soybean meals. Autoclaving does not produce the same quality soybean meals as are made in commercial plants. No soybean meals showing values below 3.5 when cresol red scores are considered were obtained with autoclaving.

EXPERIMENTS 3 TO 7

In Table 3 raw soybean meals reduced growth and increased the pancreas. Chicks fed the commercial sample testing 3.3 averaged 62g body weight at one week compared with those fed the other samples which averaged from 65—68g. however, effects on pancreas weights were not significant. In all 5 experiments body weights of chicks fed the sample testing 3.3 were always lower than the body weights of those fed the other commercial soybean meal samples. Similarly in Table 3, poults responded in the same way that

TABLE 2

Cresol red test on chick and poult response for laboratory prepared soybean meals

Soy Treatment	*Cresol red absorbed (mg/gm meal)*	*3 week body wt. (g)**		*Pancreas mg/100g body wt.*		*Feed/gain*	
		Chick	Poult	Chick	Poult	Chick	Pou
Raw	2.55	166a	214c	823	711	2.76a	2.2
5 mins x 5 lbs pressure (105°C)	3.50	229b	411a	403	323	2.00a	1.5
5 mins x 15 lbs pressure (112°C)	3.95	225b	418a	379	335	2.09ac	1.5
10 mins x 15 lbs pressure (112°C)	4.00	321b	406ab	284	326	2.01ac	1.5
20 mins x 15 lbs pressure (112°C)	4.10	233b	400ab	400	352	2.30ac	1.5
40 mins x 15 lbs pressure (112°C)	4.25	226b	379b	416	333	2.49b	1.5

* Columns with the same subscript are not significant at 5% level

TABLE 3

Cresol red test on chick and poult response for commercially available soybean meals

Sample source	*Cresol red score*	*3 week body weight (g)*		*Pancreas mg/100 g body wt.*		*Feed/gain**
		Chick	**Poults**	**Chicks**	**Poults**	**Chicks**	**Poults**
Raw	2.55	137a	180x	890a	720x	2.70a	2.18x
Commercial	3.30	213b	336xyz	490b	350y	2.03b	1.50y
,,	3.50	217b	375xy	486b	340y	2.02b	1.51y
,,	3.60	218b	340xyz	446b	312y	2.01b	1.52y
,,	3.85	216b	358xy	503b	356y	2.00b	1.54y

* Columns with the same subscript are not significant at 5% level

the chicks did. Raw soybean meals reduced growth and enlarged pancreas of poults. Turkey poults fed samples testing 3.3 averaged 130g body weight at one week as compared with those fed on the other samples which averaged from 133-138g. Only slight pancreas weight differences were observed in poults fed the commercial smaples. As was reported with chicks, body weights of poults fed samples testing 3.3 were always lower than body weights of those fed the other commercial samples.

The results of samples collected during 1971-74 is shown in Table 4. Those collected from the 1971 crop averaged 3.51 while those collected from the 1972 crop averaged 3.66. In 1973 those collected in October and November averaged 3.51 and those in January 1974 averaged 3.64. Unheated soybean meals averaged 2.55 (Table 3). Nine of the samples collected in early winter 1973 were found to have a cresol red score below 3.5. Over all out of 111 samples, 17 were found to be below 3.5. A few of these were positive to the

TABLE 4

Results of cresol red test on commercial samples of soybean meals during a four — year period

Date sample was collected	**Number samples**	**Samples below 3.5**	
	Season		
14/9/71	Early fall	99	3
15/12/72	13	2	
18/6/73	Winter	5	0
23/2/73	Winter	13	0
29/3/73	Late winter	9	0
23/4/73	Early spring	10	3
29/10/73	Late fall	13	4
19/11/73	Early winter	17	5
21/1/74	Winter	11	0
19/2/74	Winter	11	0

sniff test (Sunde, 1972) indicating rather serious underheating.

DISCUSSION

Chicks and poults are very sensitive to improperly heated soybean meal especially during the first week of age. The lower limit for poults and chicks is still uncertain but it appears that a 3.5 — 3.55 mg cresol red absorption/g meal is very near the lower range for chicks and poults during early periods of growth. The advantages of the cresol red technique are, apart from being rapid and requiring only modest equipment, this method also detects underheating as well as overheating of soybean meals.

Out of 111 soybean meal samples collected seventeen were found to be below 3.5 when evaluated by the cresol red test Why samples obtained during the late months of 1973 were affected is not certain. Possibly the processors are being "pushed" to extract more beans early than later in the crop season. In spite of the soybean meal samples obtained throughout the state showi&ng considerable variation, soybean meal is still probably the most consistent protein source available for livestock.

REFERENCES

BORNSTEIN, S. and B. LIPSTEIN 1963. The influence of age of chicks on their sensitivity to raw soybean oil meal. *Poultry Sci.* **42:** 61.

BRAY, T.J. 1964. Pancreatic hypertrophy in laying pullets induced by unheated soybean meal. *Poultry Sci.* **43:** 382.

INGRAM, G.R., RIESEN, W.W., GRAVENS, W.W. and ELVEHJEM, C.A. 1949. Evaluating soybean oil meal protein for chick growth by enzymatic release of amino acids. *Poultry Sci.* **28:** 898.

MORGAN, E.J. Jr. SOMERS, J. and LARMAND, E 1978. I. Full fat soybeans for growing and finishing large white turkeys. *Poultry Sci.* **52:** 1936.

NATIONAL RESEARCH COUNCIL, NATIONAL ACADEMY OF SCIENCES, 1971. *Nutrient requirements of poultry. No. 1, 6th ed.* Washington, D.C.

OLOMUCKI, E, and BORNSTEIN, S. 1960. The dye absorption test for the evaluation of soybean meal quality. *J. Assoc. Offic. Agric Chem* **43:** 440.

SAXENA, H.C., JENSEN, L.S. and MCGINNIS, J. 1961. Growth inhibition by raw soybean meal for chicks and turkey poults. *Poultry Sci.* **40:** 1452.

SUNDE, M.L. 1972. Personal communication.

TIAN-FUFF SHEN, BIRD, H.R. and SUNDE, M.L. 1970. Cooked full-fat soybeans for turkey poults. *Poultry Sci.* **49:** 1738.