

# The Performance of Broiler Chicks Fed Diets Containing Extruded Cottonseed Meal Supplemented with Lysine<sup>1</sup>

M. H. Henry,\* G. M. Pesti,\*<sup>2</sup> R. Bakalli,\* J. Lee,† R. T. Toledo,† R. R. Eitenmiller,† and R. D. Phillipst

\*Department of Poultry Science, and †Departments of Food Science and Technology, University of Georgia, Athens, Georgia 30602

**ABSTRACT** Three experiments were conducted to test the hypothesis that extruding cottonseed meal (CSM) with supplemental lysine improves its feeding value by detoxifying gossypol. The performance of 1-wk-old straight-run Peterson × Arbor Acres broiler chicks fed diets containing 20% feed-grade or extruded CSM was compared with that of control chicks fed corn and soybean meal-based broiler rations. All diets were formulated to meet minimum NRC requirements. Lysine levels were adjusted by addition of synthetic lysine at rates of 0.5 to 2.0% of the protein in CSM.

In all experiments, weight gain, feed intake, and feed conversion ratio (FCR) of broilers at 21 d were significantly affected by the diets. Feeding feed-grade and extruded CSM resulted in decreased body weight gain, increased feed intake, and inefficient feed utilization. When 2% lysine was added to feed-grade or extruded CSM, the body weight gains of chicks were not significantly

different from those fed the control diet. The FCR of chicks fed feed-grade and extruded CSM plus 2% lysine at 21 d was significantly better than that of chicks fed feed-grade or extruded CSM alone. Abdominal fat pads (as a percentage of body weight) were significantly increased by the inclusion of CSM with or without the addition of lysine ( $P \leq 0.019$ ). Liver, spleen, and heart weights were not affected by the presence of 20% CSM in the diet. The effects of CSM on plasma iron level was not consistent. Only in Experiment 1 did CSM cause a significant reduction in plasma iron. The hemoglobin contents and hematocrit values of blood from chicks fed diets with 20% CSM were not significantly different from those of the controls. The extrusion process reduced the free gossypol in CSM, but the total gossypol level was not changed, and chick performance was not improved. However, this study shows that, with adequate supplemental lysine, CSM can be used in broiler diets without a reduction in performance.

(Key words: broiler, cottonseed meal, lysine, soybean meal, extrusion)

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## INTRODUCTION

Cottonseed meal (CSM) is a byproduct of the process used to extract oil from cotton seeds and contains 41 or 44% crude protein, depending on the extent of hull separation prior to the oil extraction process. Although CSM is high in protein, its utilization in poultry feed as a protein supplement is limited due to low lysine levels and by the presence of a toxic substance, gossypol. Problems related to lysine level and nutrient density are easily rectified by addition of synthetic lysine to poultry feed. However, solutions to the problems related to gossypol in CSM have been elusive. Gossypol, a naturally occurring metabolite of cotton that is concentrated in the seeds, is associated with reduced performance and increased mortality in chickens (Couch et al., 1955; Smith, 1970). However, several

feeding trials have shown that chick performance is not significantly affected when the dietary level of free gossypol is lower than 250 mg/kg of feed (Heywang and Kemmerer, 1966; Hermes et al., 1983). Other studies have shown that several factors, such as age, strain of chickens, dietary iron, and dietary lysine may affect birds' tolerance of gossypol (Heywang and Bird, 1955; Clawson and Smith, 1966; Martin, 1990). The quantity of CSM that can be incorporated into the diet depends largely on the amount of gossypol in the meal.

In order to utilize CSM in poultry rations great efforts have been devoted to devising practical methods to reduce and detoxify the free gossypol. The expander-solvent extraction process, used by most companies to extract oil from cotton seed, results in CSM with 0.03 to 0.14% free gossypol and 1.09 to 1.16% bound gossypol (Proctor et al., 1968; Vix et al., 1971; Yu et al., 1993). Several other solvent extraction processes have been used to reduce the gossypol levels in CSM, but none are commercially viable (Hron, et al., 1996). Another technique used to detoxify free gossy-

**Abbreviation Key:** CSM = cottonseed meal; FCR = feed conversion ratio.

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<sup>2</sup>To whom correspondence should be addressed: gpesti@uga.edu.

pol in swine and poultry rations is addition of a soluble iron compound (Clawson and Smith, 1966). Husby and Kroening (1991) recommended that iron in the diet binds gossypol in the gut and reduces the problems related to iron deficiencies because of the formation of gossypol-iron complexes in blood (Danke and Tillman, 1965; Braham et al., 1967; Tone and Jensen, 1974; Skutches et al., 1974; Reynolds and Tone, 1988). Extensive feeding studies have shown that addition of ferrous sulfate to rations containing CSM reduced the adverse effects of gossypol on swine and poultry (Panigrahi et al., 1989; Barraza et al., 1991; Panigrahi and Morris, 1991; Boling et al., 1998). Although the addition of ferrous sulfate has reduced the adverse effects of gossypol, it is not widely accepted by the poultry industry.

Free gossypol also binds to lysine under extreme temperatures and reduces the free gossypol available for absorption (Tanksley and Knabe, 1981; Frank, 1985; Yu et al., 1996). However, extreme heat also reduces protein quality, thus limiting the usefulness of this method (Batterham et al., 1990; van Barneveld et al., 1994; Yu et al., 1996). The extrusion process, an efficient technology for heating dry materials under controlled conditions without reducing protein quality, may be a practical method to reduce the free gossypol in CSM.

The objective of this research was to determine if an extruded CSM product could be produced for use in poultry feed. It is well documented that gossypol binds to lysine and reduces its absorption. Therefore, lysine was added to CSM as a percentage of its protein content to improve feeding value, presumably by detoxifying gossypol. The performance of 1-wk-old straight-run broiler chicks was measured with diets containing commercial 44% protein cottonseed (feed grade) or extruded CSM to determine the suitability of incorporating this product in the feed.

## MATERIALS AND METHODS

A 44% crude protein CSM was obtained from a commercial oil processing plant that used the expander-solvent (hexane) extraction method to remove the oil. A proximate analysis was performed on CSM samples prior to extrusion, according to the Association of Official Analytical Chemists (AOAC, 1990) procedures. The CSM contained 44.5% crude protein, 11% crude fiber, 7.2% ether extract, 9% ash, and 9% moisture. The metabolizable energy reported by the NRC (1984) for CSM was 1,857 kcal/kg, and this value was used in the formulation of the diets. The amino acid levels in the CSM sample used were 5.1% arginine, 1.82% glycine, 1.80% serine, 1.27% histidine, 1.48% isoleucine, 2.75% leucine, 1.95% lysine, 0.75% methi-

onine, 2.40% phenylalanine, 1.46% threonine, 0.43% tryptophan, 2.02% valine, 1.28% tyrosine, and 0.79% cysteine.<sup>3</sup> CSM was extruded in a model MPF30 twin screw extruder<sup>4</sup> at 150 C barrel temperature and a feed rate of 10 kg/h in Experiment 1 and at 125 C barrel temperature at a feed rate of 12.5 kg/h in Experiment 2. In Experiment 3, CSM was extruded under both conditions. In all experiments, L-lysine was added to CSM prior to extrusion, except for one treatment in Experiment 3, in which lysine was added after extrusion at the time of mixing. Availability data based on chromic oxide balance are not included in this report.

Day-old Peterson × Arbor Acres broiler chicks were used in all experiments. Chicks were housed in Petersime battery brooders with 24 h fluorescent light. Feed and water were provided ad libitum for the duration of the experiments. A corn and soybean meal-based starter ration was fed to chicks from 1 to 7 d of age. At 7 d, chicks were weighed, and chicks within 2 SD of the mean body weight and with no physical deformities were selected. The average body weights on Day 7 were 149, 152, and 163 g for Experiments 1, 2, and 3, respectively. Four, six, and six pens of eight chicks each were randomly assigned to each dietary treatment in Experiments 1, 2, and 3, respectively. Body weights were measured at 0, 7, 14, and 21 d; residual feed was measured at 7, 14, and 21 d, and feed conversion ratio (FCR) was calculated. At termination, blood samples from three randomly chosen chicks per pen were drawn via cardiac puncture, and chicks were killed by cervical dislocation. Hearts, livers, spleens, and abdominal fat pads were removed and weighed. The livers were kept on ice for 4 h and then stored at -20 C for lipid analysis. Immediately after blood samples were collected, they were divided into two fractions. One fraction was used for determination of hematocrit and hemoglobin and iron levels in the blood. The remaining fraction was centrifuged and plasma was removed and stored at -20 C for iron analysis.

Control and experimental diets formulated and fed to chicks in Experiment 1 are shown in Table 1. The diets fed in Experiments 2 and 3 were the same as in Experiment 1 except for lysine levels and extrusion conditions.

## Tissue, Blood, and Gossypol Analyses

Fat content of the livers from Experiments 1 and 2 were determined using the procedure of Folch et al. (1957). Iron levels (in whole blood and plasma) were measured by flame atomic absorption using a Perkin Elmer 5000 atomic absorption spectroscope<sup>5</sup> (Anonymous, 1982). Packed cell volume (hematocrit) was measured by the microcapillary centrifuge technique described by Lucas and Jamroz (1961). Total hemoglobin level of the blood samples was determined using a Sigma Diagnostic Kit.<sup>6</sup> Free gossypol was determined by the AOCS method (AOCS, 1989), and total gossypol was measured using the HPLC method of Hron et al. (1999).

## Statistical Analysis

The experimental unit was the pen mean. All data from the three experiments were analyzed by analysis of vari-

<sup>3</sup>University of Missouri, Columbia, MO 65211.

<sup>4</sup>APV Baker, Ltd. (Model MPF 1700-30) Newcastle-under-Lyme, Staffs, UK ST5 7RG.

<sup>5</sup>Perkin Elmer Corp., Norwalk, CT 06859-0012.

<sup>6</sup>Sigma Co., St. Louis, MO 63178.

TABLE 1. Composition of the control and experimental diets

Ingredient	Control	Feed-grade CSM <sup>1</sup>	Extruded CSM	Extruded CSM + 2% Lysine <sup>2</sup>
Corn	53.586	48.187	48.187	48.187
Soybean meal (48%)	33.889	16.308	16.308	16.308
Cottonseed meal (44.5%)	...	20.000	20.000	...
Cottonseed meal + 2% Lysine	...	...	...	20.000
Poultry fat	5.989	5.989	5.989	5.989
Poultry byproduct meal	3.000	3.000	3.000	3.000
Deflourinated phosphate	1.745	1.745	1.745	1.745
Limestone	0.653	0.653	0.653	0.653
Common salt	0.400	0.400	0.400	0.400
Vitamin premix <sup>3</sup>	0.250	0.250	0.250	0.250
DL-Methionine	0.188	0.188	0.188	0.188
L-Lysine	...	0.087	0.087	0.087
Mineral premix <sup>4</sup>	0.100	0.100	0.100	0.100
Chromic oxide	0.100	0.100	0.100	0.100
Bacitracin	0.050	0.050	0.050	0.050
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.050	0.050	0.050	0.050
Composition by calculation				
Protein, %	23.000	23.000	23.000	23.000
ME, Kcal/g	3.200	3.200	3.200	3.200
Methionine	0.538	0.499	0.499	0.499
Methionine + Cysteine, %	0.899	0.948	0.948	0.948
Lysine, %	1.223	1.099	1.099	1.275
Threonine	0.849	0.803	0.803	0.803
Composition by analyses				
Protein, %	22.5	23.6	22.5	23.9

<sup>1</sup>Cottonseed meal.

<sup>2</sup>Lysine added on a percentage of protein based in CSM.

<sup>3</sup>Vitamin premix provides the following per kilogram: vitamin A, 5,500 IU from all-*trans*-retinyl acetate, cholecalciferol, 1,100 IU; vitamin E, 11 IU from all-*rac*- $\alpha$ -tocopherol acetate; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline Cl, 220 mg; vitamin B<sub>12</sub>, 6.6  $\mu$ g, vitamin B<sub>6</sub>, 2.2 mg; menadione, 1.1 mg (as menadione sodium bisulfate); folic acid, 0.55 mg; d-biotin, 0.11 mg; thiamine, 1.1 mg (as thiamine mononitrate); ethoxyquin, 125 mg.

<sup>4</sup>Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5.

ance using the general linear models procedure of SAS software (SAS Institute, 1985). In Experiments 1 and 3,  $P < 0.05$  was considered significant, and means were separated using Duncan's new multiple-range test. In Experiment 2, the dose-response relationships between performance and the level of lysine added to CSM were determined by regression analyses for each CSM type. Orthogonal contrasts were used to determine the differences between the main effects in Experiment 3.

## RESULTS

### *Effect of Extrusion on Gossypol*

The effect of extrusion on gossypol levels in CSM is shown in Table 2. Extrusion of CSM under either set of conditions resulted in a reduction in the free gossypol detected. However, if lysine was added to the CSM before extrusion, the amount of free gossypol detected was similar

TABLE 2. Gossypol content of cottonseed meal (CSM) before and after extrusion

Type of CSM <sup>1</sup>	Extrusion conditions (flow rate and barrel temperature)	Supplemental lysine (% of protein in CSM)	Free gossypol			Total gossypol <sup>2</sup>
			(+)-Gossypol	(-)-Gossypol	(%)	
Commercial CSM		0	0.075	0.520	0.312	0.832
Extruded CSM	10 kg/h at 150 C	0	0.044	0.550	0.339	0.889
Extruded CSM	12.5 kg/h at 125 C	0	0.049	0.562	0.334	0.896
Extruded CSM + Lysine	10 kg/h at 150 C	2	0.064	0.554	0.340	0.894
Extruded CSM + Lysine	11.5 kg/h at 125 C	2	0.072	0.540	0.322	0.862

<sup>1</sup>All samples were analyzed in triplicate (n = 3) from random sampling (CV was less than 1.0%).

<sup>2</sup>Total gossypol is a sum of the (+) and (-) -gossypol.

**TABLE 3. Performance and plasma iron level of chicks fed diets with 20% feed-grade or extruded cottonseed meal (CSM) (Experiment 1)**

Treatment	Body weight gain <sup>1</sup> 7 to 21 d (g)	Feed intake <sup>1</sup> 7 to 21 d (g)	FCR <sup>1,2</sup> 7 to 21 d (g/g)	Fat pad <sup>3</sup> (% BW)	Plasma iron <sup>3</sup> (mg/L)
Control (corn and soybean)	632 ± 15 <sup>a</sup>	904 ± 15	1.432 ± 0.027 <sup>c</sup>	1.18 ± 0.06 <sup>c</sup>	1.09 ± 0.012 <sup>a</sup>
Feed grade cottonseed meal	628 ± 13 <sup>a</sup>	945 ± 14	1.506 ± 0.023 <sup>b</sup>	1.56 ± 0.07 <sup>a</sup>	1.03 ± 0.028 <sup>ab</sup>
Extruded CSM	575 ± 23 <sup>b</sup>	901 ± 14	1.570 ± 0.044 <sup>a</sup>	1.39 ± 0.09 <sup>b</sup>	1.03 ± 0.039 <sup>ab</sup>
Extruded CSM + 2% Lys	628 ± 8 <sup>a</sup>	942 ± 22	1.452 ± 0.013 <sup>bc</sup>	1.37 ± 0.05 <sup>bc</sup>	0.92 ± 0.036 <sup>b</sup>

<sup>a-c</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Based on four replicate pens of eight chicks each per treatment.

<sup>2</sup>FCR = Feed to gain ratio.

<sup>3</sup>Based on four replicate pens of eight chicks each per treatment.

to the commercial CSM. The total gossypol and the percentage of gossypol isotypes were not affected by extrusion.

**Experiment 1.** Twenty-one day body weights ( $P < 0.03$ ) and feed conversions ( $P < 0.01$ ) were significantly affected by dietary treatment (Table 3). Results from the 21-d weights indicated that the diets had significant effects on weight gain ( $P = 0.02$ ) and feed conversion ( $P < 0.01$ ) (Table 3). Chicks fed diets formulated with extruded CSM and 2% supplemental lysine had weight gains similar to those fed the corn and soybean meal-based diet. The addition of 2% lysine to the CSM during extrusion resulted in FCR that were not statistically different from that of the control. The feed consumption of chicks was not affected by the inclusion of CSM or the addition of 2% lysine. The inclusion of CSM in the diets resulted in higher carcass fat as indicated by fat pad as a percentage of body weight (Table 3). Plasma iron levels were reduced in chicks fed extruded CSM with supplemental lysine ( $P < 0.05$ ) (Table 3). The liver, heart, spleen, liver fat, hemoglobin, and hematocrit values were not significantly affected by the use of feed grade or extruded CSM in the diets (data not shown).

**Experiment 2.** Body weight gain, feed intake and FCR of chicks were affected by the diets at 21 days of age (Table

4). Chicks fed CSM diets without supplemental lysine had lower body weight gains and poorer feed efficiency than controls. Chicks fed CSM diets with supplemental lysine had performances similar to those of chicks fed the corn and soybean meal-based control diet. A significant linear dose-related increase in body weight gain was observed at 21 d, when graded levels of lysine were added to extruded and feed-grade CSM ( $P < 0.0001$  and  $P < 0.03$ , respectively). Feed conversion of chicks fed extruded CSM plus 0.5, 1.0, or 2% lysine were not different from those of chicks fed corn and soybean meal-based diets (Table 4). The addition of lysine to extruded and feed-grade CSM resulted in a significant linear dose-related decrease in feed conversion ratios of chicks at 21 d,  $P < 0.01$  and  $P < 0.03$ , respectively (Figure 1). The fat pads of chicks fed diets with CSM were heavier than those of chicks fed the control diet (Table 4). Neither extrusion nor the addition of lysine to CSM resulted in significant reductions in the weight of the fat pads. The diets had no significant effects on liver weight, bursa weight, blood hemoglobin, and hematocrit value (data not shown).

**Experiment 3.** Twenty-one day body weight gain ( $P < 0.03$ ), feed intake ( $P < 0.04$ ), and feed conversion ( $P = 0.001$ ) were significantly affected by dietary treatments (Table 5).

**TABLE 4. Performance of chicks fed diets with 20% feed-grade or extruded cottonseed meal (CSM) (Experiment 2)**

Treatments	Supplemental lysine <sup>1</sup> (%)	Body weight gain <sup>2</sup> 7 to 21 d (g)	Feed intake <sup>2</sup> 7 to 21 d (g)	FCR <sup>2,3</sup> 7 to 21 d (g/g)	Fat pad (% body weight)
Control		638 ± 7.1	912 ± 7.6	1.431 ± 0.012	1.02 ± 0.11
Feed grade CSM	0	601 ± 8.2	942 ± 9.4	1.568 ± 0.028	1.48 ± 0.16
Feed grade CSM	1	640 ± 9.1	988 ± 15.3	1.539 ± 0.025	1.37 ± 0.14
Feed grade CSM	2	665 ± 3.2	973 ± 12.2	1.456 ± 0.020	1.28 ± 0.11
Extruded CSM	0	624 ± 15.3	942 ± 9.5	1.513 ± 0.028	1.44 ± 0.13
Extruded CSM	0.5	622 ± 7.0	932 ± 10.9	1.498 ± 0.015	1.42 ± 0.15
Extruded CSM	1	630 ± 11.1	922 ± 16.0	1.476 ± 0.025	1.40 ± 0.09
Extruded CSM	2	655 ± 8.9	879 ± 18.5	1.444 ± 0.021	1.44 ± 0.13
Analysis of variance	d.f.	(P)			
Source	2	0.105	0.036	0.002	0.02
Lysine	3	0.001	0.060	0.002	0.885
Source × lysine	1	0.144	0.014	0.480	0.719

<sup>1</sup>Percentage of protein in CSM.

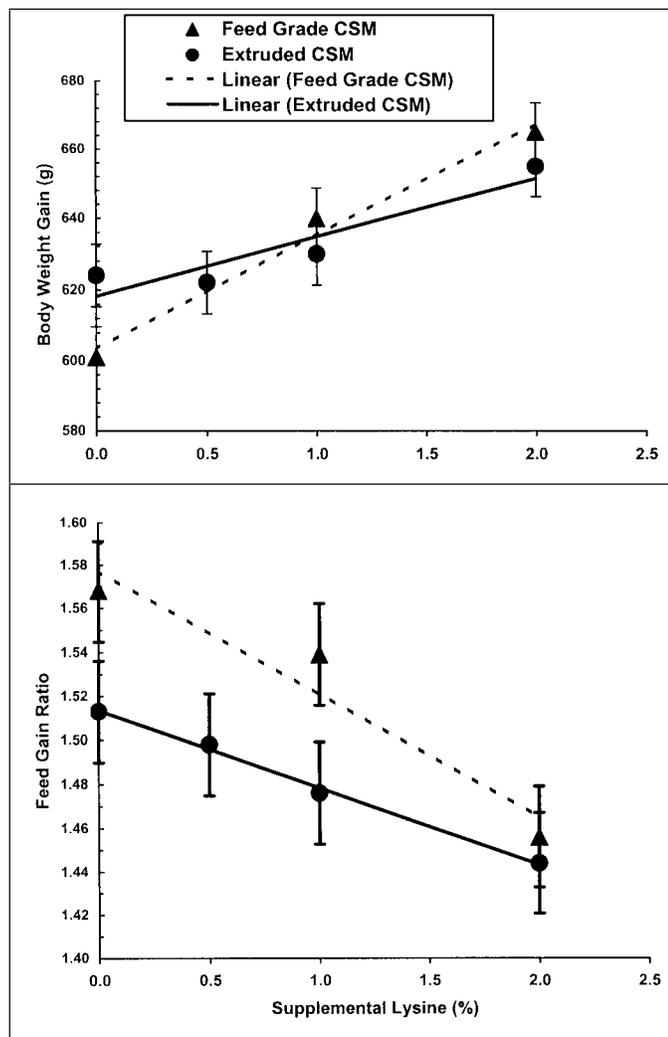
<sup>2</sup>Based on six replicate pens of eight chicks per treatment.

<sup>3</sup>FCR = feed conversion ratio.

However, there were no differences in body weight gain and feed conversion of chicks fed extruded CSM with supplemental lysine and those fed corn and soybean meal-based control diet. Feed intake and FCR of chicks fed extruded CSM were significantly lower than those of chicks fed feed-grade CSM ( $P > 0.05$  and  $0.02$ ). The addition of lysine (2% of CSM protein) resulted in significant improvements in body weight gain and FCR of broiler chicks. High temperature extrusion (150 C) resulted in reduced body weight gain and poor feed conversion compared to mild temperature extrusion (125 C). Lysine added to CSM after extrusion caused improvement in body weight gain but did not improve feed conversion of broiler chicks.

## DISCUSSION

In the present study, feed formulated with 20% CSM to meet the NRC (1994) recommended level for all nutrients



**FIGURE 1.** The effect of supplemental lysine on body weight gain (upper) and feed gain ratio (lower) of chicks fed diets formulated with 20% feed-grade (—▲—) or extruded (—●—) cottonseed meal (CSM). Supplemental lysine results in significant linear increase in body weight gain and decrease feed gain ratio. The following values were obtained from regression analysis: a) extruded CSM:  $r^2 = 0.195$ ,  $P < 0.04$  for BW and  $r^2 = 0.204$ ,  $P < 0.03$  for feed conversion; b) feed-grade CSM:  $r^2 = 0.723$ ,  $P < 0.001$  for BW and  $r^2 = 0.40$ ,  $P < 0.01$  for feed conversion.

resulted in chick weight gains equivalent to the diets with soybean meal as the major protein source but with decreased feed efficiency. Similar results were reported by Watkins et al. (1993) with CSM constituting 30% of the ration. Other studies have shown that CSM had a negative effect on chick performance (El-Boushy and Raterink, 1989; Fernandez et al., 1994, 1995). The negative effects of CSM on chick performance observed in these studies were associated with the presence of gossypol (Couch et al., 1955; Heywang and Bird, 1955; Heywang and Kemmerer, 1966; El-Boushy and Raterink, 1989), low lysine level (Grau, 1946; Anderson and Warnick, 1966), and reduced protein digestibility (Fernandez et al., 1994, 1995).

The present study also shows that the negative effects of CSM on feed efficiency can be reversed by adequate lysine supplements. Addition of 2% lysine (% protein basis) to the feed-grade and extruded feed-grade CSM, in all three experiments, resulted in FCR similar to the chicks fed corn and soybean meal-based diets. Whether the supplements are added before or after extrusion or without extrusion does not appear to be important. This point could be tested further because differences of 0.02 or 0.03 FCR can be important commercially but difficult to detect in experiments of this size.

This report is the first to show that the poor FCR associated with feeding chicks diets formulated with CSM as the protein supplement can be overcome by increasing the lysine level of the diet. The diet that was formulated with CSM and 2% supplemental lysine had a total lysine level of 1.275%, which was higher than previous studies (Watkins et al., 1993; Baber et al., 1995; Fernandez et al., 1995). Watkins et al. (1993) reported that the inclusion of low gossypol CSM up to 30% of diet had no adverse effect on body weight, but feed efficiency was adversely affected. In that same study, feed efficiency was not improved when lysine was added to replace the small portion assumed lost during processing. Fernandez et al. (1995) formulated diets based on the total digestible amino acids and was able to improve weight gain and feed efficiency of chicks fed diets with 20% CSM. The results of the present study indicate that supplemental lysine may be effective in reducing gossypol toxicity and that broiler diets formulated with CSM require significantly higher lysine levels than the NRC (1994) requirement and those that might be expected from calculating digestible lysine levels for the feed.

The second objective of this study was to detoxify the gossypol by extruding CSM in the presence of added lysine. Gossypol levels in extruded and commercial CSM are shown in Table 2. The free gossypol in the commercial cottonseed used in the present feeding trials was less than the 0.12% (wet basis) reported for standard (42% protein, wet basis) expander-solvent extracted CSM (Anonymous, 1997). The 44% protein cottonseed meal used in the present study contained very low gossypol levels that can be attributed to the process for producing the high protein CSM. Extrusion under each set of conditions reduced free gossypol values compared to the unextruded CSM. Total gossypol remained about the same, indicating that extrusion increased the level of bound gossypol. However, when

TABLE 5. Performance of chicks fed diets with 20% feed grade or extruded cottonseed meal (CSM) (Experiment 3)

Feed	Extrusion (rate/temperature) <sup>1</sup>	Supplemental lysine <sup>2</sup>	Body weight gain (7 to 21 d) <sup>3</sup> (g)	Feed intake (7 to 21 d) <sup>3</sup> (g)	FCR <sup>4</sup> (7 to 21 d) <sup>3</sup> (g:g)
Control/corn and soybean	...	...	642 ± 13.9 <sup>ab</sup>	900 ± 16.5 <sup>c</sup>	1.404 ± 0.026 <sup>c</sup>
Feed grade CSM	...	...	617 ± 22.8 <sup>bc</sup>	973 ± 16.1 <sup>a</sup>	1.584 ± 0.038 <sup>a</sup>
Feed grade CSM	...	2%	652 ± 8.4 <sup>ab</sup>	949 ± 12.1 <sup>ab</sup>	1.455 ± 0.014 <sup>ab</sup>
Extruded CSM	10/150	0	592 ± 19.6 <sup>c</sup>	928 ± 9.8 <sup>abc</sup>	1.576 ± 0.044 <sup>a</sup>
Extruded CSM	10/150	2%	655 ± 6.6 <sup>ab</sup>	942 ± 12.6 <sup>abc</sup>	1.437 ± 0.012 <sup>bc</sup>
Extruded CSM	12.5/125	0	633 ± 13.3 <sup>abc</sup>	926 ± 18.9 <sup>bc</sup>	1.462 ± 0.004 <sup>bc</sup>
Extruded CSM	12.5/125	2%	666 ± 11.3 <sup>a</sup>	946 ± 13.9 <sup>ab</sup>	1.421 ± 0.006 <sup>c</sup>
Extruded CSM <sup>5</sup>	12.5/125	2%	635 ± 11.4 <sup>abc</sup>	952 ± 8.4 <sup>ab</sup>	1.503 ± 0.002 <sup>b</sup>
Orthogonal contrasts			(P)		
Control vs. CSM			0.689	0.005	0.003
Feed-grade vs. extruded CSM			0.685	0.043	0.018
Extrusion conditions			0.025	0.542	0.006
Supplemental lysine			0.002	0.789	0.001

<sup>a-c</sup>Means within columns with no common superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Rate in kg/h and temperature in C.

<sup>2</sup>Lysine was added after CSM was extruded on a percentage of protein basis.

<sup>3</sup>Based on six replicate pens of eight chicks per treatment.

<sup>4</sup>FCR = feed conversion ratio.

<sup>5</sup>Lysine was added after extrusion of CSM at time of feed mixing.

lysine was added to the CSM prior to extrusion, the free gossypol values were the same as in the unextruded cottonseed, indicating that the added lysine might have been reacting with the reagents in the AOCS (1989) assay for free gossypol. The total gossypol, which was determined using a HPLC procedure, remained about the same in all treatments. We believe that lysine in the free form interferes with the spectrometric reading and that gossypol bound to the free amino acid is easily extracted as suggested by Tchiegang and Bourely (1997). Even with this dramatic decrease in free gossypol in the CSM, the performance of chicks fed extruded CSM was not equivalent those fed corn and soybean meal-based diets. However, extruded and feed-grade CSM with supplemental lysine resulted in increased body weight gain and feed efficiency in a dose-dependent manner, which clearly demonstrates that in formulating diets with CSM, the amino acid levels (lysine, threonine and methionine) are as important as the gossypol level in CSM. Previous studies with oilseed meals (Parsons et al., 1992; Fernandez et al., 1994; Zhang and Parsons, 1996) showed that overheating resulted in decreased protein solubility and chick performance. In the present study, CSM extruded under higher temperatures resulted in significantly poorer weight gains and feed conversion than CSM extruded at lower temperatures. However, the benefits gained from the reduction in free gossypol level may be offset by the decrease in protein quality by the extrusion process.

Although supplemental lysine and extrusion both significantly improved weight gain and feed efficiency, they were not able to prevent the increase in carcass fat associated with the use of CSM as the major protein source. The increase in carcass fat in chicks fed rations formulated with CSM as the major protein source may be attributed to the protein quality. Diets formulated with high ratios of calorie to protein resulted in high carcass fat on chickens (Don-

aldson et al., 1956). Fuller (1976) also stated that the ratio of amino acids to calories can change the carcass composition. The results of this study suggest that diets containing CSM, even with adequate lysine, did not provide birds with the same levels of available amino acids as soybean meal. Another possible explanation for the higher carcass fat in birds fed diets with CSM is the fiber content and energy level of CSM. The CSM metabolizable energy used in diet formulation was taken from NRC (1984) publication, and thus, the ME level of the diets with CSM may be greater than calculated. High energy:protein ratio increases carcass fat. Similar carcass values were reported by Watkins et al. (1993). The decrease in plasma iron levels observed may be attributed to reduction in available iron due to the formation of a gossypol-iron complex, as suggested by Clawson and Smith (1966). Although the use of iron to detoxify gossypol may be more economical, it does not result in improved feed utilization. The use of iron may also affect the utilization of other trace minerals and thus is not widely accepted by the poultry industry.

The results of these experiments indicate that broiler diets formulated with CSM require higher lysine levels to obtain performance comparable to soybean meal but will yield carcasses with slightly higher body fat. The higher level of lysine required is probably due primarily to the unavailability of the lysine bound to gossypol during the oil extraction and heating of CSM. Extrusion of CSM is not necessary for use in broiler diets.

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