Comparative Performance of Broilers Fed Diets Containing Processed Meals of BT, Parental Non-BT Line or Commercial Cotton Seeds

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ABSTRACT : An effort was made to assess comparative production performance in broiler chickens fed diets containing solvent extracted cottonseed meal (CSM) processed from BT and Parental Non-BT lines. Processed meal of national check and commercial produce cottonseeds were also used for comparison. The free gossypol contents were 0.02, 0.02, 0.44 and 0.03% in meals of BT, Parental Non-BT, national check and commercial produce cottonseeds, respectively. Day-old broiler chicks (n=243) were divided to 27 groups of 9 each. Nine dietary treatments (iso-nitrogenous, 23% CP and iso-caloric, 2,800 kcal ME/kg) were formulated viz., D1 (control, soybean meal-SBM based), D2 and D3 (BT CSM at 10% of diet with and without additional iron), D4 and D5 (non-BT CSM with and without additional iron), D6 and D7 (national check CSM with or without additional iron), and D8 and D9 (commercial produce CSM with or without additional iron at 2 ppm for every 1 ppm of free gossypol, respectively). Each dietary treatment was offered to three replicated groups up to 6 weeks of age. At the end of 6 weeks of age, 10 birds were taken out randomly from each treatment and were sacrificed to study carcass traits, organs' yield and histo-pathological changes in vital organs. The broiler chickens received CSM processed from BT (D2, 1,753 g and D3, 1,638 g) and Parental Non-BT (D4, 1,653 g and D5, 1,687 g) with or without additional Fe grew at same rate as observed in soybean meal (solvent ext.) based diet (D1, 1,676 g). The feed intake and feed conversion efficiency (feed: gain) in these dietary treatments (BT, non-BT line based diets) also did not differ significantly ($p>0.05$) from control diet. Similar observation was also observed in dietary treatments (D8 and D9) containing solvent extracted cottonseed meal of commercial produce origin. However, a decrease ($p<0.05$) in body weight gain and feed intake was observed in D6 containing national check CSM with high gossypol content. Addition of Fe in the diet (D7) improved ($p<0.05$) feed intake and weight gain but not to the extent as observed in diets containing BT, parental non-BT, and commercial produce CSM or control. However, any type of CSM did not affect feed conversion efficiency when fed with or without additional iron. The carcass characteristics in terms of dressing percentage, liver weight and heart weight was not significantly ($p>0.05$) different between the treatments. The eviscerated yields emanated from diets containing either BT, non-BT or commercial produce were statistically similar to control. However, eviscerated yield of broilers fed national check CSM with or without iron supplementation was lower ($p<0.05$) than BT cotton with Fe supplementation and commercial produce CSM. The study envisaged that BT, parental non-BT and commercial produce solvent extracted cottonseed meal can be included at 10% in soybean meal based broiler diet replacing soybean meal and rice bran without additional iron. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 57-62)***

Key Words : Cottonseed Meals, BT Cotton, Broilers, Growth Performance, Carcass Traits, Pathology

INTRODUCTION

The BT cotton hybrid genetically implanted with a gene drawn from certain soil bacteria (*Bacillus thuringiensis* or shortly BT) is capable of resisting the damage from cotton bollworms and all pests of insect family called Lepidoptera. The larval stage of the insect(s) attacks the bolls and when the BT protein (contained in the gene of plant tissue) is ingested, it sticks to the gut (throat) of the larva and the gut wall breaks down making it impossible to feed. In BT cotton, damage to bolls is minimized and need for insect sprays against the bollworm too are greatly reduced, saving the farmer from use of costly poisonous chemicals. There are no adverse impacts on other (non-target) insect pests and so sucking pests of cotton, which attack in early stages, have to be managed as usual. This also means that the BT cotton does not kill non-target beneficial fauna. The BT cotton is also safe to other (non-target, non-insect) organism like birds, fish, animals and humans as well. There is "no difference in allergenic potential between BT and non-BT cotton entries" also. BT cotton is raised along with few rows of the same hybrid not implanted with BT gene - called non-BT or Refuge Rows-a strategy claimed to prevent resistance building up in the target insects against the toxic principle of BT (Badrinarayanan, 2002).

BT Cotton has now been cleared by the government of India for propagation in the country in the central and southern cotton zones. Contradictory reports are available indicating the safe/effective level of cottonseed meal (Phelps, 1966; Waldroup, 1981). Raw cottonseed meals beyond 4-5% in diet significantly reduce average weight gains and feed intake in broiler chickens (Atuahene et al., 1986; Flemming, 1996). On the other hand, broilers fed on diets between 10 and 20% raw cotton seed meal had similar body weight, feed intake and feed conversion efficiency (Walkins et al., 1993; Watkins and Waldroup, 1995; Golian, 1994; Phelps, 1966). In the present study efforts were made

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to study the feed characteristics of processed meal of BT cotton in comparison to Parental Non-BT, national check and commercial produce cottonseed.

MATERIALS AND METHODS

Selection of chicks and experimental design

Two hundred and forty three (243) thrifty and bright looking unsexed, day-old broiler chicks were selected, wing banded, weighed and randomly distributed into 27 groups of 9 chicks each. The experiment was conducted following completely randomized design having 9 dietary treatments with 3 observations (replicates) in each. They were vaccinated against Ranikhet and Infectious Bursal Disease at their proper age.

Housing and brooding

Chicks from day-old were housed in battery brooder cages upto 3 weeks of age and thereafter shifted to battery cages fitted with feeder, waterer and dropping trays.

Selection of feed ingredients

The feed ingredients were analysed for proximate composition, calcium and phosphorus. The four cottonseed (BT cotton, non-BT cotton, national check cotton and commercial produce of cotton) samples, supplied by Maharashtra Hybrid Seeds Company Ltd. (Mahyco), New Delhi, were processed suitably in the laboratory for preparation of their respective solvent extracted meals (undecorticated). The BT cotton seed is the transgenic cotton seed containing Cry I AC gene derived from BT (*Bacillus thuriniegenes)* and non BT cotton seed is the non transgenic one whereas other two varieties are the commercially available ones. The proximate composition of different cottonseed meals is given in Table 1. While their gossypol contents are given in Table 2.

Feeds and feeding

Nine dietary treatments (isonitrogenous, 23% crude protein and isocaloric, 2,800 kcal ME/kg) were formulated viz., D1 (control, soybean meal (SBM) based), D2 and D3 (BT CSM at 10% of diet with and without additional iron, respectively), D4 and D5 (non-BT CSM with and without additional iron respectively), D6 and D7 (national check CSM with or without additional iron, respectively) and D8 and D9 (commercial produce CSM with or without additional iron at 2 ppm for every 1 ppm of free gossypol as in Table 3). Each dietary treatment was offered to 27 broiler chicks in three replicated groups of 9 each upto 6 weeks of age. They were supplied fresh drinking water daily *ad lib.* Body weight of individual broiler chicks was recorded weekly. The feed intake of chicks was recorded at weekly intervals from 0-6 week of age by offering weighed quantity of feed and weighing their residues. The mortality of birds was recorded and when it occurred, weighed and sent for postmortem examination. The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain.

Table 1. Proximate composition (% on dry matter basis) of cottonseed meals (CSM)

	Moisture	CP	EE	CF	Ash	NFE
BT CSM	7.22	34.40	0.91	11.12	5.32	48.25
Non BT CSM	3.09	33.18	0.50	12.65	5.06	48.61
National check	5.48	35.20	0.85	15.57	5.10	43.28
Comm. CSM	7.43	35.17	0.77	16.65	5.07	42.34

Table 2. Protein and Gossypol Content (% on dry weight basis) in CSM

	Protein		Gossypol, total		Gossypol, free	
	Seeds	CSM	Seeds	CSM	Seeds	CSM
BT CSM	27.1	34.40	1.23	0.74	1.14	0.02
Non BT CSM	25.2	33.18	1.52	0.58	1.39	0.02
National check	25.4	35.20	1.59	0.70	1.35	0.44
Comm. CSM	-	35.17	-	0.72	-	0.03

Table 3. Ingredient (%) and chemical composition of experimental diets

Ingredient	Control	CSM
Maize	54.5	56
Soybean	29	23.7
Rice bran	6.2	0
Cotton seed meal	0	10
Fish meal	8	8
Lime stone	1	1
Di calcium phosphate	1	1
Trace mineral premix[a]	0.1	0.1
Vitamin premix[b]	0.1	0.1
Salt	0.1	0.1
Total	100	100
Chemical composition (dry matter basis)		
ME, kcal/kg	2,802	2,802
CP, %	23.02	23.02
Lys, %	1.21	1.18
Met., %	0.52	0.53
Ca %	1.25	1.30
P (total)	0.89	0.91
P (Av. P)	0.48	0.49
CF	4.20	4.35

[a] Supplied mg/kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4.
[b] Supplied per kg diet: Vit. A, 8,250 IU; Vit. D_3, 1,200 ICU; Vit. K, 1 mg; Vit. E, 40 IU; Vit. B_1, 2 mg; Vit. B_2, 4 mg; Vit. B_{12}, 10 mg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.
* Calculated value.

Carcass traits

At the end of 6th week of age, 10 broiler chicks picked up randomly from each treatment groups. They were starved for 12 h (indeed drinking water was supplied *ad lib.)*, and were sacrificed as per standard procedure for evaluation of carcass characteristics including the defeathered yield, eviscerated carcass yield, weight of the liver, heart and gizzard of chicks.

Laboratory analyses

The representative samples of feed ingredients were analyzed for proximate composition (moisture, crude protein, ether extract, crude fibre, total ash and nitrogen free extract), calcium and phosphorus (AOAC, 1990). The processed cotton seed meal samples were analyzed for total and free gossypol content following AOCS, 1989.

Gross and histopathology changes

The tissues of the target organs (intestine, liver, spleen, heart and kidney) collected from sacrificed birds at the end of 6 weeks of experimental feeding were examined for gross and histo-pathological changes.

Statistical analysis

Data were subjected to analyses of variance following one way classification of completely randomized design. The means of different dietary treatments were tested for statistical significance using Duncan's multiple range tests (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Body weight gain

The body weight gains at 0-4 weeks and 0-6 weeks of age are given in Table 4. The data of 0-4 weeks or 0-6 weeks period revealed that broiler chickens received solvent extracted cottonseed meal (CSM) processed from BT (D2, 1,753 g and D3, 1,638 g) and Parental Non-BT (D4, 1,653 g and D5, 1,687 g) cottonseeds with or without additional Fe grew at same rate as observed in soybean meal (solvent ext.) based diet (D1, 1,676 g).

The rapidly growing chicks are highly susceptible to toxic effect of feed, if any and manifested by growth depression. As BT, non-BT and commercial produce CSM did not depress body weight either at 0-4 or 0-6 weeks of age, it indicated that those ingredients at 10% level did not exert any toxic effect. However, a significant decrease ($p<0.01$) in body weight gain was observed in D6 containing national check CSM with high gossypol content. Addition of Fe in the diet (D7) improved ($p<0.05$) weight gain but not to the extent as observed in diets containing BT, parental non-BT, commercial produce CSM or control. Lower body weight gain in D6 might be attributed to higher free gossypol content in National check variety (0.44%). Addition of iron at 2 ppm per 1 ppm gossypol though ameliorated toxic effect of gossypol but was unable to curb its toxicity completely.

Feed intake and feed conversion ratio

The feed intake in Table 4, the dietary treatments (BT, non-BT line based diets) did not differ significantly ($p>0.05$) from control diet. A similar observation for feed intake was also observed in different dietary treatments as for those of body weight gain. Feed intake recorded in D8 and D9 (with commercial produce CSM) was also similar to that in control. The result indicates that the cottonseed meals from BT, non-BT or commercial produce were equally palatable. However, a significant depression in feed intake was observed in D6 containing CSM of National check, any which might be due to lower body weight of birds in this group. Supplementation of Fe improved feed intake (D7).

Any type of CSM did not affect feed conversion efficiency when fed with or without additional iron both at 0-4 or 0-6 (Table 4) weeks of age. Therefore, it indicated that feed utilization efficiency was not affected by gossypol.

Contradictory reports are available indicating the safe/effective label of cottonseed meal. Raw cottonseed meals beyond 4-5% in diet significantly reduce average weight gains and feed intake in broiler chickens. (Atuahene et al., 1986; Flemming, 1996). On the other hand broilers fed on diets between 10 and 20% raw cotton seed meal had similar body weight, feed intake and feed conversion

Table 4. Production Performance of broilers from 0-4 and 0-6 wk of age

Diet	Treatment		0-4 wk			0-6 wk			
			Wt gain (g/b)	Feed intake (g/b)	FCR	Wt gain (g/b)	Feed intake (g/b)	FCR	Mortality (%)
1	SBM	Control	848.3^{a}	1,637.2^{a}	1.93	1,676.1^{a}	3,472.4^{a}	2.07	-
2	CSM	BT	881.6^{a}	1,648.5^{a}	1.87	1,753.0^{a}	3,472.3^{a}	1.98	7.4
3	CSM	BT+Fe	815.3^{a}	1,550.5^{b}	1.90	1,637.8^{a}	3,277.9^{a}	2.05	-
4	CSM	Non BT	835.7^{a}	1,615.9^{ab}	1.93	1,653.1^{a}	3,444.8^{a}	2.08	-
5	CSM	Non BT Fe	829.7^{a}	1,628.1^{ab}	1.97	1,686.9^{a}	3,463.9^{a}	2.05	7.4
6	CSM	National check	573.9^{c}	1,158.0^{d}	2.00	1,149.0^{c}	2,370.5^{c}	2.06	7.4
7	CSM	National Fe	715.7^{b}	1,413.5^{c}	1.98	1,456.1^{b}	2,830.0^{b}	1.95	-
8	CSM	Comm.	829.0^{a}	1,646.7^{a}	1.99	1,620.6^{a}	3,475.4^{a}	2.15	-
9	CSM	Comm. Fe	834.0^{a}	1,616.7^{ab}	1.94	1,683.9^{a}	3,381.4^{a}	2.01	3.7
		SEM	18.89	31.04	0.015	36.02	75.62	0.02	-

^{a,b,c,d} Values in coloum bearing different superscripts differ significantly ($p<0.05$).

efficiency (Watkins and Waldroup. 1995; Golian. 1994; Phelps. 1966). Therefore in the present study all the meals tested at 10% level replacing protein of soybean meal and rice bran. Addition of cotton seed meals at the rate of 10% in diet replacing soybean meal caused no depression in body weight gain or feed intake except in the dietary treatment containing National check variety of cotton seed meal with high amount of gossypol. Reports are available indicating that lysine is the first limiting amino acid in cottonseed meal (Phelps, 1966; Ryan et al., 1986). However in the present study, depression in growth and feed intake was not observed in the diets containing processed meal from BT, Parental Non-BT and commercial produce cottonseeds. Therefore lysine was not limiting amino acid in these processed meals when incorporated with soybean meal and fishmeal. Moreover the gossypol contents in these meals (0.02, 0.02 and 0.03%, respectively) were far below the levels reported to be deleterious by several groups of research workers as reviewed by Phelps (1966). Song et al. (1996) also did not observe any adverse effect on body weight gain and feed utilization in rats or quails fed diets with cottonseed meals from BT-transgenic cotton plants in 28 and 8 days toxicity trials. The high amount of fiber, gossypol, or cyclopropene fatty acids have been attributed to lower feed intake and body weight gain in cotton seed meal based diet (Phelps et al., 1965). However, the decrease in feed intake and weight gain, in the present study, was not attributed to the high amount of fibre or cyclopropene fatty acids as all the processed meals contained similar amount of fibre and cyclopropene fatty acids are almost totally eliminated from the cottonseeds during the oil extraction (Phelps et al., 1965). Therefore, depression of growth and feed intake in diet containing national check variety might be due to higher content of free gossypol (0.44%) in it contributing to 0.044% in diet. Reports have shown depressed weight gains and/or feed intakes when free gossypol levels fed to poultry were between 140-756 ppm (0.014-0.076%) (Milligan and Bird. 1951), 200-400 ppm (0.02-0.04%) (Richardson and Blaylock, 1950), 240-360 ppm (0.024-0.036%) (Heywang et al., 1952), or greater than 480 ppm (0.048%) (Lipstein and Bornstein, 1964) or 600 ppm (0.06%) (Couch et al., 1955). Supplementation of iron at 2 ppm for every 1 ppm of free gossypol, to this diet improved the gain in weight and feed intake significantly from that with iron un-supplemented group. The results of the present experiment corroborated well with the earlier findings (El Boushy and Raterink, 1989; Watkins et al., 1993). It has further been shown that the adverse effects of gossypol can be avoided by adding $FeSO_4$ to CSM in a 1:1 ratio by weight of iron to free gossypol to form a harmless iron gossypol complex (Clawson and Smith, 1966). However the supplemented iron at 2 ppm for every 1 ppm of free gossypol, was unable to overcome the deleterious effect of gossypol completely as envisaged from the data and growth of feed intake in dietary treatment D6 that was statistically lower than the control diet or diets containing processed meal from BT, Parental Non-BT and commercial produce cottonseeds. This might be due to lesser available lysine because of binding to gossypol. Gossypol is a polyphenolic compound that is located inside the pigment glands of cottonseeds (Ryan et al., 1986). This compound can form a Schiffs base with the epsilon amino group of lysine and render it indigestible and unavailable.

Carcass characteristics

The carcass characteristics (Table 5) in terms of dressing percentage and giblets yield were not significantly ($p>0.05$) different between the treatments. The eviscerated yields emanated from diets containing either BT, non-BT or commercial produce were statistically similar to that of control. However, eviscerated yield of broilers fed national check CSM with or without iron supplementation was significantly ($p<0.05$) lower than BT cotton with Fe supplementation and commercial produce CSM. The yield of different organs when expressed on unit live weight did not differ significantly ($p>0.05$).

Carcass traits and weight of vital organs did not differ in dietary treatments containing processed meal from BT.

Table 5. Carcass yield of broilers at 6 weeks of age

Diet	Treatment		Defeathered Yield (%)	Eviscerated yield (%)	Giblets (g/1,000 g)	Gizzard (g/1,000 g)	Heart (g/1,000 g)	Liver (g/1,000 g)
1	SBM	Control	89.57	64.93[ab]	47.16	21.08	4.55	21.52
2	CSM	BT	90.21	65.09[ab]	45.58	18.98	4.6	21.99
3	CSM	BT+Fe	89.24	65.89[a]	45.41	19.14	4.83	21.43
4	CSM	Non BT	90.04	64.73[ab]	49.96	21.37	5.07	23.50
5	CSM	Non BT Fe	89.58	65.05[ab]	48.51	22.06	4.68	21.76
6	CSM	National check	90.38	63.83[b]	47.82	20.82	4.79	22.20
7	CSM	National Fe	90.45	63.63[b]	52.23	23.90	5.12	23.20
8	CSM	Comm.	90.94	66.31[a]	50.54	21.78	5.12	23.62
9	CSM	Comm. Fe	90.73	66.26[a]	47.94	21.84	4.62	21.46
		SEM	0.16	0.22	0.50	0.33	0.079	0.32

[ab] Values in column bearing different superscripts differ significantly ($p<0.05$).

Parental Non-BT and commercial produce cottonseeds. However, eviscerated yield of broilers fed national check CSM with or without iron supplementation was lower. This might also be due to lower body weight gain in this group due to presence of high amount of gossypol. Atuahene et al. (1986) found significant differences among treatment means for dressing percentage, liver and viscera weights, along with depressed weight gain and decreased feed intake in experimental diets containing 5, 7.5 or 10% cottonseed meal.

Gross and histopathology

The tissues of the target organs (intestine, liver, spleen, heart and kidney) did not reveal any gross pathological changes in any dietary treatment. The histopathological examination of tissues revealed no changes in organs such as liver, kidney, spleen and intestine in broilers fed on soybean meal or cotton seed meals processed from either BT, non-BT or commercial produced cotton seeds upto 6 weeks of age. Liver tissues of birds fed raw national check cottonseed meal revealed severe degenerative changes including necrotic focci, sinusoidal congestion and dilatation of hepatic cord along with mild fatty changes. The kidney tissues of the same group showed hemorrhages and tubular degeneration, while no adverse changes were observed in spleen and intestinal tissues. The supplementation of iron in diet containing national check cottonseed meal reduced the severity of pathological changes.

The histo-pathological changes observed in tissues of liver and kidney of birds fed national check variety might be attributed to the higher gossypol content (0.44% free gossypol), Iron supplementation in diet D7 reduced the severity of the pathological lesions. Moreover, no such pathological changes were observed in low gossypol containing cottonseed meals viz., BT (0.02%), non-BT (0.02%) or commercial produce (0.03% gossypol) cottonseed meal, which also confirms the role of gossypol in pathological changes. Deleterious effects of gossypol in blood and bone of chicks characterized by reduction of haemoglobin levels, red blood cells and bone marrow cells have also been reported (Rigdon et al., 1958). No pathological changes in organs such as liver, kidney, intestine and testes of rats were also observed by Chen-Song et al. (1996) when cottonseed meal (gossypol content <0.02%), containing protein from BT-transgenic cotton resistant to insects, was fed.

Mortality

Total of seven birds died (Table 4) during the whole experimental period, which is equivalent to 2.89%, an insignificant event. The mortality of birds was 2, 2, 2 and 1 in dietary treatments D2, D5, D6 and D9, respectively. However, the pathological changes observed on post-mortem examination of dead birds during the experimental period did not attribute to the dietary treatments except in D6.

The results indicated that the cottonseed meals with gossypol content (0.44% in meal or 0.044% in mash) did not influence the mortality of birds. Contradictory reports are also available on the effect of gossypol on mortality pattern. Increasing levels of gossypol have shown to be correlated with increased mortality in some trials (Lillie and Bird, 1950; Couch et al., 1955) but not in others (Milligan and Bird, 1951; Eagle and Davies, 1957).

The study envisaged that low free gossypol containing cottonseed meals like BT, parental non-BT and commercial produce solvent extracted cottonseed meal could be included at 10% in soybean meal based broiler diets containing fish meal by replacing soybean meal and rice bran in the diet.

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