FINAL REPORT

Broiler chicken performance as influenced by feeding diets containing transgenic Bt cottonseed meal

Project Leader

Dr A. K. Shrivastav
Principal Scientist & Head
Nutrition &Feed Technology Division

Associates

Dr Pramod K Tyagi
Senior Scientist
Dr Praveen K Tyagi
Senior Scientist
Dr A.V. Elangovan
Senior Scientist

Study Centre

Central Avian Research Institute
Izatnagar (UP), 243122, India
Fax : 091-0581- 2301321,Tel: 2303223
E - mail : akscari@rediffmail.com

Sponsored by

JK AGRI GENETICS Limited
1-10-177 4th Floor
VarunTowers, Begumpet
Hyderabad – 500 016

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A study was conducted to evaluate and compare the feeding value of diets containing cottonseed meals obtained from three types of cotton seeds i.e. transgenic Bt (JKC-738), its normal isoline (non –Bt) and commercial (NH-44) seeds obtained from J.K. Seeds, Hyderabad on broiler chicken performance and carcass yield in a 7 weeks feeding trail. The three types of cotton seeds were first processed for solvent extraction to obtain solvent extracted cottonseed meals (CSM). The total and free gossypol (%) contents of different types of seeds were 3.27 and 1.13 in Bt, 3.87 and 1.35 in non-Bt, 2.66 and 0.78% in commercial (NH-44) type. The corresponding values of total and free gossypol contents of meals (solvent extracted) were 1.25 and 0.07, 1.25 and 0.04, and 0.56 and 0.04 % in BT, non-BT or commercial types of meals, respectively.

Day-old broiler chicks (n=160) were divided into 16 groups of 10 each. Four experimental diets (iso-nitrogenous- 23% CP and 21% CP and iso-caloric- 2900 and 2950 kcal ME/kg for 0-4 and 4-7 weeks for starting and finishing phases, respectively) were formulated. Diet D_1 was a typical corn-soyabean meal based control diet. Three more diets were prepared by incorporating 10% each of Bt CSM (D_2), non-Bt CSM (D_3), and commercial CSM (D_4) in the control diet. The calcium, available phosphorus and all the limiting amino acids for poultry namely lysine, methionine, cystine, threonine and arginine were similar in all the diets in both growth phases (0-3 weeks starting and 3-6 weeks finishing phases). Each dietary treatment was offered to four replicated groups of birds from day old to 7 weeks of age. The birds were reared in battery cages with group wise brooding, feeding and watering facilities. All management and vaccination practices were kept identical for all the dietary treatments. Birds were weighed at weekly intervals and data of feed intake were also recorded simultaneously. At the end of 7th week of age, 8 birds per treatment (2 birds/replicate) were sacrificed to study the effect of feeding CSM types on different carcass traits and development of digestive and immune organs.

Body weight gain, feed intake and feed conversion efficiency, did not differ statistically ($P<0.05$) either at starting (0-4 wks), and overall (0-7 wks) phases. However, during finishing (4-7 wks), significantly ($P<0.01$) lower feed intake with better ($P<0.05$) FCR was observed in chicks fed Bt cotton diet. The carcass traits also remained statistically ($P<0.05$) similar due to various dietary treatments except for drum sticks ($P<0.05$) and thigh ($P<0.01$) cut up parts.

The study envisaged that transgenic Bt cottonseed meal (solvent extracted) containing low free gossypol could safely be included up to 10% level in maize- soybean meal based diet up to 0-7 weeks of age of broiler chicken without adversely affecting their growth performance and carcass traits.

Key words: Broiler chicken, performance, carcass traits, transgenic Bt cottonseed meal

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Cotton (*Gossypium* spp.) is a leading commercial crop grown world over for its valuable fiber. India ranks first in the world accounting for 20% of the total area planted under cotton. However, even with highest area under cotton, nine million hectares, India ranks only third with only 13% in production of cotton. India's average yield is only 319 kg/ha lintas compared to world average of 603kg/ha. Cotton is highly susceptible to insects, especially to the larvae of lepidopteran pests, which is impacting cotton production. Fifty percent of the total insecticides consumed in the country are used only for cotton crop. The total loss due to damage to cotton crop is estimated to be more than Rs.1200 crores. The chemical control to suppress these insect pest are proving ineffective as these pests have developed high level of resistance for most of such chemicals used for the control of bollworm complex. Such a high level of resistance requires repeated application of insecticides leading to heavy expenditure, crop failures, and viscous cycle of debt for farmers. Therefore, it has been argued that adoption of Bt cotton could help in protecting the crop against potentially the most damaging bollworms and thus reduce the risk of crop failures. Bt cotton, a transgenic plant, produces an insect controlling protein Cry1A(c), the gene for which has been derived from the naturally occurring bacterium, *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k.). The cotton hybrids containing Bt gene produces its own toxin for bollworm attack thus significantly reducing chemical insecticide use and providing a major benefit to cotton growers and the environment. Incorporation of Bt gene holds promise in cotton crop by controlling bollworm, increasing yields and reducing insecticide needs that are environmentally unsafe. According to estimates, the area under Bt cotton in the country is still negligible at over 92,000 hectare out the total of over 9 million hectare under cotton. In 2002-03, the first year of its approval for commercial cultivation, the area under Bt cotton at 38,038 hectare was just 0.51 per cent of the area under cotton during that period.

Commercial cottonseed meal (CSM) available in the market has somewhat less protein than soybean meal and like groundnut meal, is not a good source of lysine. Unless synthetic lysine is added to the diet of poultry, use of cottonseed meal is usually limited to 4-5%. Gossypol is a compound found in cottonseed meal, which at high levels can depress growth and can cause the egg yolk to be olive green, and for the yolks to turn brown and the whites to turn pink on storage. The feasibility of CSM utilization in poultry rations, therefore, largely depends upon the concentration of gossypol in CSM. Reports have shown depression in weight gains and/or feed intakes when free gossypol levels fed to poultry were between 140-756 mg/kg (Milligan and Bird, 1951), 200-400mg/kg (Richardson and Blaylock, 1950), 240-360 mg/kg (Heywang *et al.*, 1952), or greater than 480 mg/kg (Lipstein and Bornstein, 1964) or 600 mg/kg (Couch *et al.*, 1955). Still, contradictory reports are available in the literature regarding the safe/effective level of CSM in poultry rations (Phelps, 1966; Waldroup, 1981). Raw cottonseed meals beyond 40-50g/kg in diet significantly reduced average weight gains and feed intake in broiler chickens (Atuahene *et al.* 1986; Flemming 1996). On the other hand, broilers fed on diets having 100 to 200g/kg raw cottonseed meal had similar body weight, feed intake and feed conversion efficiency (Walkins *et al.*, 1993; Watkins and Waldroup, 1995; Golian, 1994; Phelps 1966).

The government of India has now cleared the cultivation of transgenic Bt cotton in the central and southern cotton zones of the country and as a result, CSM derived from transgenic Bt cottonseeds is available for use in poultry rations. However, before incorporating such transgenic crop in poultry rations, the nutritional quality of transgenic Bt CSM *vis-à-vis* commercial CSM should be evaluated. The results of some of the earlier trials conducted in this laboratory (Elangovan *et al.*, 2003; Mandal *et al*, 2004)), envisaged that Bt, parental non-Bt and commercial CSM (solvent extracted) can safely be included at 100 g/kg in soybean meal based broiler diet replacing soybean meal and rice bran without addition of iron. The present study was carried out to evaluate the nutritional value of diets containing cottonseed meal obtained from transgenic Bt cotton hybrid seeds (JKC-738 from J.K.Agri Genetics Ltd., Hyderabad) resistant to Lepidopteron insect pests by transforming with Bt-Cry1Ac gene (truncated version) of *Bacillus thuringiensis* on broiler chicken performance and carcass yield.

Materials and methods

The proposed research work was conducted at the Avian Nutrition and Feed Technology Division, Central Avian Research Institute, Izatnagar, India. The biological trial was conducted from 25th August to 13th October 2004. The details of materials and methods employed in the study are presented hereunder:

Birds and experimental design

One hundred and sixty day-old straight run crossbred broiler chicks selected randomly from a larger population were wing banded, weighed and randomly distributed into 16 groups of 10 chicks each. The experiment was conducted following completely randomized design having four dietary treatments with four observations (replicates) in each.

Housing and brooding

The broiler chicks from their first day of age were housed in battery cages. The cages were fitted with feeder, waterer and dropping trays along with electrical brooding arrangements. Standard management practices for brooding, vaccination schedule and 24 h light to encourage feed intake were followed. The brooder temperature was maintained at 35°C during 1st week of age and gradually reduced to 25°C at 4 weeks of age.

Procurement of feed ingredients

All the feed ingredients, other than cottonseed meals, were procured from feed storage and processing unit, Central Avian Research Institute. They were analyzed for proximate composition (AOAC, 1990), calcium (Talpatra *et al.*, 1940) and phosphorus (AOAC, 1990). Three types of cotton seeds i.e. transgenic Bt (JKC-738), its normal isoline (non – Bt) and commercial (NH-44) were obtained from J.K.Agri Genetics Ltd., Hyderabad. The transgenic Bt (JKC-738) cottonseeds, resistant to Lepidopteron insect pests, were prepared by transforming with Bt-Cry1Ac gene (truncated version) of *Bacillus thuringiensis.* All the three types of seeds were processed in the laboratory for the preparation of their respective solvent extracted meals (undecorticated). The ground seeds were expelled applying pressure and residual oil was extracted in laboratory using petroleum ether as the fat solvent. The different cottonseeds and their respective meals were analysed for crude protein (AOAC, 1990) and total and free gossypol (AOCS, 1989) contents.

Experimental diets

Four experimental diets (iso-nitrogenous; 23 and 21% crude protein and iso-caloric; 2900 and 2950 kcal ME/kg for starting phase i.e. 0-21 days and finishing phase i.e. 21-42 days of age, respectively) were formulated (Table 1) following standard nutritient requirement specifications for growing broiler chickens. Diet D_1 was a typical corn-soyabean meal based control diet. Three more diets were prepared by incorporating 10% each of Bt CSM (D_2), non-Bt CSM (D_3), and commercial CSM (D_4) in the control diet. The limiting amino acids lysine, methionine, cystine, threonine and arginine were similar in all the diets in both growth phases (0-21 days starting and 21-42 days finishing phases).

Feeding/watering schedule

Each experimental diet was offered as mash *ad libitum* to four replicated groups of birds in both the phases of production i.e. starting and finishing phases. The chicks were provided fresh drinking water at all the time.The birds were reared in battery cages with group wise brooding, feeding and watering facilities. All management and vaccination practices were kept identical for all the dietary treatments.

Body weight and feed intake record

Body weight of individual broiler chicks was recorded weekly, while the feed intake of chicks, allotted in replicates, was recorded at weekly interval up to 7 weeks of age. The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately. The mortality of birds was recorded as and when it occurred, weighed and sent for post mortem examination.

Carcass characteristics

At the end of the 7-week feeding trial, two birds were picked randomly from each replicate (i.e. 8 birds/ treatment). These birds were starved for 12-hr before sacrifice as per standard procedure for evaluation of carcass characteristics. The yield of defeathered weight, eviscerated weight, different digestive and immune organ weights viz., proventriculus, gizzard, heart, liver, spleen, bursa, thymus, and abdominal fat was recorded. The carcass was cut up to standard parts viz. back, breast, thigh, drum stick, neck and wings and measurements were recorded. All these parameters were expressed as percentage of live weight.

Statistical analysis

The data were subjected to analysis of variance following one-way classification of completely randomized design (Snedecor and Cochran, 1989). The means of different dietary treatments were tested for statistical significance using Duncan's multiple range tests (Duncan, 1955).

Crude protein and gossypol contents of cotton seed/meal types

The three types of cotton seeds i.e. transgenic Bt (JKC-738), its normal isoline (non –Bt) and commercial (NH-44) seeds were first solvent extracted in the laboratory to obtain respective solvent extracted cottonseed meals (CSM). The different cottonseeds and their respective meals were analysed for crude protein and total and free gossypol contents (Table 2) before experimental diets formulation. The protein, total and free gossypol (%) contents of different types of seeds were 26.0, 3.27 and 1.13 in Bt, 27.2, 3.87 and 1.35 in non-Bt, and 24.6, 2.66 and 0.78% in commercial (NH-44) type, respectively. The corresponding values of crude protein, total and free gossypol contents (%) of meals (solvent extracted) were 36.9, 1.25, 0.07, in BT, 33.3, 1.25 and 0.04 non-BT and 34.7, 0.56 and 0.04 % in commercial types of meals, respectively. The crude protein content in all the types of CSMs was within normal range and free gossypol content was also within the reported safe inclusion levels of cottonseed meals for broiler chicken.

Growth performance and survivability

The average body weight gains of chicks in different dietary treatments during starting (0-3 weeks of age), finishing (4-7 weeks of age) and over all (0-7 weeks of age) phases are given in Table 3 & Fig.1. Body weight gains of the chicks under different dietary treatments did not differ statistically (P>0.05) during different growth phases of age. The broiler chicks grow at a very rapid rate and are highly sensitive to the presence of anti-nutrients or toxicants in feed that may be immediately manifested in their growth depression. The solvent extracted transgenic Bt, non –Bt and commercial cottonseed meals when included at10% of the diet did not depress body weight either at 0-3,4-7 or 0-7 weeks of age, indicating no toxic effects in birds. Feed intake and the efficiency of feed utilization (Table 3; Fig.2 & 3) of birds also did not differ significantly due to dietary treatments except during 4-7 weeks of age wherein chicks fed on diet containing 10% Bt CSM showed lower feed intake (P<0.01) than the other groups and superior FCR (P<0.05) than the other CSM fed groups. These results showed that palatability of feed was not affected due to inclusion of CSM types in diet of broiler chicken. Earlier work from this laboratory (Johri *et al*., 2001, Elangovan *et al*., 2003 ; Mandal *et al*. 2004) also showed that cottonseed meal containing transgenic genes and having low gossypol content could be included in growing broiler chicken diet without any deleterious effects. The mortality rate of birds in different dietary treatments was also well within normal range that on post-mortem examination did not reveal any pathological changes attributable to the dietary treatments. The results indicated that the cottonseed meals with gossypol content (0.04-0.07% in meal) and presence of Bt transgenic gene 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 gossypol content of CSM is detrimental in selecting its safe inclusion level in broiler diet and that's why contradictory reports are available indicating the safe/effective level of inclusion of cottonseed meal in poultry diets. 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*., 1855). Atuahene *et al*. (1986) and Flemming *et al*. (1996) reported that raw cottonseed meals beyond 4-5% in diet significantly reduced average weight gains and feed intake in broiler chickens. On the other hand, broilers fed diets between 10 and 20% CSM had no effect on growth performance (Watkins and Waldroup, 1995; Golian, 1994; Phelps 1966). In the present study, all the meal types were tested at 10% level replacing soybean meal in diet that caused no depression in body weight gain or feed intake as compared to the control diet in which soybean meal was used. Similarly, BT, Parental Non-BT and commercial cottonseed meals containing low free gossypol (Elangovan *et al*., 2003;Mandal *et al*., 2004) did not exert depression of growth and feed intake. In 28 and 8-day toxicity trials, 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.

Carcass characteristics

Various carcass characteristic parameters viz. feather loss, blood loss, yields of giblet, gizzard, heart and liver did not differ due to the dietary treatments (Table 4) indicating that either the presence of Bt gene or gossypol content in CSM types had no effect on these carcass traits of broiler chickens. The major cut up parts (back, breast, drum sticks, neck, thigh and wings) of carcass samples of broilers also did not differ due to treatments (Table5) except for drum stick weight which was significantly higher ($P<0.05$) in control diet than comm. CSM type diet and thigh weight being higher ($P<0.01$) in non Bt and comm. CSM type groups than control and Bt CSM type groups. In the earlier work (Elangovan, 2003), carcass characteristics in terms of dressing percentage and giblets yield were not significantly ($P>0.05$) different due to either BT, non-BT, commercial cottonseed meal or control SBM diet, similarly the yield of different organs when expressed on unit live weight also did not differ significantly ($P>0.05$). 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.

Development of digestive and immune organs

As shown in Table 6, the relative weights (% live weight) of proventriculous, brusa, spleen, thymus and that of abdominal fat were not affected by various dietary treatments. Contrary to these findings, the yield of immune organs such as bursa was significantly higher for BGII CSM group and thymus yield was significantly lower ($P<0.01$) in control and comm. CSM fed group (Elangovan *et al.*, 2003).

Therefore, in the present experiment, as all the dietary treatments were nutritionally balanced properly to meet the requirements of growing broilers, it is envisaged that either the gossypol content or transgenic gene/protein in Bt CSM types had any deleterious effect on growth performance, mortality and various carcass characteristics of growing broiler chickens. This indicates that Bt cottonseed meal is as safe and nutritious to chickens as commercial cotton with low free gossypol.

Conclusion

The study envisaged that transgenic Bt cottonseed meal (solvent extracted) containing low free gossypol could safely be included up to 10% level in maize- soybean meal based diet up to 0-7 weeks of age of broiler chicken without adversely affecting their growth performance and carcass traits.

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Table 1.Ingredient and nutritient composition (%) of starter and finisher (4-7 weeks) diets

	Starter (0-4 weeks) diets				Finisher (4-7 weeks) diets			
	D_1	D_2	D_3	D_4	D_1	D_2	D_3	D_4
	Control	Bt	Non-Bt	Comm.	Control	Bt	Non-Bt	Comm.
Ingredient composition								
Maize,yellow	61.2	58.52	57	57.5	66.32	63.93	62.93	63.23
Rice bran,deoiled	1	0	0	0	1.3			
Soybean meal	34.5	27.2	28.46	28.05	29.1	21.7	22.7	22.4
Bt CSM		10				10		
Non-Bt CSM			10				10	
Commercial CSM				10				10
Tallow	0	0.9	1.2	1.1	0	1	1	1
Limestone	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
DCP	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Trace mineral premix*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0	0.07	0.03	0.04	0	0.07	0.07	0.07
Methionine	0.1	0.11	0.11	0.11	0.08	0.09	0.09	0.09
Total	100	100	100	100	100	100	100	100
Nutrient composition								
ME,kcal/kg	2901	2897	2901	2899	2949	2955	2945	2948
CP	23.0	23.1	23.1	23.1	21.0	20.98	20.99	21.01
Lysine	1.29	1.20	1.21	1.20	1.13	1.04	1.07	1.06
Methionine	0.49	0.49	0.49	0.49	0.45	0.45	0.45	0.45
Thrionine	0.95	0.87	0.89	0.88	0.86	0.78	0.79	0.79
Cystine	0.43	0.36	0.37	0.36	0.39	0.32	0.33	0.33
Arganine	0.46	0.41	0.41	0.41	0.46	0.41	0.41	0.41
Total Ca	1.03	1.00	1.00	1.00	1.01	0.98	0.99	0.98
Available P	0.49	0.46	0.46	0.46	0.47	0.44	0.44	0.44

**Trace mineral premix supplied mg / kg diet:Mg, 300; Mn,55; I, 0.4; Fe, 56; Zn, 30; Cu, 4.*

***Vitamin premix supplied per kg diet :Vit.A, 8250 IU; Vit.D_3,, 1200 ICU; Vit.K, 1mg; Vit.E, 40 IU; Vit. B_1 ,2mg; Vit. B_2 4mg; Vit.B_{12} ,10mcg; niacin, 60mg; pantothenic acid, 10mg; choline, 500mg.*

Table 2. Crude protein and gossypol contents (% on dry weight basis) in CSM types

CSM type	Protein		Gossypol, total		Gossypol, free	
	Seed	Meal	Seed	Meal	Seed	Meal
Bt (JKC-738)	26.0	36.9	3.27	1.25	1.13	0.07
Non Bt	27.2	33.3	3.87	1.25	1.35	0.04
Commercial (NH-44)	24.6	34.7	2.66	0.56	0.78	0.04

Table 3. Growth performance of broiler chicks (0-7 weeks)

Dietary treatment	Body weight gain (g/b)			Feed intake (g/b)			FCR		
	0-4wk	4-7wk	0-7wk	0-4wk	4-7wk	0-7wk	0-4wk	4-7wk	0-7wk
Control	790.8	990.3	1783.7	1217.3	2209.3a	3426.6	1.54	2.23ab	1.92
Bt CSM	764.6	952.1	1713.1	1220.2	2018.4b	3238.6	1.59	2.12b	1.89
Non Bt CSM	781.8	948.8	1738.9	1187.8	2299.8a	3487.7	1.52	2.44a	2.01
Comm.CSM	807.9	987.6	1798.4	1262.1	2339.5a	3601.6	1.57	2.38a	2.01
SEM	8.18	16.28	22.21	16.59	41.10	51.60	0.018	0.046	0.026
Stat.	NS	NS	NS	NS	P<0.01	NS	NS	P<0.05	NS

Fig 1. Live weight gain (g/b)

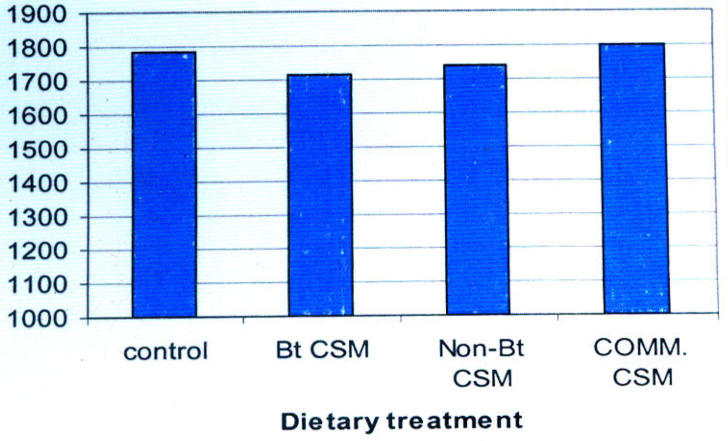

Fig 2. Feed intake (g/b)

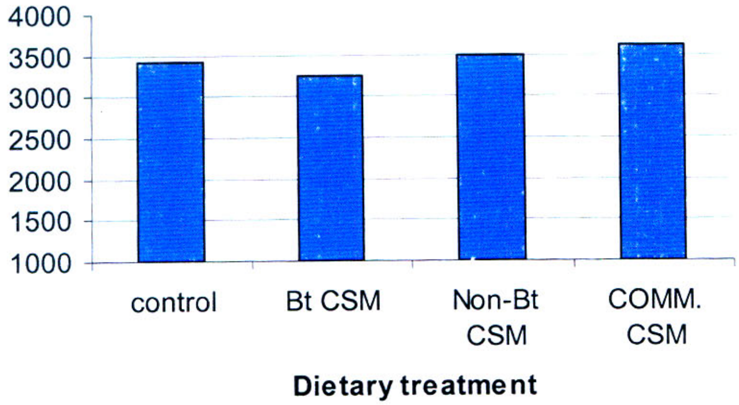

Fig 3. Feed : gain

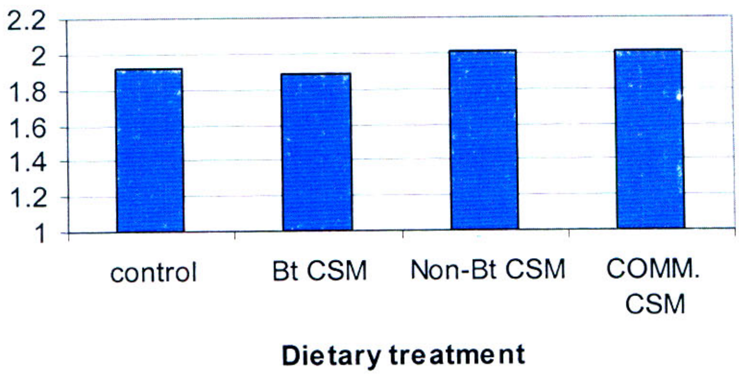

Table 4. Carcass traits (% of live weight) of broilers

Treatment	Feather + blood loss	Blood loss	Eviscerated yield	Giblet	Gizzard	Heart	Liver
Control	11.26	4.32	70.67	4.20	1.90	0.411	1.89
Bt CSM	10.74	3.74	71.75	4.40	2.15	0.440	1.80
Non-Bt CSM	10.61	3.89	72.03	4.45	2.09	0.451	1.91
Comm. CSM	10.95	3.70	71.79	4.26	1.81	0.503	1.93
SEm	0.20	0.174	0.236	0.081	0.066	0.013	0.033
Stat.	NS	NS	NS	NS	NS	NS	NS

Table 5. Cut up parts (% of live weight) of broilers

Treatment	Back	Breast	Drum stick	Neck	Thigh	Wings
Control	15.24	35.32	10.82^{a}	4.40	10.52^{b}	8.85
Bt CSM	15.29	16.32	10.49^{ab}	4.74	10.50^{b}	9.24
Non-Bt CSM	15.78	16.72	10.44^{ab}	4.50	11.35^{a}	9.46
Comm. CSM	14.90	16.90	9.71^{b}	4.15	11.98^{a}	9.27
SEm	0.180	4.95	0.147	0.101	0.168	8.63
Stat.	NS	NS	$P<0.05$	NS	$P<0.01$	NS

Table 6. Digestive and immune organs (% of live weight) of broilers

Treatment	Abdominal fat	Proventriculus	Bursa	Spleen	Thymus
Control	0.697	0.411	0.213	0.171	0.196
Bt CSM	0.947	0.368	0.199	0.164	0.208
Non-Bt CSM	0.823	0.434	0.190	0.130	0.208
Comm. CSM	0.897	0.404	0.211	0.177	0.205
SEm	0.069	0.016	0.0098	0.0092	0.0098
Stat.	NS	NS	NS	NS	NS

Feeding Trial in Progress

Measurement of Body Weight