

## **EVALUATION OF THREE TRANSGENIC CORN HYBRIDS (BT CORN) IN BROILER GROWTH TRIAL**

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### **ABSTRACT**

The aim of this study was to evaluate the effect of including three transgenic corn hybrids in broiler diets with the corresponding isogenic corn diets on broiler performance, blood characteristics and histological status for both liver and kidney. Two hundred and fifty two chicks (Ross 308) were fed for 35 days with diets containing Bt or isogenic corn. Both Bt and isogenic corn were analyzed for chemical composition and total aflatoxin contents. The analysis has shown that the amino acid balance for the tested corn was relatively similar to their crude protein content. The diets were formulated to be iso-caloric and iso-nitrogenous and covering the chick requirements as recommended by the management guide data (Ross). The different in chemical composition between corn used in this study is considered as a natural variation. Also, the results showed that both Bt and isogenic corn were free from aflatoxins. The results of the growth study showed no differences in performance parameters. No differences were detected in blood characteristics or histological structure of both kidneys and liver between groups fed the three transgenic corn and their corresponding isogenic corn. Accordingly, it is clear that the transgenic corn had no deleterious or unintended effects on production traits of broiler chickens in this study.

**Keywords:** Bt corn, broiler, growth trial

### **INTRODUCTION**

Agriculture biotechnology has produced a number of new varieties of crop plants with enhanced features, such as protection against common pests, tolerance to herbicides, and improved quality traits. Between 1996 and 2004 the area planted with a genetically modified (GM) crops increased from 2 to 80 million ha (James, 2005). In 2010, the total area cultivated with GM increased to 148 million ha (Glandorf, 2012).

Before commercialization, the produced transgenic crop must undergo estimation of many food safety parameters. The coarse framework of "Substantial Equivalence" is used to compare transgenic crops of the first generation with their isogenics' counterparts (OECD, 1993). Scientific bodies and expert panels proposed guidelines for nutritional and safety assessment of feeds (and food) from GM (EFSA, 2004; ILSI, 2003, 2004; OECD, 2003), these includes assessing whether the key nutrients or anti-nutrients in the plant components used for feed or food have been changed, and whether these modification had affected both performance and physiological status of farm animals.

One of the crops that have been introduced to gene modification is corn. This modification includes protection against common pests, tolerance to herbicides and improved quality traits.

Corn is the largest component of broiler feeding regimens that were designed to provide maximal body weight gain in a short time.

Bt30K08, Bt30K09 and Bt30K84 are three hybrids of Bt corn that were originated to produce a new generation of corn that has self protection against insect damage.

Although the transgenic protein is present in corn grain in part per billion (Brake *et al.*, 2003) it was of interest to determine whether transgenic corn grains had any unexpected effects on poultry, either as a direct effect of a transgenic protein in the diet or as a result of any unintended compositional changes in the grain that may have altered its nutritional value.

The purpose of this study was to evaluate whether feed comprised of transgenic grain derived from Bt30K08, Bt30K09 and Bt30K84 had any adverse effects on broiler chickens performance when compared with feed comprised of grain derived from a nontransgenic isolate (30K08, 30K09 and 30K84).

## **MATERIALS AND METHODS**

### **Corn**

Both transgenic and nontransgenic isolate corn was brought from Pioneer Company. The presences of gene modification in transgenic corn were confirmed by PCR.

### **Experimental system and chicks**

Two hundred and fifty two chicks (Ross 308) one-day-old were brought from a commercial farm (El- Wadee). The trial was carried out at a poultry house in Nubaria, Regional Center for Food and Feed, Alexandria, Egypt. The chicks were randomly divided into six groups each group composed of three replicates and each replicate contained 14 chicks per pen. The trial was conducted under controlled lighting period (24hrs.).

### **Corn Analyses**

Corn samples were taken from both transgenic and nontransgenic isolate for proximate analyses, determination of potassium, magnesium, calcium, phosphorus, fatty acids and amino acids contents. Determination was done following the analytical procedures in the AOAC (2005). Vitamins B1 and B2 were determined according to NMKL 1996- method no 189.2 while vitamin E was determined according to Leth and Sondergaro (1983). Total Aflatoxin screening was done according to AOAC (2005). The chemical evaluations of test materials are shown in Table 1.

### **Diet Formulation**

Based on the initial proximate analyses (Table 1), different corn was assumed to have the same nutritive value. To compensate for the difference in percentage of crude protein between corn sources, nonnutritive filler (silica) was added.

The experimental diets were formulated to cover the chick requirements as recommended by the management guide data (Ross). The diets were formulated to be isonitrogenous and isocaloric. All diets were fortified with complete vitamin and mineral mixes. Composition of diets for

starter (0-14 d), grower (15-28 d) and finisher periods (29-35d) are shown in Tables 2, 3 and 4 respectively.

Feed and water were provided *ad-libitum* throughout the whole experimental period.

The parameters body weight, body weight gain, feed intake and feed conversion ratio were examined to reveal the effect of including nontransgenic and transgenic corn on growth performance. These parameters being measured at age 14, 28 and 35 days.

**Table (1): Chemical evaluation for both transgenic and non transgenic corn. (as fed basis).**

Analyses	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
<b>Proximate analyses %</b>						
Ash	0.90	0.90	0.70	0.80	0.80	0.90
Crude fiber	7.00	7.10	7.20	7.00	7.00	6.90
Protein	8.24	8.73	8.12	8.70	8.35	9.06
<b>Mycotoxin analyses</b>						
Total Aflatoxin	ND <sup>1</sup>	ND	ND	ND	ND	ND
<b>Amino acids%</b>						
ASP	0.60	0.67	0.55	0.67	0.66	0.71
THR	0.27	0.30	0.27	0.32	0.30	0.33
SER	0.43	0.45	0.37	0.43	0.43	0.46
GLU	1.68	1.72	1.54	1.66	1.65	1.81
PRO	0.75	0.81	0.77	0.82	0.75	0.81
GLY	0.29	0.33	0.29	0.36	0.33	0.34
ALA	0.66	0.69	0.59	0.62	0.62	0.67
VAL	0.35	0.38	0.37	0.42	0.38	0.40
ISO	0.23	0.24	0.23	0.25	0.24	0.26
LEU	1.09	1.09	0.99	1.00	1.01	1.11
TYR	0.37	0.39	0.37	0.39	0.35	0.39
PHE	0.40	0.42	0.37	0.39	0.39	0.43
HIS	0.20	0.21	0.22	0.26	0.23	0.25
LYS	0.19	0.24	0.20	0.27	0.26	0.28
ARG	0.37	0.42	0.35	0.44	0.41	0.43
CYS	0.19	0.18	0.15	0.20	0.18	0.21
MET	0.17	0.19	0.19	0.20	0.16	0.17
<b>Fatty acids%</b>						
Palmitic	12.10	12.50	12.60	12.40	12.60	12.40
Stearic	2.70	2.60	2.50	2.70	2.70	2.80
Oleic	36.00	35.50	38.00	39.10	37.80	37.20
Linoleic	46.50	47.00	45.00	44.40	45.10	45.70
Linolenic	0.60	0.50	0.60	0.60	0.70	0.70
Arachidic	0.50	0.50	0.40	0.40	0.50	0.50
<b>Minerals %</b>						
K	0.34	0.35	0.37	0.33	0.34	0.35
Mg	0.14	0.16	0.15	0.14	0.14	0.15
Ca	0.04	0.04	0.03	0.04	0.04	0.05
P	0.21	0.21	0.23	0.24	0.23	0.23
<b>Vitamins (mg/100g)</b>						
B1	0.004	0.005	0.005	0.004	0.005	0.005
B2	0.0004	0.0005	0.0004	0.0004	0.0004	0.0004
E	0.420	0.460	0.450	0.430	0.450	0.490

<sup>1</sup> ND: not detected.

At 35 days of age, 12 birds from each treatment were randomly selected to study the effect of both non transgenic and transgenic corn on

blood parameters. Where, three ml blood were obtained from the wing vein using a 3 ml syringe and 23 gauge needle. The blood were transferred immediately into sterile tubes containing Heparin and centrifuged at 3000 rpm for 15min. to separate the plasma. The plasma was collected and kept in a deep freezer for subsequent chemical analysis.

Serum uric acid, creatinine, AST and ALT were determined using DiaSys FS Kits and protocol.

At the end of the fifth weeks of age, ten random birds from each group were slaughtered, immediately after slaughtering both kidneys and liver were collected and stored in 10% formalin solution for morphological measurements.

**Statistical analysis**

The obtained data were subjected to a one way analysis of variance using the linear model (GLM) of SAS (SAS institute, 1991). Means were compared using Duncan's new multiple range test ( $P < 0.05$ ) (Duncan, 1955).

**Table (2): Composition of broiler starter diets (0-14 d) with different levels of both transgenic and non transgenic corn. (as-fed basis).**

Ingredients (%) and compositions	Starter					
	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
	%					
Ground yellow corn	60.495	61.195	60.495	61.195	60.695	61.495
Soybean meal (48%)	28.000	28.000	28.000	28.000	28.000	28.000
Corn gluten meal	6.500	5.800	6.500	5.800	6.300	5.500
Vegetable oil	0.550	0.520	0.550	0.520	0.540	0.520
Dicalcium phosphate	2.120	2.120	2.120	2.120	2.110	2.120
Limestone	0.760	0.760	0.760	0.760	0.770	0.760
Vit. & Min mixture <sup>1</sup>	0.400	0.400	0.400	0.400	0.400	0.400
Cholinchlorid	0.075	0.075	0.075	0.075	0.075	0.075
Salt	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine HCl <sup>2</sup>	0.440	0.450	0.440	0.450	0.440	0.450
DL-Methionine <sup>2</sup>	0.260	0.280	0.260	0.280	0.270	0.280
Calculated values %						
Crude protein	23.170	23.150	23.170	23.090	23.140	23.150
Metabolizable energy (Kcal/Kg).	3029	3024	3029	3025	3028	3024
Lysine	1.400	1.400	1.400	1.400	1.400	1.400
Methionine	0.600	0.600	0.660	0.660	0.660	0.660
Methionine + Cystine	1.040	1.040	1.040	1.040	1.040	1.040
Calcium	1.000	1.000	1.000	1.000	1.000	1.000
Available Phosporus	0.500	0.500	0.500	0.500	0.500	0.500

<sup>1</sup> Vitamin-mineral mixture supplied per kg of diet: It. (A), 12000 I.U., Vit. (D<sub>3</sub>), 2000 I.U; Vit. (E), 10 mg; Vit. (K<sub>3</sub>), 2mg; Vit. (B<sub>1</sub>), 1 mg; Vit. (B<sub>2</sub>), 5 mg; Vit. (B<sub>6</sub>), 1.5 mg; Vit. (B<sub>12</sub>), 10 µg; Biotin, 50 µg; Choline chloride, 500 mg; Pantothenic acid, 10 mg; Niacin, 30 mg; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Iron, 30 mg; Copper, 10 mg; Iodine, 1 mg; Selenium, 0.1 mg and Copalt, 0.1mg.

<sup>2</sup> Lysine and Methionine were added according to management recommendation guide data (Ross).

**Table (3): Composition of broiler grower diets (15-28 d) with different levels of both transgenic and non transgenic corn. (as-fed basis).**

Ingredients (%) and compositions	Grower					
	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
Ground yellow corn	61.085	61.085	61.015	61.085	61.085	60.34
Soybean meal (48%)	26.550	25.600	26.550	25.600	26.350	25.600
Corn gluten meal	6.000	6.000	6.000	6.000	6.000	6.000
Vegetable oil	1.830	2.020	1.830	2.020	1.830	1.990
Dicalcium phosphate	1.710	1.710	1.710	1.710	1.710	1.710
Limestone	1.330	1.330	1.330	1.330	1.330	1.330
Vit. & Min mixture <sup>1</sup>	0.400	0.400	0.400	0.400	0.400	0.400
Cholinchlorid	0.075	0.075	0.075	0.075	0.075	0.075
Salt	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine HCl <sup>2</sup>	0.370	0.410	0.440	0.370	0.380	0.410
DL-Methionine <sup>2</sup>	0.250	0.260	0.250	0.250	0.250	0.260
Sand	—	0.710	—	0.760	0.190	1.485
Calculated values %						
Crude protein	22.100	22.010	22.040	21.990	22.090	22.100
Metabolizable energy (Kcal/Kg).	3104	3101	3104	3101	3100	3100
Lysine	1.300	1.300	1.300	1.300	1.300	1.300
Methionine	0.570	0.570	0.570	0.570	0.570	0.570
Methionine + Cystine	1.000	1.000	1.000	1.000	1.000	1.000
Calcium	1.000	1.000	1.000	1.000	1.000	1.000
Available Phosphorus	0.500	0.500	0.500	0.500	0.500	0.500

<sup>1</sup> Vitamin-mineral mixture supplied per kg of diet: Vit. (A), 12000 I.U., Vit. (D<sub>3</sub>), 2000 I.U; Vit. (E), 10 mg; Vit. (K3), 2mg; Vit. (B1), 1 mg; Vit. (B2), 5 mg; Vit. (B6), 1.5 mg; Vit. (B12), 10 µg; Biotin, 50 µg; Choline chloride, 500 mg; Pantothenic acid, 10 mg; Niacin, 30 mg; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Iron, 30 mg; Copper, 10 mg; Iodine, 1 mg; Selenium, 0.1 mg and Copalt, 0.1mg.

<sup>2</sup> Lysine and Methionine were added according to management recommendation guide data (Ross).

**Table (4): Composition of broiler finisher diets (29-35 d) with different levels of both transgenic and non transgenic corn. (as-fed basis).**

Ingredients (%) and compositions	Finisher					
	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
Ground yellow corn	65.000	65.000	64.995	65.500	64.500	64.000
Soybean meal (48%)	23.000	23.100	23.100	23.100	23.100	23.100
Corn gluten meal	5.000	4.500	5.000	4.500	5.000	4.200
Vegetable oil	2.995	3.040	2.910	2.875	3.100	3.545
Dicalcium phosphate	1.890	1.890	1.890	1.890	1.890	1.890
Limestone	0.680	0.680	0.680	0.680	0.680	0.680
Vit. & Min mixture <sup>1</sup>	0.400	0.400	0.400	0.400	0.400	0.400
Cholinchlorid	0.075	0.075	0.075	0.075	0.075	0.075
Salt	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine HCl <sup>2</sup>	0.320	0.330	0.310	0.330	0.310	0.330
DL-Methionine <sup>2</sup>	0.240	0.250	0.240	0.250	0.240	0.260
Sand	—	0.335	—	—	0.305	1.120
Calculated values %						
Crude protein	20.050	20.120	20.110	20.150	20.070	20.070
Metabolizable energy (Kcal/Kg).	3212	3200	3206	3203	3206	3208
Lysine	1.160	1.160	1.160	1.160	1.160	1.160
Methionine	0.510	0.510	0.510	0.510	0.510	0.510
Methionine + Cystine	0.920	0.920	0.920	0.920	0.920	0.920
Calcium	0.900	0.900	0.900	0.900	0.900	0.900
Available Phosphorus	0.450	0.450	0.450	0.450	0.450	0.450

<sup>1</sup> Vitamin-mineral mixture supplied per kg of diet: Vit. (A), 12000 I.U., Vit. (D<sub>3</sub>), 2000 I.U.; Vit. (E), 10 mg; Vit. (K<sub>3</sub>), 2mg; Vit. (B<sub>1</sub>), 1 mg; Vit. (B<sub>2</sub>), 5 mg; Vit. (B<sub>6</sub>), 1.5 mg; Vit. (B<sub>12</sub>), 10 µg; Biotin, 50 µg; Choline chloride, 500 mg; Pantothenic acid, 10 mg; Niacin, 30 mg; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Iron, 30 mg; Copper, 10 mg; Iodine, 1 mg; Selenium, 0.1 mg and Copalt, 0.1mg.

<sup>2</sup> Lysine and Methionine were added according to management recommendation guide data (Ross).

## RESULTS

### Chemical composition of transgenic and non transgenic corn hybrids.

The chemical composition (Table 1) of both transgenic and non transgenic corn showed slight variations among these samples of corn hybrids. No appreciable differences in the amino acids, fatty acids, minerals and vitamin profile were detected among the different samples of corn hybrids.

**Growth performance and mortality percentage**

The effect of corn sources on growth performance is shown in Table 5. Results revealed that there was no significant effect of corn sources on body weight, body weight gain, feed intake and feed conversion at different growth periods. No mortality occurred during the whole experimental periods.

**Blood characteristics**

The effect of corn sources on blood characteristics is shown in Table 6. No detected effect on serum uric acid, serum creatinine, AST and ALT was obtained either on transgenic or non transgenic corn diets.

**Histopathology**

Figures 1 and 2 showed the effect of feeding poultry transgenic corn and the non transgenic corn hybrids on kidney histological structure. No histological changes were recognized in kidneys structure.

Figures 3 and 4 showed the effect of feeding poultry transgenic corn and the non transgenic corn hybrids on liver histological structure. No histological changes were recognized in livers structure.

**Table (5): Effect of different corn sources on growth performance**

Item	Corn sources					
	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
<b>Starter period (1-14 days of age)</b>						
Live body weight (g)	335	339	352	360	343	348
Body weight gain (g)	292	296	309	317	300	305
Feed intake (g)	385	400	404	421	409	409
Feed conversion (Feed / gain)	1.32	1.35	1.31	1.33	1.36	1.34
<b>Grower period (15-28 days of age)</b>						
Live body weight (g)	1130	1137	1146	1161	1151	1124
Body weight gain (g)	795	798	794	801	808	776
Feed intake (g)	1315	1345	1330	1350	1370	1293
Feed conversion (Feed / gain)	1.65	1.69	1.68	1.69	1.70	1.67
<b>Finisher period (29-35 days of age)</b>						
Live body weight (g)	1579	1575	1613	1643	1638	1634
Body weight gain (g)	449	438	467	482	487	510
Feed intake (g)	839	834	865	905	933	961
Feed conversion (Feed / gain)	1.87	1.90	1.85	1.88	1.92	1.88
<b>Overall experimental period (1-35 days of age)</b>						
Live body weight	1579	1575	1613	1643	1638	1634
Body weight gain (g)	1536	1532	1570	1600	1595	1591
Feed intake (g)	2539	2579	2599	2676	2712	2663
Feed conversion (Feed / gain)	1.65	1.68	1.66	1.67	1.70	1.67

**Table (6): Effect of different corn sources on blood characteristics**

Items	Bt30K08	30K08	Bt30K09	30K09	Bt30K84	30K84
Uric acids (mg/dl)	3.9	3.7	3.5	3.5	3.6	3.5
Creatinine (mg/dl)	0.34	0.35	0.35	0.32	0.33	0.32
AST (U/L)	247	248	243	246	243	246
ALT (U/L)	31	33	34	36	34	36

## DISCUSSION

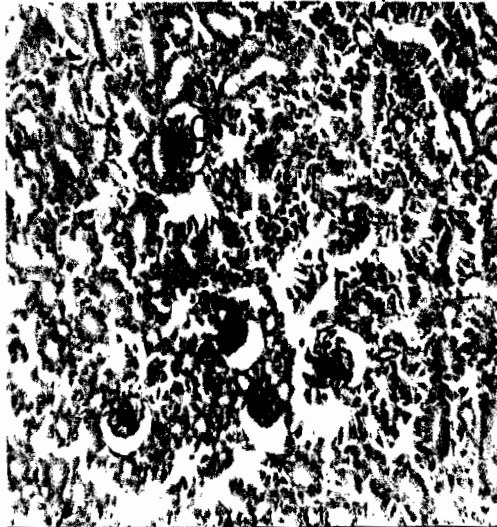
Before commercialization, the produced transgenic crop must undergo estimation of many food safety parameters. Field trials that compare between the nutritional value and effects of GM corn and that of conventional corn on the animal productive performance, is one of the ways to estimate the safety of food (EFSA, 2004; ILSI, 2003, 2004; OECD, 2003).

Piva *et al.*, (2001) reported that birds fed diets containing Bt corn were slightly heavier than birds fed the non-Bt isoline, while results from other studies indicated no impact of Bt corn for final body weight and body weight gain in broilers (Halle *et al.*, 1998; Brake and Vlachos, 1998; Mirales *et al.*, 2000; Taylor *et al.*, 2001 a, b).

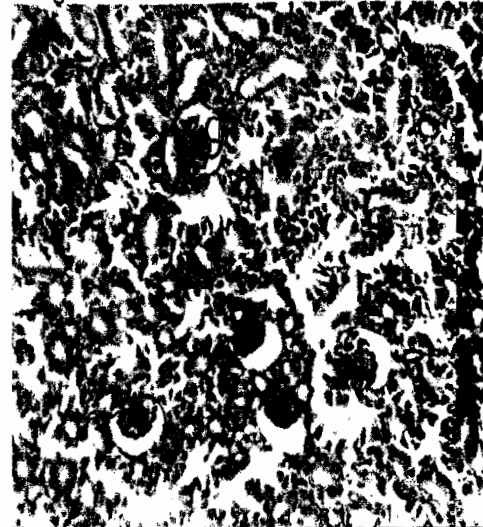
The present performance results show no statistical differences between birds fed Bt corn and the isogenic corn hybrids.

Results showed no differences in blood characteristics between birds fed diets contain Bt corn and those fed the isogenic corn. This agrees with the results of growth performance.

Feeding birds with Bt corn had no effect on the histological status of both kidney and liver compared to those fed isogenic corn.

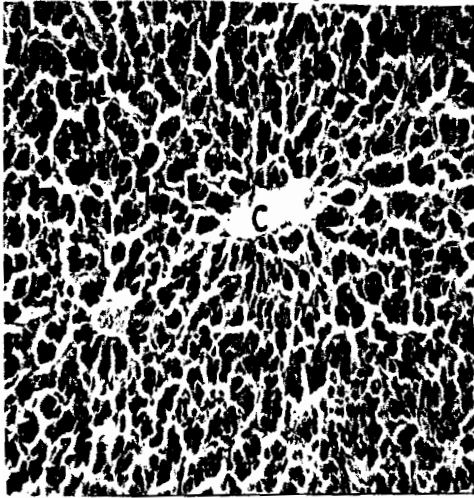


**Fig. (1)** Normal histological structure of kidneys for birds fed non-transgenic corn.

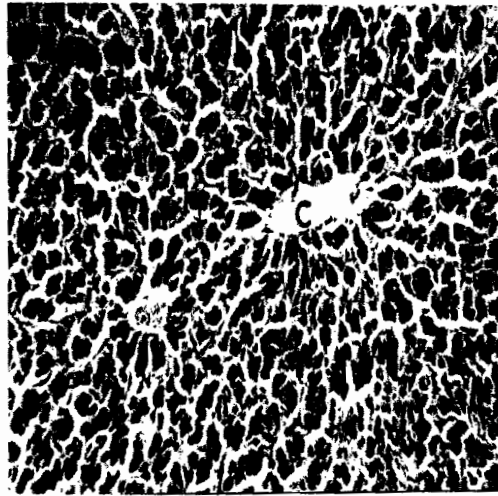


**Fig. (2)** Normal histological structure of kidney for birds fed transgenic corn.





**(3) Normal histological structure of livers  
for birds fed non-transgenic corn.**



**Fig. (4) Normal histological structure of livers  
for birds fed transgenic corn.**

### **CONCLUSION**

It could be concluded that grains from the genetically enhanced corn products evaluated in this study is nutritionally equivalent in broiler diets to grains from unenhanced corn. Yet more studies must be done on the fate of DNA retained in the tissue of animal or bird fed on diets contained transgenic compounds. This will help to conduct a safety assessment with regard to the transfer of genetic information following feed consumption and to evaluate if the use of GM plants in livestock feed presents a higher risk than conventional plant use.

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***El-Afifi, T. M. et al.***

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تقيم ثلاث سلالات من الأزره المهندسه وراثيا عن طريق تجربة نمو على نجاج التسمين

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كان الهدف من الدراسه هو تقييم التأثير الحادث على كل من أداء للنمو، صفات الدم و الحاله الهستولوجيه لكل من الكبد والكلى في طيور التسمين وذلك عند تغذيتها على ثلاث سلالات من الأزره المهندسه وراثيا ومقارنة ذلك بنفس السلالات غير المهندسه وراثيا.

غذي مئتان لثان وخمسون ككتوت (Ross 308) لفترة خمس و ثلاثون يوما على علائق إحتوت على الأزره المهندسه وراثيا وغير المهندسه. تم إجراء التحاليل المتعلقه بالتركيب الكيماوي والسموم الفطريه. حيث أظهرت التحاليل أن الأحماض الأمينية للأزره المختبره متجانسه مع محتواها من البروتين الخام. تم تركيب العلائق بحيث كانت كلها متزنه في محتواها من الطاقه والبروتين وتغطي إحتياجات الكتاكيت طبقا لدليل التربية الخاص بسلاله (Ross). يرجع الإختلاف في التركيب الكيماوي بين الأزره المستخدمه في تلك الدراسه الي إختلاف طبيعي بين الأجناس. أظهرت النتائج خلو الزره سواء المهندسه وغير المهندسه من الأفلاتوكسينات. أوضحت نتائج تجربة النمو عدم وجود فروق في مقاييس الأداء. عدم وجود فروق في صفات الدم أو التركيب الهستولوجي لكل من الكلى و الكبد بين المجاميع المغذاه على السلالات المهندسه وراثيا وما يقابلها من سلالات غير مهندسه. وعليه فمن الواضح أن الأزره المهندسه وراثيا لم يكن لها تأثير ضار أو غير مقصود على الأداء الإنتاجي لطيور التسمين.

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