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DETECTION OF GENETICALLY MODIFIED INGREDIENTS IN POULTRY MIXED DIETS IN NINEVEH PROVINCE BASED ON PCR TECHNIQUE

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ABSTRACT

Biotechnology was applied in the agricultural field to improve the growth of some plants and develop the herbicide tolerance. These derived crops, especially soybeans, were used as genetically modified ingredients in some livestock feeding, especially in poultry feed. The current study aimed to evaluate the presence of transgenic ingredients in some poultry mixed diets in Nineveh province through the detection of genetically modified regulators, including both 35S promoter and Nopaline synthase terminator, as well as the detection of Roundup Ready soybean in poultry mixed diets. Results revealed that 62% of poultry mixed diet samples were positive to the presence of Roundup Ready soybean gene and 71% of them positive to the 35S promoter gene existence, while 83.3% of imported soybean meal used to prepare poultry mixed diets were positive to the existence of Roundup Ready soybean gene indicating the addition of transgenic ingredients in rations used to feed poultry in Nineveh province. The study showed that all local soybean meal examined are free from genetically modified regulatory elements. These results demonstrated the possibility of using polymerase chain reaction technique as sensitive, reproducible and easy applicable to detect the genetic modification in poultry feeds.

Keywords: Genetically Modified Ingredients, Roundup Ready Soybean, Poultry mixed diets

INTRODUCTION

The introduction of genes into plants was started since1980 (Fraley *et al*., 1983), by the application of biotechnology to insert foreign genes into the genomes of other organisms to improve a target trait (Flachowsky *et al*., 2005). The production of genetically modified crops increased rapidly by cultivation of these yields around the world, which resulted in a high concern about the risk of these transgenic ingredients on consumer and animal health. Soybean is one of the main crops that get awareness in agriculture biotechnology (Flachowsky, 2017). The dominant transgenic crop was the plant named Roundup Ready soybean (RR), produced by Monsanto in both Brazil and Argentina, to produce plants that tolerate the herbicide glyphosate by gene introduction for 5-enolpyruvylshikimate-3-phosphate synthase from the microorganism in a plant named *Agrobacterium tumefaciens* (Berdal and Holst-Jensen, 2001; Owen and Zelaya, 2005). The Roundup Ready soybean was approved for use in food and animal feedstuffs, including poultry, as a good source of protein with high nutritional value in ration, especially the essential amino

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acids. More than 90% of the transgenic plants grown are used for feeding farm animals (Flachowsky *et al*., 2012).

Gene expression in plants can be detected using viruses derived from the plants as a pathogen through the recognition of transcriptional promoters derived from the plant viruses, especially the Cauliflower Mosaic Virus (CaMV), which is used to improve crops commercially with high tolerance to insecticide (Gatehouse, 2008; Flachowsky, 2013) as well as the *Agrobacterium tumefaciens* nopaline synthase terminator indicating the presence of genetic modification in plants. The prevalence of using genetically modified plants around the world generates a question about their safety. The possible risks associated with transgenic plants may be express antibiotic resistance and allergic effects (Bertoni and Marsan, 2005; Duke and Cerdeira, 2005).

In Ninevah province, there are various types of rations used in poultry feeding. Ingredients in these feeds are from different origins, some of them are imported, like soybean meals. Therefore, this is the first study which was conducted to detect the existence and distribution of genetically modified ingredients in poultry feeds used in Nineveh province, represented by 35S promoter gene, nos terminator gene in relation to the Roundup Ready soybean gene using PCR technique as a sensitive reliable method.

MATERIALS AND METHODS

Ethical approvement

The study was conducted according to the institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul and included an authorized ID of UM.VET. 2024.045. Mosul, Iraq.

Samples

Thirty-two poultry mixed diets and soybean meal samples were obtained from different sources in Nineveh province. Samples were collected randomly from feed manufacturing companies and poultry farms during September 2024 till January 2025. Twenty collected samples were poultry mixed diets, while twelve samples were from both imported and local soybean meal. All the samples were transported to the laboratory for further analysis.

DNA Extraction

According to the DNA extraction kit provided by (Add bio, Korea). Twenty-five mg of grounded feedstuffs were held in microcentrifuge tube and mixed with 20 µl proteinase K and 100 µl of lysis buffer by vortex and incubated at 65 ºF with inversion tubes twice to three times for 10 minutes. Centrifugation was done at 13000 rpm for five minutes. 200 µl of supernatant transferred to a new tube mixed with 200 µl of binding solution for 15 sec. Ethanol was added (200 µl) mixed by vortex for 15 seconds and inverted tubes.by spin column the solution was transferred with centrifugation at 13000 rpm for one min. After that, remove the supernatant, the DNA washed twice with washing solution 1 and 2 and repeat the centrifugation, spin tube hydrated by addition centrifugation. DNA was elated with 100 µl for one minute and the extracted DNA was stored at -20°C

Qualitative PCR

Three genes were used to screen the residence of genetically modified ingredients in the extracted DNA from animal feedstuff samples including 35S, nos and RR soybean genes, by using primers (Macrogen/Korea). The 35S gene has a molecular weight of 123 bp, the nos gene is 118 bp and the RR gene with a molecular weight of 172 bp (Table 1).

PCR Reactions

PCR assay was employed for the detection of GMO genes including the promoter 35S, terminator nos and RR genes. A total volume of 25 µl as a final product used for pcr reaction. The mixture consists of 1 µl of each pair of primers forward and reverse each 10 pmol/ µl followed by adding 12.5 µl of master mix (GeNetBio, Korea) then 2

µl of DNA template followed by adding 8.5 µl of nuclease-free water.

PCRs reactions were done by applying a program that included: first step at 95ºC for 5 minutes followed by second step (95ºC for 30 s at 35 cycles) then a third step at (60 ºC for 30 S), fourth step at (72 ºC for 45 s), ended by the fifth step at (72 ºC for 10 min). Amplicons were analyzed using gel electrophoresis (1.5% agarose gel) and DNA ladder 100 bp.

Table1: Primers sequences used to identify 35S, nos and RR genes of genetic modification in poultry mixed diets

Genes	Primers	Primer Sequence (5′– 3′)	Amplicon size [bp]	References
35S	35S -F	CCACGTCTTCAAAGCAAGTGG	123	Lipp *et al.*, 2001
	35S -R	CCTCTCCAAATGAAATGAACTTCC		
nos	nos -F	GCATGACGTTATTTATGAGATGGG	118	
	nos-R	GACACCGCGCGCGATAATTTATCC		
RR	RR-F	CAT-TCC-CGG-CGA-CAA-GTC	172	Meyer *et al.*, 1996
	RR -R	TTG-ATG-ACG-TCC-TCG-CCT-TC		

RESULTS

The distribution percentage of the genetically modified ingredients in poultry mixed diets and soybean meal samples used in feeding of poultry in Nineveh province revealed the presence of 35S promoter gene in 71% of poultry mixed diets and in 29% of imported soybean meal samples (Figure 1), whereas the screening of specific Roundup Ready soybean gene in poultry mixed diets revealed that 62% of mixed diet samples and 38% of imported soybean meal samples were positive for the existence of genetically modified elements (Figure 2), while the local soybean samples revealed negative results for genetically modified ingredients.

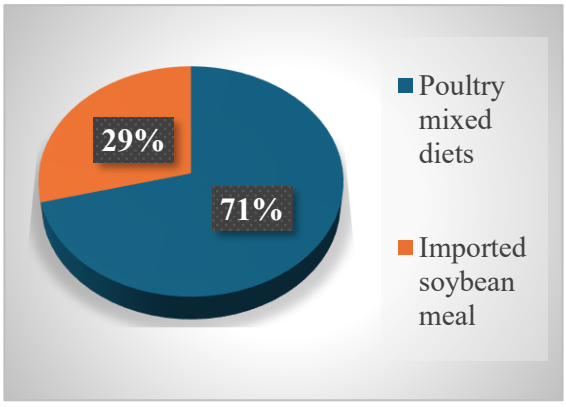

Figure 1: Distribution of 35S promoter gene in poultry mixed diets and soybean meal in Nineveh province.

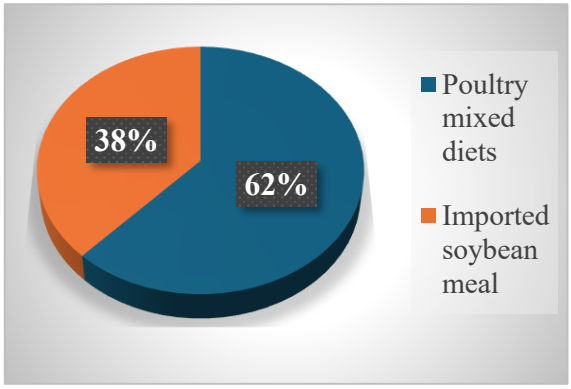

Figure 2: Distribution of Roundup Ready soybean gene in poultry mixed diets and soybean meal in Nineveh province

Out of 20 poultry mixed diet samples used in the current study, 5 (25%) and 2 (66.67%) were positive for 35S promoter gene existence in mixed feedstuffs and imported soybean meal samples respectively (Table 2). The screening of specific genetically modified ingredients represented by Roundup Ready soybean gene in feed and soybean meal samples showed that imported soybean meal 5 (83.3%) followed by poultry mixed diets 8 (40%) were positive to the recognition of RR gene (Table 2). Also, the study revealed that local soybean meal samples were negative to the presence of genetically modified elements. All study samples were negative to the presence of nos terminator gene at 118 bp using the PCR method.

Table 2: Genetically modified ingredients of poultry mixed diets and soybean meal in Nineveh province

Feeds	No.	P-35 S		T-Nos		RR	
		No.	%	No.	%	No.	%
Poultry mixed diets	20	5	25	0	0	8	40
Imported Soybean meal	6	2	66.67	0	0	5	83.3
Local Soybean meal	6	0	0	0	0	0	0
Total	32	7	21.88	0	0	13	40.63

The detection of genetically modified ingredients in poultry mixed diets using PCR technique revealed the PCR products with amplicon size 123 bp for the presence of 35 S promoter as genetically modified elements. Out of 20 samples of poultry mixed diets analyzed, five samples were positive for the 35S gene, and only eight mixed diet samples were positive for the Roundup Ready soybean gene presence with a product size of 172 bp, as shown in (Table 3). Gel electrophoresis displays the amplification of PCR products of 35S promoter gene for genetic modification in poultry mixed diets and imported soybean meal samples at 123 bp, as shown in (Figure 3) and the amplification of PCR products of RR gene in poultry mixed diets and imported soybean meal at 172 bp as shown in (Figure 4). The positive results referred to the sensitivity and reliability of PCR technique. These results confirmed the presence of some genetically modified additives in poultry mixed diet ingredients used to prepare poultry rations in Nineveh province.

Table 3: Screening of genetically modified ingredients in poultry mixed diets in Nineveh province

Poultry Mixed diets samples	P-35 S	T-Nos	RR
MD1	-	-	-
MD2	-	-	+
MD3	-	-	-
MD4	+	-	+
MD5	-	-	-
MD6	+	-	+
MD7	-	-	-
MD8	-	-	-
MD9	-	-	-
MD10	+	-	+
MD11	-	-	+
MD12	-	-	-
MD13	-	-	+
MD14	-	-	-
MD15	-	-	-
MD16	+	-	+
MD17	-	-	-
MD18	-	-	-
MD19	+	-	+
MD20	-	-	-

(+: present) (-: Absent)

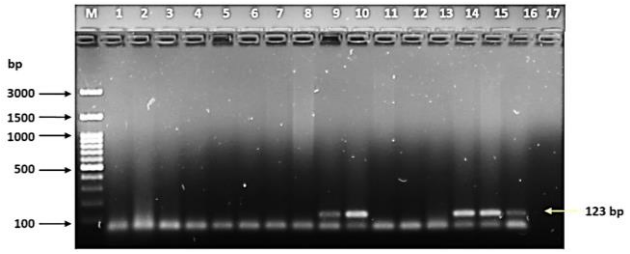

Figure 3: Gel-electrophoresis displaying the amplified product of 35S promoter gene for GM ingredients in poultry mixed diets, Lane 9-10, 14-16 positive samples with a product size 123 bp, Lane 1-8, 11-13, represents negative samples, Lane 17 represents the negative control. Lane M is the DNA marker 100 bp.

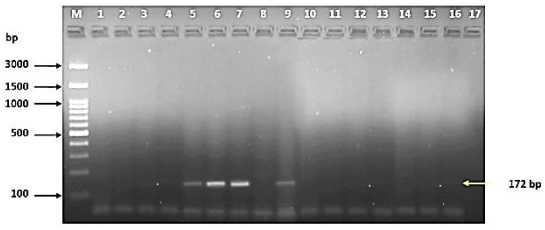

Figure 4: Gel-electrophoresis displaying the amplified product of RR soybean gene for GM ingredients in poultry mixed diets, Lane 5,6,7,9 positive samples with a product size 172 bp, Lane 1-4,5, 8,10-16 represents negative samples, Lane 17 represents the negative control. The Lane M is the DNA marker 100 bp

DISCUSSION

A variety of herbicide-tolerant crops have been added in farm animal feed, although many articles indicate the lack of adverse effects of genetically modified soybean and corn on animal well-being (Taylor *et al*., 2003; Czerwiński *et al*., 2015), the use of these crops for livestock animals is still a subject of public debate (Flachowsky, 2013), therefore concerns have been expressed to regarding their safety in human consumed products from poultry feed transgenic ingredients (Tufarelli *et al*., 2015). Previous studies reported the existence of genetic modification in some ingredients used to prepare animal feeds, including soybean meal and corn (Vijayakumar *et al*., 2009). Most of them use CaMV 35S and nos genes as indicators to detect genetic modification in plants (Fraiture *et al*., 2015; Khumnirdpetch *et al*., 2001).

Out of 20 poultry mixed diets examined in the present study, only eight samples were positive for transgenic Roundup Ready soybean gene. So, 40% of the total samples examined were positive for genetic modification, none of the local soybean meals found positive for genetic modification. These results are supported by a local study in Karbala city that the soybean was genetically modified (Al-Khafaji *et al*., 2023) and disagreement with those reported by (Siew-Ping and Yoke-Kqueen, 2011) that 70% of animal feedstuffs in Malaysia were positive for the presence of Roundup Ready soybean gene using PCR method. Also (Yoke-Kqueen, 2006) reported that 92.3% of feedstuffs were positive to the existence of transgenic soybean gene (RR). The current study deals with the detection of genetically modified elements represented by 35S promoter and nos terminator genes and revealed that 25% of poultry mixed diet samples were positive to the 35S promoter gene, whereas all feed samples showed no detection of the nos terminator gene. These data close to some extent to the results obtained by (Oraby *et al.,* 2005) who examined 24 different food samples of agriculture crops in Egypt and found three positive products for 35S promoters. These results could be contributed to that some of these ingredients are imported from countries that permit the addition of these ingredients in animal feeds, especially Brazil and Argentine based on the category of that genetically modified soybean produces traits elevating the nutritional value of poultry through reducing inhibition of trypsin in soybean and increasing protein of soybean (Stein *et al*., 2008). However, some countries argue the negative sequel, such as in Europe, is contrary to transgenic crops used in human food and animal feedstuffs (Tait, 2000; Atherton, 2002; Nawaz *et al*., 2019).

One fear is that foreign DNA could get into animal feed products consumed by humans (Turnbull *et al*., 2021). Other concerns may be the presence of antibiotic resistance genes in animal products, or may be from allergenicity of newly generated proteins (Tait, 2000; Atherton, 2002). Therefore, the presence of transgenic soybeans in animal feedstuffs is highly significant and important all over the world (Matovu and Alçiçek, 2021; Sieradzki *et al*., 2021). Our study revealed that the genetically modified ingredients in poultry feeds arising from importation of these ingredients from different sources cultivated the transgenic crops on a large scale. Therefore, there is a necessity to monitor the soybean added to poultry rations and confirm it is free from genetic modifications.

CONCLUSION

It was concluded that some poultry mixed diet samples used in Nineveh province contain some transgenic ingredients arising from imported soybean meal added as a source of protein, and the PCR technique is suitable to identify the resident of foreign DNA in plants exposed to genetic modification by biotechnology. Actually,

there is a need for further recognition to determine the level of genetically modified ingredients in poultry feeds, and monitoring is necessary to restrict the distribution of transgenic ingredients in animal feeds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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الكشف عن المواد المعدلة وراثيا في أعلاف الدواجن في محافظة نينوى بتقنية تفاعل البلمرة المتسلسل

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طبقت التقنيات الأحيائية في مجال الزراعة لتحسين نمو النباتات وتطوير المقاومة للمبيدات الحشرية، واستخدمت المحاصيل المعدلة وراثيا وخاصة الصويا كمواد معدلة وراثيا في تغذية بعض الحيوانات الحقلية وخاصة أعلاف الدواجن .هدفت الدراسة الحالية إلى تقييم وجود المواد المعدلة وراثيا في بعض علائق الدواجن في محافظة نينوى من خلال الكشف عن ادلة التعديل الوراثي والمتضمنة كل من جينات 35S المحفز و Nopaline synthase الناهي بالإضافة إلى التحري عن وجود جين الصويا المعدلة وراثيا في بعض خلطات أعلاف الدواجن .أظهرت النتائج ان ٦٢٪ من خلطات أعلاف الدواجن موجبة لاحتوائها على جين الصويا المعدلة وراثيا وان ٧١٪ منها موجبة لتواجد جين دليل التعديل الوراثي 35S المحفز ، بينما كانت ٨٣،٣٪ من الصويا المستوردة والمستخدمة في خلطات أعلاف الدواجن موجبة لتواجد جين الصويا المعدلة وراثيا مما يؤكد إضافة المواد المعدلة وراثيا في علائق الدواجن في محافظة نينوى. وأوضحت الدراسة ان الصويا المحلية خالية من جينات عناصر التعديل الوراثي، وتشير هذه النتائج إلى امكانية اعتماد تقنية تفاعل البلمرة المتسلسل كطريقة حساسة ومعتمدة وسهلة للكشف عن التعديل الوراثي في أعلاف الحيوانات