Digestive fate of transgenic DNA and protein in livestock tissues fed genetically modified feed ingredients: A review

^{*1,2}Salisu, I. B., ¹Amin, A. B., ²Ali, Q., ¹Tijjani, A. and ¹Ibrahim, A. A.

¹Department of Animal Science, Faculty of Agriculture, Federal University Dutse, PMB 7156 Dutse, Jigawa State-Nigeria

²Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, 87-West Canal Bank Road Lahore-53700, Pakistan

Corresponding author: salisu.ib@fud.edu.ng; +2347066006080, or +923174632425,

Abstract

The digestive fate of ingested transgenic DNA and/or proteins in livestock fed a diet containing genetically modified (GM) feed is at present the topic of scientific discussion. A number of studies have investigated the possible transfer and accumulation of novel DNA and/or proteins from transgenic plants in tissues samples of various livestock species, but the data reported are sometimes controversial. Therefore, this study aimed to evaluate the potential transfer and accumulation of transgenic DNA/ proteins from transgenic feed ingredients to livestock through reviewing scientific publications on experimental studies in large and small ruminants, monogastric and fish. Most of the studied experiments were conducted in Europe, and in majority these experiments, transgenic maize or soybean, or both, expressing insect resistant or herbicide tolerant or combination of both, were fed to the experimental animals. More than 50% of the experimental studies evaluated, reported the detection of transgenic DNA/protein from a number of analyzed samples of various livestock species. Transgene detection either in form of DNA or protein was more prevalence in the experimental studies when monogastric animals or fish were used as the experimental animals. Although, these transgenes were also detected in the tissues, organs and products of large and small ruminants, yet the detection frequency was less when compare with that in non-ruminants and fish. Thus overall, findings from this study indicated that transgenic DNA is not completely degraded during feed processing and digestion and that DNA fragments could be detected by well-established detection methods such as PCR or qPCR in a number of organs and tissues of livestock animals.

Keywords; Genetically modified (GM) feed, Transgenic DNA/protein, DNA transfer, Digestibility, Polymerase chain reaction (PCR)

Introduction

In recent years, the biotechnological advances, based on recombinant DNA technology, have allowed the production of a great deal of novel crop varieties, popularly called genetically modified (GM) crops or transgenic crops. Over the past few years, the adoption of these GM crops for human and animal consumption is increasing frequently. Various animal feeding studies, with transgenic crops were conducted so far and based on outcomes of these studies, a substantial equivalence

between the present GM crops and conventional varieties was established as majority of these experimental studied did not reveal any substantial changes in feed digestibility, performance, or health in animals fed GM feeds compared with animals fed non-GM feeds. Scientist have also evaluated the digestibility and possible transfer of the transgenic DNA (tDNA) from transgenic crops to livestock tissues and their products intended for human consumption, and no traces of tDNA have been identified in any of the accessed tissue

or products of these animals consumed GM feeds. Conversely, some studies have Shockingly reported the trace of very small DNA fragments from transgenic crops in tissues from pigs, sheep, goats and fish. These contradictory findings have resulted to considerable public debate concerning the safe use of GM crops in livestock feeding. In order to update our knowledge on this topic, this review aimed to evaluate a number of published experimental studies in refereed scientific journals that focused on fate of recombinant DNA/protein digestibility and its potential transfer to livestock tissues and livestock derived products consumed by humans. The digestive fate of tDNA/protein and its possible transfer to livestock and their product was and summarized and highlighted.

Material and methods

A structured literature searches in Scopus ([http:// www.scopus.com/home.url](http://www.scopus.com/home.url)) and Web of Science ([http:// webofscience.com/](http://webofscience.com/)) were performed using five different search strings; Assessing the possibility of GM DNA transfer from GM feed to livestock, Carry-over of DNA from GM feed to livestock, Detection of recombinant plant / endogenous DNA in GM fed animals, Digestibility of transgenic DNA in livestock tissues fed GMO-based diet, Fate of transgenic DNA in animal tissues. Publications relevant to our “search string” were selected based on abstract screening. Other related peer-reviewed papers from the reference list in the selected publications were also considered. Publications studies involved non-livestock species (e.g. rodents and other wild animals) were overlooked. However, any study which involves the use of fish as a model was as well included. Besides, publications which are only written in English were used in this review. For

evaluating the digestibility and the possible transfer of transgenic DNA/protein to livestock tissues, experimental studies which used control groups were only selected. A number of the published experimental studies that assessed the effect of GM feeds on health parameters (e.g. haematology, serum chemistry, histopathology and immunology) and performance parameters (e.g. average daily weight gain, body weight, feed conversion), or reproductive traits (e.g. litter size, birth weight) were come across, but we only focused on those studies which were more specific to transgenic DNA/protein digestibility and its potential transfer to livestock tissues.

Results

A total of 48 scientific publications from the period of 2001 to 2016, which reported the studies that focused on the potential transfer of transgenic DNA/proteins from GM feeds to livestock tissues were evaluated. The overview of the experimental studies that were described in these 48 publications is presented in Tables 1 and 2. Any publication in which the authors reported the use of an animal species and their offspring as experimental animals e.g. goats and their offspring, sow and their offspring or does and their litters, were regarded as one experimental groups. Similarly, animals of multigenerational studies were as well considered as one experimental group.

Figure 1 shows the distribution of the most common GM crops used in the experimental studies. Although, various GM crops have been reported to be used as additional feed ingredients in livestock diet, in this review, it was found that GM maize or GM soybean were mostly fed to the experimental animals than any other GM crops. Other GM crops such as GM cotton, GM canola and GM rape seeds were also used in some of the experimental studies,

Table 1. Fate of transgenic DNA/protein in the tissues of large and small ruminants fed GM feed

GM-feed material	Transgene present	Animal species	Tissues/sample assayed	Transgene detection	Detection method	References
GM maize & soybean	<i>Bt & herbicide tolerant genes</i>	Dairy cow	Milk	No	qPCR	(De Giacomo <i>et al.</i> , 2016)
GM maize & soybean	<i>Bt & herbicide tolerant genes</i>	Dairy cow	Milk, rumen	No	PCR	(Furgał-Dierżuk <i>et al.</i> , 2015)
GM maize & soybean	<i>Bt & herbicide tolerant genes</i>	Calf	Blood, liver, spleen, kidney, pancreas, muscle, stomach, duodenum, jejunum, colon, excreta	Yes, in stomach	PCR	(Sieradzki <i>et al.</i> , 2013)
GM maize	<i>Cry1Ab</i>	Dairy cow	blood, feces, milk, urine	Yes, in feces	ELISA	(Paul <i>et al.</i> ., 2010)
GM maize	<i>Bt gene</i>	Dairy cow	Blood	No	qPCR	(Bertheau <i>et al.</i> , 2009)
GM maize	<i>Cry1Ab</i>	Dairy cow	Blood, milk, feces, urine	NO	ELISA	(Gürtler <i>et al.</i> ., 2009)
GM maize	<i>Cry1Ab, mepsps</i>	Dairy cow	Milk	No	PCR	(Calsamiglia <i>et al.</i> , 2007)
MG maize and soybean	<i>Bt & herbicide tolerant genes</i>	Dairy cow	Milk	Yes	PCR	(Agodi <i>et al.</i> ., 2006)
GM maize	<i>Glufosinate resistant gene</i>	Dairy cow	Milk	No	PCR & ELISA	(Phipps <i>et al.</i> ., 2005)
GM cotton	<i>cry1Ac, cry2Ab & cp4 epsps</i>	Dairy cow	Milk	No	PCR & southern blot	(Castillo <i>et al.</i> ., 2004)
GM maize and soybean	<i>cry1a[b] cp4 epsps</i>	Beef & dairy cow	blood, feces, intestinal and rumen content, milk	Yes, in all samples	PCR	(Phipps <i>et al.</i> ., 2003)
GM soybean	<i>cp4 epsps</i>	Dairy cow	Mil	NO	PCR	(Phipps <i>et al.</i> ., 2002)
GM maize	<i>CryIA(b)]</i>	Beef & dairy cow	blood, feces, intestinal content, milk, muscle, liver & spleen	Yes	PCR	(Einspanier <i>et al.</i> , 2001)
GM maize	<i>Bt gene</i>	Sheep	GIT, liver, lymph nodes & rumen fluid	NO	PCR	(Trabalza-Marinucci <i>et al.</i> , 2008)
GM rapeseed	<i>cp4 epsps</i>	Sheep	digesta, faeces & blood	No	PCR & qPCR	(Alexander <i>et al.</i> , 2006)
GM canola	<i>cp4 epsps</i>	Sheep	GIT and GIT content, kidney, liver, spleen	Yes, in GIT and GIT content	PCR & southern blot	(Sharma <i>et al.</i> ., 2006)
GM maize	<i>CryIA(b)</i>	Sheep	feces, oral cavity, rumen fluid	Yes, in rumen fluid	PCR	(Duggan <i>et al.</i> ., 2003)
GM soybean	<i>cp4 epsps</i>	Goat	Blood, liver, kidney, skeletal muscle, spleen, heart & milk	Yes, in blood & milk	PCR	(Tudisco <i>et al.</i> ., 2010b)
GM maize		Goat	Milk	NO	PCR & qPCR	(Rizzi <i>et al.</i> ., 2008)

PCR=Polymerase chain reaction, qPCR=Real-time polymerase chain reaction, ELISA= Enzyme link immunosorbent assay

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Table 2. Fate of transgenic DNA/protein in the tissues on non-ruminants/fish fed GM feed

GM-feed material	Transgene present	Animal species	Tissues/sample assayed	Transgene detection	Detection method	References
GM maize and soybean	<i>Bt</i> & <i>herbicide tolerant genes</i>	Broiler	Blood, liver, spleen, breast muscle, gizzard, duodenum, Jejunum, ileum, caecum, cloaca & excreta	Yes, in gizzard & duodenum	PCR	(Sieradzki <i>et al.</i> , 2013)
GM maize and soybean	<i>Cry 1A(b)</i> & <i>cp4 epsps</i>	Broiler	Gizzard, duodenum, jejunum, ileum, caecum, blood, liver, spleen, breast & muscle	Yes, in Gizzard	PCR	(Świątkiewicz <i>et al.</i> , 2010)
MG maize and soybean	<i>Cry 1A(b)</i> & <i>cp4 epsps</i>	Broiler	Kidney & Liver	Yes, in liver	Multiplex – PCR	(Řehout <i>et al.</i> ., 2008b)
GM soya	<i>Herbicide tolerant gene</i>	Broiler	Blood	Yes	PCR	(Řehout <i>et al.</i> ., 2008a)
GM maize	<i>Bt gene</i>	Broiler	Muscle	No	PCR	(Nemeth <i>et al.</i> ., 2004)
GM maize	<i>cry1Ab gene</i>	Broiler	kidney, liver, muscle, spleen	No	PCR & southern blot	(Jennings <i>et al.</i> ., 2003)
GM maize	<i>cry9C</i>	Broiler	Blood, liver, muscle	No	PCR & ELISA	(YONEMOCHI <i>et al.</i> , 2002)
GM corn	<i>phyA2 gene</i>	Laying hens	Blood, heart, gizzard liver, spleen, kidney, breast muscle, & eggs	Yes in gizzard	PCR & western blot	(Ma <i>et al.</i> , 2013)
GM maize and soybean	<i>Bt</i> & <i>herbicide tolerant genes</i>	Laying hens	Blood, liver, spleen, breast muscle, gizzard, duodenum, Jejunum, ileum, caecum, cloaca , excreta & eggs	Yes, in gizzard & duodenum	PCR	(Sieradzki <i>et al.</i> , 2013)
GM maize and soybean	<i>Bt</i> & <i>herbicide tolerant genes</i>	Pig	Blood, liver, spleen, muscle, stomach, duodenum, jejunum & excreta	Yes, in stomach, duodenum	PCR	(Sieradzki <i>et al.</i> , 2013)
GM maize and soybean	<i>Bt</i> & <i>herbicide tolerant genes</i>	Pig	Blood	No	PCR	(Świątkiewicz <i>et al.</i> , 2013)
GM maize	<i>cry1Ab</i>	Pig	Blood, GIT content, heart, kidney, liver, muscle & spleen	Yes, in GIT	PCR	(Walsh <i>et al.</i> ., 2012)
GM maize	<i>Cry1Ab</i>	Pig	Blood & feces	No	PCR	(Buzoianu <i>et al.</i> , 2012)
GM maize	<i>cry1Ab</i>	Pig	Blood, GIT content, heart, kidney, liver, muscle, & spleen	Yes in GIT	PCR & ELISA	(Walsh <i>et al.</i> ., 2011)
GM maize and soybean	<i>Bt</i> & <i>herbicide tolerant genes</i>	Pig	stomach, duodenum, ileum, caecum colon, blood, liver, spleen, lung & muscle	Yes, in stomach & duodenum	PCR	(Świątkiewicz <i>et al.</i> , 2011)
GM maize	<i>cry9C</i>	Pig	Blood, liver & muscle	NO	PCR & ELISA	(Yonemochi <i>et al.</i> , 2010)

GM canola	<i>cp4 epsps</i>	Pig	GIT content, and GIT tissues kidney, liver & spleen	Yes, in the whole GIT content & GIT tissues	PCR & southern blot	(Sharma <i>et al.</i> , 2006)
GM maize	Cry1A(b)	Pig	blood, kidney, liver, muscle, spleen	Yes, in blood, kidney, liver & spleen	PCR & southern blot	(Mazza <i>et al.</i> , 2005)
GM maize	<i>Bt gene</i>	Pig	Muscle	No	PCR	(Nemeth <i>et al.</i> , 2004)
GM maize	Cry1A	Pig	blood, GIT plus content, liver, muscle, lymph nodes, & spleen	NO	PCR	(Klotz <i>et al.</i> , 2002)
GM maize	Cry1Ab	Pig	Muscle	No	PCR, southern blot & ELISA	(Weber and Richert, 2001)
GM soybean	<i>Herbicide tolerant genes</i>	Rabbits	Blood, liver, kidney, heart, stomach, jejunum, muscle & adipose tissue	Yes, in stomach digesta & feces	qPCR	(Morera <i>et al.</i> , 2016)
GM soybean	<i>Herbicide tolerant genes</i>	Rabbits	Blood, liver, kidney, heart & muscle	NO	PCR	(Tudisco <i>et al.</i> , 2006)
GM soybean	<i>Herbicide tolerant genes</i>	Fish (salmon)	GTI	NO	qPCR/In situ hybridization	(Sanden <i>et al.</i> , 2011)
GM maize & soybean	<i>Bt & herbicide tolerant genes</i>	Fish (Zebra)	brain, GIT, liver, muscle	Yes, in GIT & liver	qPCR	(Sissener <i>et al.</i> , 2010)
GM soybean	<i>Herbicide tolerant genes</i>	Fish (trout)	Blood, brain, GI tract contents, kidney, liver, muscle & spleen	Yes, in the whole GI tract content, kidney, leucocytes, muscle	Nested PCR	(Chainark <i>et al.</i> , 2008)
GM soybean	<i>Herbicide tolerant genes</i>	Fish (trout)	Muscle	Yes, in muscle	Nested PCR	(Chainark <i>et al.</i> , 2006)
GM maize & soybean	<i>Bt & herbicide tolerant genes</i>	Fish (salmon)	Blood, GIT content, kidney & liver	Yes, in blood, GIT content, kidney & liver	PCR	(Nielsen <i>et al.</i> , 2005)
GM soybean	<i>Herbicide tolerant gene</i>	Fish (salmon)	brain, GIT content, liver & muscle	Yes, in mid and distal intestinal content, pyloric content & stomach	PCR	(Sanden <i>et al.</i> , 2004)

PCR=Polymerase chain reaction, qPCR=Real-time polymerase chain reaction, ELISA= Enzyme link immunosorbent assay

however, the percentage use of these crop was very minimal when compare to those of GM maize and soybean. Additionally, more than 95% (Tables 1 and 2) of the GM crops used in these experimental studies were engineered to possess a single or binary

insect resistant trait, herbicide tolerant trait and sometime combination of both traits. Only one study was found with GM corn expressing a trait other than insect resistant or herbicide tolerant trait (Table 2).

Figure 1. Distribution of genetically modified (GM) crops in livestock diet

From the result of these studies, it was noticed that most of the experimental studies were conducted in dairy cows and pigs and only few studies were conducted in farm animals like calves, goats, laying hens

and rabbits (Fig. 2 A). Furthermore, majority of the studies were conducted in Europe, with only few studies from Argentina, China, Japan, and the USA. Within Europe, most of the studies were done in Italy and Poland (Fig. 2 B).

Figure 2. Livestock species distribution and country wise distribution of the reviewed studies

The traceability of transgenic DNA and proteins in the organs, tissues and products of livestock animals is depicted in (Fig. 3A) below. Based on the result obtained from this study, it was found that transgenic DNA and protein could be detected in the organs, tissues or products of the experimental animals. Out of 48 studies evaluated, more than fifty percent (58.3%) of the experimental studies (Tables 1 and 2), reported the detection of transgenic DNA/proteins either in the tissues, organs or products of the experimental animals using various molecular detection technique depicted in (Fig. 3B). The possibility of transgenic DNA/protein detection was also evaluated with respect to livestock species. Transgene detection

either the inform of DNA or protein was more prevalence in the experimental studies when monogastric animals or fish were used as the experimental animals (Fig.3A). Although, these transgenes were also detected in the tissues, organs and products of large and small ruminants, yet the detection frequency was less when compare with that in non-ruminants and fish (Fig.3A). Thus, overall findings from this study indicated that dietary DNA is not completely degraded during feed processing and digestion and that DNA fragments could be detected by PCR or variant of PCR such as M-PCR, N-PCR and qPCR (Fig. 3B) in a number of organs and tissues of livestock animals.

Figure 3. Transgene detection frequency in the analyzed tissues of the experimental animals (A) and distributions of the molecular method used for the transgene detection (B).

Note; M_PCR= Multiplex-PCR, N_PCR=Nested-PCR, S. blot = Southern blot, W. blot= Western blot, Int. hybridization=In situ hybridization

Discussion

Currently, the detection and fate of transgenic DNA and/or proteins in livestock feed and their tissues has been emphasized as an essential issue in the ongoing debate over the use of transgenic crops in livestock feed as well as human food products. Livestock and humans are continuously exposed to diverse sources of exogenous DNA as part of their diet. DNA and proteins are constituents of almost all feedstuffs and foods. Scientists have reported that both human and animals have the historic ingestion of DNA and proteins of plant and animal origin, without any real evidence of related health problems. The DNA consists of genetic code and is therefore found in almost every cell in plants and animals. In addition, DNA in plants and animals is commonly comprised of the similar four basic units known as nucleotides. As such, the digestive fate of dietary sources of DNA like the naturally occurring DNA from plants, animals, and other organisms and the inserted DNA in GM crops are the same. Following ingestion, all types of DNA (i.e. indigenous plant DNA or transgenic DNA) is rapidly broken down within the gastrointestinal (GI) tract of both animals and humans through the action of digestive enzymes and microbial activities into small fragments and nucleotides. Studies have revealed that, in ruminants, more than 85% of plant DNA consumed is degraded to nucleotides within 4 h and absorbed into rumen microbes or passed into the duodenum.

This study was conducted to investigate the digestive fate of transgenic DNA/protein in the digestive tract and other tissues of farm animals fed genetically modified feed by reviewing scientific publication from referee journals. Out of 48 experimental studies reviewed, more than 50% of these studies confirmed that transgenic DNA/protein is not completely degraded in

the animal tissues and is easily detectable when standard molecular detection methods are applied. These experimental studies, reported the detection of transgenic DNA/protein in a number of analyzed samples (e.g. rumen fluid, GTI and GIT contents, blood, liver, kidney, heart, Gizzard and animal faeces among others) when transgenic maize or soybean or both were included in the diet of the experimental animals. The finding from this study revealed that transgenic DNA may undergo the similar fate as DNA from conventional feed but resistant to mechanical, chemical, and enzymatic activities and also to microbial processes of the digestive tract of livestock since it is not completely degraded. This deduction was in accordance with previous report, that small plant DNA fragments can survive after digestion due to their interaction with some dietary compound in the upper digestive tract and that some could enter the intestinal epithelium and be absorbed by the host organism. Since the transgenic DNA is not different from other sources of DNA in the diet, if plant DNA is absorbed, it is very likely that transgenic DNA may also be absorbed. Studies have also shown that intestinal microflora of the small bowel of some human ileo-stomists was capable of acquiring and harboring DNA sequences from transgenic soybean even if gene transfer from the bacteria to the mammalian cells did not occur. However in those studies, no harmful effects have been identified either in the farm animals or humans. Contrarily, other studies indicated the evidence of complete degradation of free DNA in ruminant and monogastric GIT upon its release from partially or completely digested feeds. These controversial findings, continued to fuel public concerns regarding the use of transgenic crops in livestock feeding mainly due to the potential risk of horizontal transfer of transgenic

DNA from a transgenic diet to farm animals and derived products intended for human consumption. Despite these controversies, scientific evidence indicates that the potential existence of plant DNA fragments, transgenic or not, in animal tissues and fluids presents no health risks for animals and human. Since the size of individual nucleotides or the sequences of the DNA fragments absorbed is so small as compare with that of the intact transgenes that they are not likely to transfer genetic information. And in any case, the chances of transfer and functional integration of DNA transgenic or not, from ingested feed or food is very rare. Moreover, no gene or DNA fragments absorbed by vertebrates through intestinal mucosa have ever been identified in the genome of animals and humans. Also the United Nations Food and Agriculture Organization, the World Health Organization, and the US Food and Drug Administration have declared that the ingestion of DNA from various sources including DNA introduced in to the GM crops did not indicate any health or safety concerns to both human and animals. These decisions are based mainly due to the previous history of safety related with the ingestion of DNA by human and animals.

Conclusion

Based on this study, it was concluded that transgenic DNA may exhibited similar stability and persistence in the gastrointestinal tract of livestock animals. Feed-derived foreign DNA fragments are not completely degraded and may possibly be taken up into organs and tissues of farm animals through the GI tract. Nevertheless, the potential existence of DNA fragments, transgenic or not, in animal tissues and fluids presents no health risks for animals and human. Transgenic DNA detection in livestock is dependent on a number of factors, including matrix effects, limit of

detection (LOD), transgene quantity in the feed, feed digestibility, and differences in the digestive physiology among livestock. Overall, the finding of our study provide some information on the digestive fate of feed transgenic DNA in farm animals, given that the persistence of DNA after dietary exposure is one of the important aspect of risk assessment for genetically modified foodstuffs.

References

- Agodi, A., Barchitta, M., Grillo, A. and Sciacca, S. 2006.** Detection of genetically modified DNA sequences in milk from the Italian market. *International journal of hygiene and environmental health* 209, 81-88
- Alexander, T. W., Reuter, T., Aulrich, K., Sharma, R., Okine, E. K., Dixon, W. T. and Mcallister, T. A. 2007.** A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production. *Animal Feed Science and Technology* 133, 31-62.
- Alexander, T. W., Reuter, T., Okine, E., Sharma, R. and Mcallister, T. A. 2006.** Conventional and real-time polymerase chain reaction assessment of the fate of transgenic DNA in sheep fed Roundup Ready® rapeseed meal. *British journal of nutrition* 96, 997-1005.
- Beever, D., Glenn, K., and Phipps, R. 2003.** A safety evaluation of genetically modified feedstuffs for livestock production; the fate of transgenic DNA and proteins. *ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES* 16, 764-772.
- Beever, D.E., and Kemp, C. 2000.** "Safety issues associated with the DNA in animal feed derived from

- genetically modified crops. A review of scientific and regulatory procedures", in: *Nutrition Abstracts and Reviews. Series A, Human and Experimental*), 197-204.
- Bertheau, Y., Helbling, J., Fortabat, M., Makhzami, S., Sotinel, I., Audéon, C., Nignol, A., Kobilinsky, A., Petit, L., and Fach, P. 2009.** Persistence of plant DNA sequences in the blood of dairy cows fed with genetically modified (Bt176) and conventional corn silage. *Journal of agricultural and food chemistry* 57, 509-516.
- Buzoianu, S.G., Walsh, M.C., Rea, M.C., O'donovan, O., Gelencser, E., Ujhelyi, G., Szabo, E., Nagy, A., Ross, R.P., and Gardiner, G.E. 2012.** Effects of feeding Bt maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material. *PLoS One* 7, e47851.
- Calsamiglia, S., Hernandez, B., Hartnell, G., and Phipps, R. 2007.** Effects of corn silage derived from a genetically modified variety containing two transgenes on feed intake, milk production, and composition, and the absence of detectable transgenic deoxyribonucleic acid in milk in Holstein dairy cows. *Journal of dairy science* 90, 4718-4723.
- Castillo, A., Gallardo, M., Maciel, M., Giordano, J., Conti, G., Gaggiotti, M., Quaino, O., Gianni, C., and Hartnell, G. 2004.** Effects of feeding rations with genetically modified whole cottonseed to lactating Holstein cows. *Journal of Dairy Science* 87, 1778-1785.
- Chainark, P., Satoh, S., Hino, T., Kiron, V., Hirono, I., and Aoki, T. 2006.** Availability of genetically modified soybean meal in rainbow trout *Oncorhynchus mykiss* diets. *Fisheries Science* 72, 1072-1078.
- Chainark, P., Satoh, S., Hirono, I., Aoki, T., and Endo, M. 2008.** Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout *Oncorhynchus mykiss*. *Fisheries Science* 74, 380-390.
- De Giacomo, M., Di Domenicantonio, C., De Santis, B., Debegnach, F., Onori, R., and Brera, C. 2016.** Carry-over of DNA from genetically modified soyabean and maize to cow's milk. *J. Anim. Feed Sci* 25, 109-115.
- Duggan, P.S., Chambers, P.A., Heritage, J., and Forbes, J.M. 2003.** Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *British Journal of Nutrition* 89, 159-166.
- Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwägele, F., Jahreis, G., and Flachowsky, G. 2001.** The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology* 212, 129-134.
- Einspanier, R., Lutz, B., Rief, S., Berezina, O., Zverlov, V., Schwarz, W., and Mayer, J. 2004.** Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. *European Food Research and Technology* 218, 269-273.

- Fao, W. 2001.** Evaluation of allergenicity of genetically modified foods: report of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology. *FAO, Rome*.
- Fda, U. 1992.** Statement of policy: foods derived from new plant varieties; notice. *Federal Register* 57, 22,984-923,005.
- Flachowsky, G. 2013.** Feeding studies with first generation GM plants (input traits) with food-producing animals. *Animal nutrition with transgenic plants. CABI Biotechnology Series. CABI, Oxfordshire, UK*, 72-93.
- Flachowsky, G., Chesson, A., and Aulrich, K. 2005.** Animal nutrition with feeds from genetically modified plants. *Archives of Animal Nutrition* 59, 1-40.
- Furgal-Dierżuk, I., Strzetelski, J., Twardowska, M., Kwiatek, K., and Mazur, M. 2015.** The effect of genetically modified feeds on productivity, milk composition, serum metabolite profiles and transfer of tDNA into milk of cows. *Journal of Animal and Feed Sciences* 663, 127.
- García-Villalba, R., León, C., Dinelli, G., Segura-Carretero, A., Fernández-Gutiérrez, A., García-Cañas, V., and Cifuentes, A. 2008.** Comparative metabolomic study of transgenic versus conventional soybean using capillary electrophoresis–time-of-flight mass spectrometry. *Journal of Chromatography A* 1195, 164-173.
- Gürtler, P., Paul, V., Albrecht, C., and Meyer, H. 2009.** Sensitive analytical methods for quantification of novel DNA and protein in bovine milk–First results from a long-term feeding study in dairy cows. *Journal für Verbraucherschutz und Lebensmittelsicherheit* 3, 26-28.
- Herman, R.A., and Price, W.D. 2013.** Unintended compositional changes in genetically modified (GM) crops: 20 years of research. *Journal of agricultural and food chemistry* 61, 11695-11701.
- Jennings, J., Albee, L., Kolwyck, D., Surber, J., Taylor, M., Hartnell, G., Lirette, R., and Glenn, K. 2003.** Attempts to detect transgenic and endogenous plant DNA and transgenic protein in muscle from broilers fed YieldGard Corn Borer Corn. *Poultry Science* 82, 371-380.
- Klotz, A., Mayer, J., and Einspanier, R. 2002.** Degradation and possible carry over of feed DNA monitored in pigs and poultry. *European Food Research and Technology* 214, 271-275.
- Ma, Q., Gao, C., Zhang, J., Zhao, L., Hao, W., and Ji, C. 2013.** Detection of transgenic and endogenous plant DNA fragments and proteins in the digesta, blood, tissues, and eggs of laying hens fed with phytase transgenic corn. *PLoS One* 8, e61138.
- Mazza, R., Soave, M., Morlacchini, M., Piva, G., and Marocco, A. 2005.** Assessing the transfer of genetically modified DNA from feed to animal tissues. *Transgenic research* 14, 775-784.
- Mcallan, A. 1982.** The fate of nucleic acids in ruminants. *Proceedings of the Nutrition Society* 41, 309-316.
- Morera, P., Basirico, L., Ronchi, B., and Bernabucci, U. 2016.** Fate of transgenic deoxyribonucleic acid fragments in digesta and tissues of

- rabbits fed genetically modified soybean meal. *Journal of animal science* 94, 1287-1295.
- Nadal, A., De Giacomo, M., Einspanier, R., Kleter, G., Kok, E., Mcfarland, S., Onori, R., Paris, A., Toldrà, M., and Van Dijk, J. 2018.** Exposure of livestock to GM feeds: Detectability and measurement. *Food and Chemical Toxicology* 117, 13-35.
- Nemeth, A., Wurz, A., Artim, L., Charlton, S., Dana, G., Glenn, K., Hunst, P., Jennings, J., Shilito, R., and Song, P. 2004.** Sensitive PCR analysis of animal tissue samples for fragments of endogenous and transgenic plant DNA. *Journal of Agricultural and Food Chemistry* 52, 6129-6135.
- Netherwood, T., Martín-Orúe, S.M., O'donnell, A.G., Gockling, S., Graham, J., Mathers, J.C., and Gilbert, H.J. 2004.** Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature biotechnology* 22, 204.
- Nielsen, C.R., Berdal, K.G., Bakke-Mckellep, A.M., and Holst-Jensen, A. 2005.** Dietary DNA in blood and organs of Atlantic salmon (*Salmo salar* L.). *European Food Research and Technology* 221, 1-8.
- Paul, V., Guertler, P., Wiedemann, S., and Meyer, H.H. 2010.** Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. *Transgenic Research* 19, 683-689.
- Phipps, R., Beever, D., and Humphries, D. 2002.** Detection of transgenic DNA in milk from cows receiving herbicide tolerant (CP4EPSPS) soyabean meal. *Livestock Production Science* 74, 269-273.
- Phipps, R., Deaville, E., and Maddison, B. 2003.** Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. *Journal of Dairy Science* 86, 4070-4078.
- Phipps, R., Jones, A., Tingey, A., and Abeyasekera, S. 2005.** Effect of corn silage from an herbicide-tolerant genetically modified variety on milk production and absence of transgenic DNA in milk. *Journal of dairy science* 88, 2870-2878.
- Řehout, V., Hanusova, L., Čítek, J., Kadlec, J., and Hosnedlova, B. 2008a.** Detection of DNA fragments from Roundup Ready soya in blood of broilers. *Journal of Agrobiology* 25, 145-148.
- Řehout, V., Hanusová, L., Kadlec, J., Čítek, J., and Hosnedlová, B. 2008b.** Detection of DNA fragments from roundup ready soya and Bt maize in organs of broilers. *Journal of Agrobiology* 25, 141-144.
- Rizzi, A., Brusetti, L., Arioli, S., Nielsen, K.M., Tamagnini, I., Tamburini, A., Sorlini, C., and Daffonchio, D. 2008.** Detection of feed-derived maize DNA in goat milk and evaluation of the potential of horizontal transfer to bacteria. *European Food Research and Technology* 227, 1699-1709.
- Rossi, F., Morlacchini, M., Fusconi, G., Pietri, A., Mazza, R., and Piva, G. 2005.** Effect of Bt corn on broiler growth performance and fate of feed-derived DNA in the digestive tract. *Poultry Science* 84, 1022-1030.
- Salisu, I.B., Shahid, A.A., Ali, Q., Rao,**

- A.Q., and Husnain, T. 2018. Nutritional Assessment of Dietary Bt and Cp4EPSPS Proteins on the Serum Biochemical Changes of Rabbits at Different Developmental Stages. *Frontiers in Nutrition* 5.
- Sanden, M., Bruce, I.J., Rahman, M.A., and Hemre, G.-I. 2004. The fate of transgenic sequences present in genetically modified plant products in fish feed, investigating the survival of GM soybean DNA fragments during feeding trials in Atlantic salmon, *Salmo salar* L. *Aquaculture* 237, 391-405.
- Sanden, M., Johannessen, L., Berdal, K., Sissener, N., and Hemre, G.I. 2011. Uptake and clearance of dietary DNA in the intestine of Atlantic salmon (*Salmo salar* L.) fed conventional or genetically modified soybeans. *Aquaculture nutrition* 17, e750-e759.
- Sharma, R., Damgaard, D., Alexander, T.W., Dugan, M.E., Aalhus, J.L., Stanford, K., and Mcallister, T.A. 2006. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. *Journal of agricultural and food chemistry* 54, 1699-1709.
- Sieradzki, Z., Mazur, M., Kwiatek, K., Świątkiewicz, S., Świątkiewicz, M., Koreleski, J., Hanczakowska, E., Arczewska-Włosek, A., and Goldsztejn, M. 2013. Assessing the possibility of genetically modified DNA transfer from GM feed to broiler, laying hen, pig and calf tissues. *Polish journal of veterinary sciences* 16, 435-441.
- Sissener, N.H., Johannessen, L.E., Hevrøy, E.M., Wiik-Nielsen, C.R., Berdal, K.G., Nordgreen, A., and Hemre, G.-I. 2010. Zebrafish (*Danio rerio*) as a model for investigating the safety of GM feed ingredients (soya and maize); performance, stress response and uptake of dietary DNA sequences. *British journal of nutrition* 103, 3-15.
- Świątkiewicz, M., Bednarek, D., Markowski, J., Hanczakowska, E., and Kwiatek, K. 2013. Effect of feeding genetically modified maize and soybean meal to sows on their reproductive traits, haematological indices and offspring performance. *Bulletin of the Veterinary Institute in Pulawy* 57, 413-418.
- Świątkiewicz, M., Hanczakowska, E., Twardowska, M., Mazur, M., Kwiatek, K., Kozaczyński, W., Świątkiewicz, S., and Sieradzki, Z. 2011. Effect of genetically modified feeds on fattening results and transfer of transgenic DNA to swine tissues. *Bulletin of the Veterinary Institute in Pulawy* 55, 121-125.
- Świątkiewicz, S., Twardowska, M., Markowski, J., Mazur, M., Sieradzki, Z., and Kwiatek, K. 2010. Fate of transgenic DNA from Bt corn and Roundup Ready soybean meal in broilers fed GMO feed. *Bull Vet Inst Pulawy* 54, 237-242.
- Trabalza-Marinucci, M., Brandi, G., Rondini, C., Avellini, L., Giammarini, C., Costarelli, S., Acuti, G., Orlandi, C., Filippini, G., and Chiaradia, E. 2008. A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. *Livestock Science* 113, 178-190.

- Tudisco, R., Calabrò, S., Bovera, F., Cutrignelli, M., Nizza, A., Piccolo, V., and Infascelli, F. 2010a.** Detection of plant species-specific DNA (barley and soybean) in blood, muscle tissue, organs and gastrointestinal contents of rabbit.
- Tudisco, R., Lombardi, P., Bovera, F., Cutrignelli, M., Mastellone, V., Terzi, V., Avallone, L., and Infascelli, F. 2006.** Genetically modified soya bean in rabbit feeding: detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. *Animal Science* 82, 193-199.
- Tudisco, R., Mastellone, V., Cutrignelli, M., Lombardi, P., Bovera, F., Mirabella, N., Piccolo, G., Calabro, S., Avallone, L., and Infascelli, F. 2010b.** Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. *Animal* 4, 1662-1671.
- Tufarelli, V., Selvaggi, M., Dario, C., and Laudadio, V. 2015.** Genetically modified feeds in poultry diet: safety, performance, and product quality. *Critical reviews in food science and nutrition* 55, 562-569.
- Van Eenennaam, A., and Young, A. 2014.** Prevalence and impacts of genetically engineered feedstuffs on livestock populations1. *Journal of Animal Science* 92, 4255-4278.
- Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A., Epstein, M.M., Ross, R.P., and Lawlor, P.G. 2011.** Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. *PLoS One* 6, e27177.
- Walsh, M.C., Buzoianu, S.G., Rea, M.C., O'donovan, O., Gelencsér, E., Ujhelyi, G., Ross, R.P., Gardiner, G.E., and Lawlor, P.G. 2012.** Effects of feeding Bt MON810 maize to pigs for 110 days on peripheral immune response and digestive fate of the cry1Ab gene and truncated Bt toxin. *PLoS One* 7, e36141.
- Weber, T., and Richert, B. 2001.** Grower-finisher growth performance and carcass characteristics including attempts to detect transgenic plant DNA and protein in muscle from pigs fed genetically modified "Bt" corn. *J Anim Sci* 79, 67.
- Wiedemann, S., Lutz, B., Albrecht, C., Kuehn, R., Killermann, B., Einspanier, R., and Meyer, H.H. 2009.** Fate of genetically modified maize and conventional rapeseed, and endozoochory in wild boar (*Sus scrofa*). *Mammalian Biology-Zeitschrift für Säugetierkunde* 74, 191-197.
- Yonemochi, C., Fujisaki, H., Harada, C., Kusama, T., and Hanazumi, M. 2002.** Evaluation of transgenic event CBH 351 (StarLink) corn in broiler chicks. *Animal Science Journal* 73, 221-228.
- Yonemochi, C., Suga, K., Harada, C., and Hanazumi, M. 2010.** Tevaluation of transgenic event CBH 351 (StarLink) corn in pig. *Animal science Journal* 81, 94-101.

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