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Enhancing Animal Nutrition and Sustainability: The Vital Role of Genetically Modified Crops in Animal Feeding

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Agriculture and livestock are fundamental to the economies of developing countries. A substantial portion of crop harvests is allocated for animal feed. Thus, more technological advancements are necessary to enhance agricultural productivity and provide affordable feed. Future biotech crops are expected to play a crucial role in this area. The goal is often to introduce traits into plants that do not naturally occur in these species. These new traits may include resistance to pests, diseases, or environmental conditions, or the production of specific nutrients or pharmaceutical agents. GM crops have indirectly benefited the livestock sector by increasing the yield of feed ingredients and improving quality traits. These crops are primarily used in livestock feed rations as energy and/or protein sources. Numerous animal feeding studies have been conducted to demonstrate that genetically modified crops are as nutritious and wholesome as compared to their conventional counterparts. No biological relevant differences in animal performance, health, or animal product (meat and milk) composition had been observed in various studies conducted. Overall, no significant differences in gain, intake, and feed conversion have been reported. Since the GM crop's composition is not different from its conventional counterpart [except for the introduced transgene(s) and expressed protein(s)] and the expressed transgenic protein is rapidly digested in the digestive system, one would not expect any unintended effects. The introduction of genetically modified (GM) crops into the market undergoes extensive testing and a rigorous approval process to ensure food, feed, and environmental safety. This process includes thorough analyses before GM crops are deemed safe for commercial use. GM livestock feed is assessed for its nutritional composition and digestibility by comparing it with conventional crops. Therefore, while GM crops have the potential to enhance the efficiency of animal agriculture by improving nutritional content, reducing pesticide use, and increasing crop yields, it is essential to approach their adoption with caution.

Keywords: Livestock; ration; agriculture; productivity; nutrients.

1. INTRODUCTION

"Agriculture and livestock are fundamental to the economies of developing countries. As populations grow, the demand for livestock products like meat, milk, and eggs increases significantly. With rising urbanization and income levels in many parts of the developing world, per capita consumption of these products is expected to increase by about 2% annually" [1]. "Global meat demand is projected to rise by over 55% from current levels, with most of this increase occurring in developing countries" [2]. "A substantial portion of crop harvests is allocated for animal feed. A variety of raw materials, including soya, maize, oilseed rape, cottonseed, canola, and other grains have been used for formulating compound feeds for pigs, poultry, dairy cows, and other livestock. Approximately 90% of the world's soya production is used in animal feed" [3]. "Consequently, the demand for feed grains is anticipated to grow by 3% annually in developing countries and by 0.5% in developed countries. On average, production of 1 kg of livestock meat requires less than 3 kg of feed grain, and for production of 1 kg of milk requires less than 1 kg of feed grain" [4]. "Thus, more technological advancements are necessary to enhance agricultural productivity and provide affordable feed" [5]. Future biotech crops, whose genetic makeup has been artificially altered by inserting new genes through recombinant DNA technology, are expected to play a crucial role in this area. The goal is often to introduce traits into plants that do not naturally occur in these species. These new traits may include resistance to pests, diseases, or environmental conditions, or the production of specific nutrients or pharmaceutical agents.

"Since their introduction in 1996, genetically modified (GM) crops have been used as livestock feed. GM crops have indirectly benefited the livestock sector by increasing the yield of feed ingredients and improving quality traits. These crops are primarily used in livestock feed rations as energy and/or protein sources. Conventional crops like rapeseed and mustard oil cake can be used as protein supplements for ruminants, but the presence of glucosinolate can result in a pungent smell and bitter taste after hydrolysis by endogenous enzymes" [6,7]. "Considering the quality concerns of the feed and other aspects like pest control and herbicide resistance, GM crops have an advantage in

Table 1. Crops modified for various traits [11]

Feed Crop	Improved Traits
Alfalfa	Herbicide tolerance, modified product quality
Canola	Herbicide tolerance, modified product quality, pollination control system
Cotton	Insect resistance, herbicide tolerance
Cowpea	Insect resistance
Flax	Herbicide tolerance
Maize/corn	Modified product quality, insect resistance, herbicide tolerance, pollination control system, abiotic stress tolerance
Rice	Insect resistance, herbicide tolerance
Safflower	Modified oil/fatty acid , Antibiotic resistance
Soybean	Modified product quality, herbicide tolerance, insect resistance, altered growth/yield
Sugarcane	Insect resistance
Wheat	Herbicide tolerance

feeding trials. Genetically modified crops, also known as transgenic crops, are plants whose genetic makeup has been artificially altered by inserting new genes through recombinant DNA technology" [8]. "These crops have become increasingly prevalent in animal feeding due to their enhanced traits, such as improved nutritional content, resistance to pests, and higher yields. The use of GM crops in animal feed can reduce the need for chemical inputs, such as pesticides and fertilizers, thereby promoting environmental sustainability. Aflatoxins, toxic secondary metabolites produced by some *Aspergillus* species, for example, are a global agricultural and health issue, causing significant crop losses annually. Host-induced gene silencing, is an effective method to eliminate aflatoxin in transgenic maize. Similarly, maize plants transformed with a kernel-specific RNA interference (RNAi) gene cassette targeting the aflC gene, which encodes an enzyme in the *Aspergillus* aflatoxin biosynthetic pathway, showed no aflatoxin in kernels from these RNAi transgenic plants after pathogen infection, while nontransgenic control kernels had high toxin loads" [9].

"The benefits of genetic engineering in agriculture include increased crop yields, reduced food or drug production costs, reduced pesticide use, enhanced nutrient composition and food quality, pest and disease resistance, greater food security, and medical benefits for the growing global population. Advances have also been made in developing crops that mature faster and tolerate environmental stressors such as aluminum, boron, salt, drought, and frost, enabling plants to grow in otherwise unfavorable conditions" [10]. Other applications include producing non-protein (bioplastic) or non-industrial (ornamental plant) products. The aim of this review article is to explore the critical role of genetically modified (GM) crops in meeting the rising demand for livestock products in developing countries.

Crops have been modified for various traits, as summarized in Table 1.

2. GLOBAL SCENARIO OF GM CROPS USED FOR FEEDING LIVESTOCK

"Around 90% of GM crops were grown in developing countries like China, India, the Philippines, and South Africa, where a majority of farmers operate on smaller scales compared to their counterparts in the USA, the largest producer of GM commodity crops" [12]. "In 2010, the USA cultivated 66.8 million hectares of GM crops, followed by Brazil with 25.4 million hectares and Argentina with 22.9 million hectares" [13]. Other significant producers with over 1 million hectares include India, Canada, China, Paraguay, Pakistan, South Africa, and Uruguay. Soybean dominates as the leading GM crop in the Americas, accounting for more than half of global GM production by volume, followed by GM maize, which constitutes about one-third of global GM production, primarily from the Americas. Canada leads in GM oilseed rape production, while Brazil, India, and China contribute substantially to GM cotton production [14].

"The main GM crops modified for agronomic traits include soybean (36.5 million hectares), maize (12.4 million hectares), cotton (6.8 million hectares), and canola (3.0 million hectares)" [15]. These crops are typically modified for

Table 2. Cultivation of GMP in countries with the highest portions (by FAOSTAT 2019)

Country	GMP-Area (million ha)	Most Cultivated Plants
USA	71.5	Soybean, Maize, Cotton, Rape seed, Sugar beet, Alfalfa
Brazil	52.8	Soybean, Maize, Cotton, Sugar cane
Argentina	24.0	Soybean, Maize, Cotton, Alfalfa
Canada	12.5	Rape seed, Maize, Soybean, Sugar beet, Alfalfa,
India	11.9	Cotton
Paraguay	4.1	Soybean, Maize, Cotton
China	3.2	Cotton, Papaya
South Africa	2.7	Maize, Soybean, Cotton
Pakistan	2.5	Cotton

traits like herbicide tolerance and insect resistance. They are utilized in livestock production as sources of energy and/or protein in various forms such as whole crop (maize silage), specific components (maize grain), or co-products like oilseed meals. Oilseed meals, in particular, derived from GM crops, are extensively used in livestock feed. For instance, in 2002, it was estimated that the global production of soybean exceeded 150 million tonnes, with approximately 50% of the area planted with GM soybean, resulting in around 35 million tonnes of GM soybean meal being used in the livestock industry. Additionally, significant quantities of maize grain, canola, cottonseed meal, and maize silage are also integrated into livestock rations.

3. GM CROPS AS FEED INGREDIENTS FOR FARM ANIMALS IN INDIA

India, with over 20% of the world's livestock population growing at an annual rate of 0.66%, presents greater competition for land between human food production and animal fodder. Currently, only 4% of India's total cultivable area is dedicated to fodder production, leading to significant deficits in green fodder (35.6%), dry crop residues (10.5%), and concentrate feed ingredients (44%) [16]. Efforts to address these challenges have seen recognition since the Earth Summit in 1992, acknowledging that modern agricultural biotechnology alone cannot fully solve the complexities of future food security.

The adoption of GM technology in India began with initiatives like Monsanto's offer in the early 1990s to transfer insect-resistant Bt cotton technology [17]. This move aimed to combat significant losses faced by smallholder cotton farmers due to pests like the bollworm complex. Recently, the Indian government approved the import of 5.5 lakh tonnes of GM soymeal for poultry feed, signaling a potential shift towards wider acceptance of GM crops in livestock feed [18-20]. In Karnataka, stakeholders including the Compound Livestock Feed Manufacturers' Association of India (CLFMAI) and Karnataka Poultry Farmers and Breeders Association (KPFBA) have advocated for GM feed, emphasizing its potential benefits for meat production in India amidst declining availability of conventional soybean and maize. Numerous studies on ruminant feeding have indicated that GM crops, such as corn, soybeans, cottonseed, and alfalfa, modified for traits like insect resistance and herbicide tolerance, show no significant differences in animal performance, health, or the composition of animal products like meat and milk compared to conventional crops [21,22]. These findings support the argument that GM crops, with their specific transgenic traits, do not pose unintended effects when digested by animals.

As discussions continue around the adoption of GM soybean and maize in India's livestock feed industry, stakeholders are optimistic about convincing policymakers of the benefits of GM technology for enhancing agricultural productivity and ensuring food security in the country.

4. EFFECT OF FEEDING OF GM CROPS IN RUMINANTS

"Numerous ruminant feeding studies have been conducted to demonstrate that genetically modified crops are as nutritious and wholesome as compared to their conventional counterparts" [21, 22]. No biological relevant differences in animal performance, health, or animal product (meat and milk) composition had been observed in various studies conducted. Table 3 provides a description of studies where GM crops were fed to ruminants. "The GM crops included corn, corn silage, soybeans, soybean meal, cottonseed,

Table 3. Feeding of GM crops in ruminants

GM crop	Animal	Experimental Design	Studied Health Parameters	Results	References
1. Bt maize (MON810)	Lactating cows	GM-maize group Control group	Body condition score, Immune response	No adverse effect on performance	[23]
2.GM maize an GM soybean	10 days old bull	90 days Non-GM maize and SBM Non-GM maize and GM SBM GM maize and non-GM SBM GM maize and GM SBM	Performance parameters	No adverse effects of GM components	[24]
3. Bt cotton	Twenty crossbred (KS and KF) multiparous cow	Control Treatment	Milk yield, voluntary feed intake, milk composition	No effect on mean voluntary DM intake, Improved body weight in Bt group No effect on average milk yield, milk composition	[25]
4.Bt maize (MON810) and RR soybean (MON-40-30-2)	1-week old calves	12 weeks Group I – control, conventional soybean meal and conventional maize Group II – GM soybean meal and conventional maize Group III – conventional soybean meal and GM maize Group IV – GM soybean meal and GM maize	Haematology, Immune response	No effect of GM crops	[26]
5.Bt cottonseed	lactating Murrah buffaloes	1. 39.5% non-transgenic cottonseed in concentrate mixture 2. 39.5% Bt cottonseed in concentrate mixture	DMI, performance, blood biochemical constituents	No significant difference in the DMI, Body weight, TEC, Hb content and PCV similar. Plasma glucose, serum total proteins, albumin, globulin, triglycerides and high density lipoprotein similar	[27]
6. Bt maize (MON810) and RR soybean (MON-40-3-2)	10-day old calves	Group1-control, conventional SBM and conventional maize Group 2-GM SBM and conventional maize Group 3 – conventional SBM and GM maize Group 4– GM SBM and GM maize	Histopathology	No effect of GM feed	[28]

Table 4. Effect of feeding of GM crops in pigs

GM crop	Animal	Experimental design	Studied parameters	Results	References
1.Bt maize(MON810)	35-day old male pigs	GM maize group Control	Immune response, histopathology, serum biochemistry, organ weight	No visual effects observed on histology or serum biochemistry	[29]
2.Bt maize(MON810)andRR soybean(MON-40-30-2)	Fattening pigs	GM-maize group GM-soybean groups GM-maize AND GM-soybean group Control	Haematology	No effect of GM feed observed	[26]
3.Btmaize(MON810)and RR soybean (MON-40-3-2)	Pregnant sows and offspring	GM-maize group GM-soybean group GM-maize & GM-soybean group Control	Haematology	No effect of GM feed observed	[30]
4.Bt maize (MON810)	Offspring and sows	GM-maize group Control	Haematology, immune response, serum biochemistry, gastrointestinal microbiota, organ weight (offspring only)	Lower granulocyte count and percentage at birth in offspring from GM-fed animals	[31]

Table 5. Feeding of GM crops in poultry

GM crop	Animal	Duration of Study and Experimental Design	Studied Parameters	Results	References
1.Bt maize(Cry1Ab)	10 days Japanese quails	GM maize grp Non GM grp	body weight, hematology, serum chemistry, relative organ weight	No effect of GM feed observed	[32]
2.Bt sugarcane(Cry1Ac)	2 week old broilers	GM-sugarcane grp Control	Serum biochemistry, histopathology, organ weight	No effect of GM feed observed	[33]
3.Bt maize(MON810)andRR soybean(MON-40-30-2)	25-week old laying hens	30 weeks GM-maize grp GM-soybean grp GM-maize & GM-soybean grp Control	Histopathology	No effect of GM feed observed	[28]
4.Bt maize	Newly hatched Japanese quail	22 weeks GM-maize grp Control	Immune response	Higher serum zinc concentrations in GM-maize fed animals	[34]

GM crop	Animal	Duration of Study and Experimental Design	Studied Parameters	Results	References
6.Bt maize (MON810) and RR soybean (GTS-40-3-2)	6-week old Japanese quail	GM-maize gen. GM-soybean gen. Control gen.	Clinical examination, histopathology, organ weight	Higher relative weight of breast muscle and gizzard in GM-maize fed animals	[35]

Table 6. Negative effects of feeding GM crops

GM Crop	Animal and Experiment. Pd.	Exp. Grp.	Parameters Studied	Results	References
Bt maize (MON810)	Lactating cows (25 months)	GM-maize group Control group	Body condition score, immune response	Lower body condition	[23]
Bt maize (NK603, MON863,MON810) and RR soybean	Weaned piglets (22.7 weeks)	GM-fed maize& GM soybean group Control group	Serum biochemistry, histopathology, organ weight	Severe inflammation in stomach	[37]
Bt maize (CBH 351 Starlink)	3-month old pigs	GM-maize group Control group	Till end of fattening period Haematology, serum biochemistry, histopathology	Higher BUN Lower glucose	[38]
Bt maize (MON810)	Nulliparous sows and offspring	GM-maize group Control	Haematology, immune response, serum biochemistry, gastrointestinal microbiota, organ weight (offspring only)	Lower granulocyte percentage on day 110 of gestation in GM-fed animals as well as in offsrprings Higher serum creatinine	[39]

fodder beets and alfalfa. The GM traits included a variety of insect-protected and herbicide-tolerant traits or a combination of them. Overall, no significant differences in gain, intake, and feed conversion were reported. Since the GM crop's composition is not different from its conventional counterpart [except for the introduced transgene(s) and expressed protein(s)] and the expressed transgenic protein is rapidly digested in the digestive system, one would not expect any unintended effects" [21, 22].

4.1 Feeding of GM Crops in Pigs

Table 4 summarises the effects of feeding various genetically modified crops to different age groups of pigs. No adverse effects had been observed on the performance of the animals.

Hence, feeding trials conducted to examine the safety and efficacy of GM feeds for farm livestock indicated that there was no evidence of significantly altered nutritional composition, deleterious effects (Table 5). Animals perform in comparable manner when fed biotechnological feed ingredients.

4.2 Negative Effects of Feeding GM Crops

Feeding genetically modified (GM) crops to animals raises concerns about potential negative effects across several fronts. These crops may introduce allergens or toxins not present in their non-modified counterparts, posing risks of allergic reactions or toxicity to animals.. Moreover, GM crops often include antibiotic-resistant genes, which could transfer to gut bacteria in animals, contributing to antibiotic resistance [36]. The environmental impact is also a concern, as the cultivation of GM crops, particularly herbicide-resistant varieties, may increase herbicide use, affecting biodiversity and ecosystems crucial for animal habitats. Despite rigorous testing, the long-term effects of consuming GM crops on animal health and the environment remain uncertain, necessitating continued research and monitoring to assess these potential risks thoroughly. Table 6 shows the studies which describes negative effects on the health of animals.

5. SAFETY ASSESSMENT OF GM FEEDS IN LIVESTOCK

The introduction of genetically modified (GM) crops into the market undergoes extensive testing and a rigorous approval process to ensure food, feed, and environmental safety. This process includes thorough analyses before GM crops are deemed safe for commercial use. GM livestock feed is assessed for its nutritional composition and digestibility by comparing it with conventional crops [40]. Agronomic, phenotypic, and compositional analyses are crucial for establishing substantial equivalence between GM and conventional crops. While these comparisons do not constitute a safety assessment *per se*, they help identify any differences and similarities.

Critical safety assessments also involve studying the properties of proteins produced by introduced genes in GM crops, particularly focusing on their potential toxicity and allergenicity when used as animal feed [41]. Studies are conducted to examine the effects of feeding GM crops on animals and on animal products such as meat, milk, and eggs (Table 7). The presence of foreign DNA from transgenic crops in animal tissues and derived products like milk, meat, and eggs is also evaluated. Studies have shown that transgenic DNA is not detectable in these food products derived from animals fed with GM crops, reaffirming safety standards.

The World Health Organization (WHO) has concluded that consuming DNA, including that from GM crops, poses inherently lower or no risk, as mammals regularly consume DNA from various sources such as plants, animals, bacteria, parasites, and viruses [42]. Scientific evidence supports that transgenic DNA and proteins expressed in GM crops are rapidly degraded in the animal digestive system and during feed processing. Furthermore, studies on various animals, including beef cattle, swine, sheep, fish, dairy cows, and chickens, have consistently shown no adverse effects on animal performance when fed with GM crops.

Detection of transgenic DNA in animal tissues plays a crucial role in assessing the impact of GM crops on animal health and the environment. Techniques such as Polymerase Chain Reaction (PCR), quantitative PCR (qPCR), Southern blotting, and fluorescent in situ hybridization (FISH) are employed to detect and quantify transgenic DNA. These methods provide insights into the persistence and potential integration of transgenes in animal genomes, as well as the possibility of horizontal gene transfer to gut microorganisms or other species. Continued advancements in molecular biology techniques contribute to ongoing research and regulatory assessments aimed at ensuring the safety and environmental sustainability of GM crop adoption in agriculture.

Table 7. Effects of feeding GM crops on animal products

Type of Study	Animal Species	Tissues Sampled	Detection OF DNA		References
			Transgene	Positive Tissue	
Feeding GM maize & soybean Bt & herbicide tolerant genes	Dairy cow	Milk	No		[43]
Feeding Phytase transgenic corn	laying hens	digestive tract, blood, heart, liver, spleen, kidney, breast muscle, eggs	Yes	gizzard	[44]
Feeding Bt maize, glyphosate tolerant soya	pig	blood	No		[30]
Feeding Bt maize	cow (dairy)	blood	No		[45]
Feeding glyphosate tolerant canola	pig	GIT content, and GIT tissues kidney, liver, spleen	Yes	entire GIT content, and GIT tissues	[46]
Feeding Bt cottonseed glyphosate tolerant cottonseed	cow (dairy)	milk	No		[47]
Feeding Bt maize	chicken (broiler)	blood, GIT content, heart, kidney, liver, muscle spleen, thymus	No		[48]
Feeding Bt maize	chicken (broiler)	kidney, liver, muscle, spleen	No		[49]
Feeding Bt maize, glyphosate tolerant soya	cow (dairy)	blood, feces, intestinal and rumen content, milk	Yes	Rumen	[50]

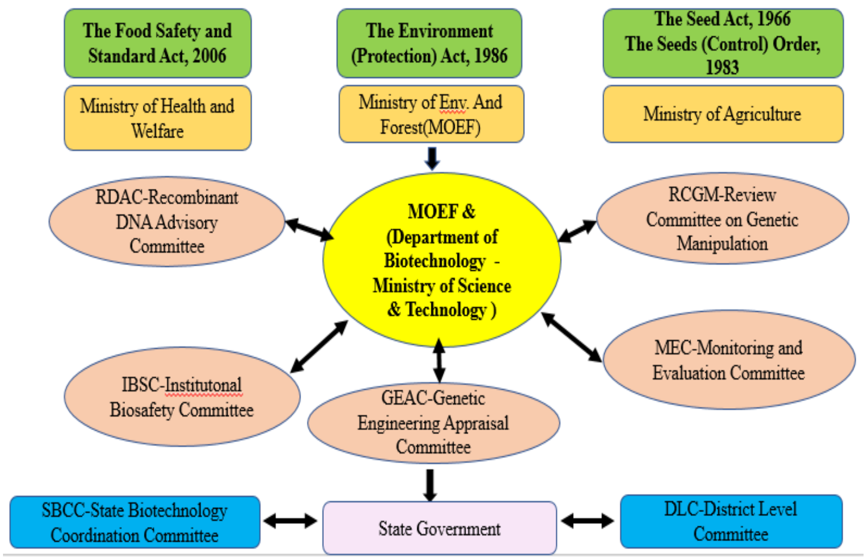

Fig. 1. Regulatory framework on GM crops in India

From several studies, it has been concluded that, transgenic DNA occasionally detected in animal fluid and organ samples. Also, no transgene DNA has been reported in animal-derived edible products such as milk or eggs.

6. COORDINATED REGULATORY FRAMEWORK ON GM CROPS IN INDIA

The National Biotechnology Board, established in 1982, issued safety guidelines in 1983 for conducting biotechnological research in laboratories and contained settings. It was elevated to the Department of Biotechnology (DBT) under the Ministry of Science and Technology (MOST) in 1986 [51]. Initially, DBT focused on global biotechnology developments, formulated safety guidelines, and promoted the use of relevant biotechnologies in India. Recognizing the need for robust biosafety assessments, biodiversity protection, and environmental risk evaluations [52], DBT transferred oversight of genetically modified organisms (GMOs), hazardous microorganisms, and transboundary movement of living modified organisms (LMOs) to the Ministry of Environment, Forest and Climate Change (MOEF).

The Government of India, under the Allocation of Business Rules 1961, assigned responsibilities for biodiversity conservation and environmental protection to MOEF (Government of India, 1961). Consequently, MOEF began regulating GMOs and their products under the Environment Protection Act (EPA) 1986. The EPA Rules 1989 were introduced under the hazardous substances section of EPA 1986, categorizing GM crops and GMOs as potentially harmful substances akin to hazardous materials impacting human, animal, or environmental health.

The regulatory framework established a tiered system with six competent authorities: Policy Advisory Committees such as the Recombinant DNA Advisory Committee (RDAC); Regulating and Approval Committees like the Institutional Biosafety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM), Genetic Engineering Approval Committee (GEAC); and Post-Monitoring Committees including the State Biotechnology Coordination Committee (SBCC) and District Level Committee (DLC). These committees were mandated to oversee different aspects of GMO regulation as outlined in the EPA Rules 1989. While DBT played a role in biosafety assessments under these rules, MOEF primarily managed post-monitoring activities at the state level.

However, the roles of the Ministry of Health and Ministry of Agriculture, crucial for regulating seed quality and human health in relation to GM crops, were not clearly defined under the EPA Rules 1989. Over time, the biosafety regulatory system evolved into a comprehensive framework involving multiple ministries, each administering specific legislative acts like the Environment Protection Act 1986 by MOEF, Seed Act 1966 and Seeds (Control) Order by Ministry of Agriculture (MOA), and Food Safety and Standards Act 2006 by Ministry of Health and Family Welfare (MOH&FW). The EPA Rules 1989 remained central to biosafety regulations for GM crops, while other laws focused on food safety and seed quality.

Guidelines for safety in biotechnology, including specific guidelines for GM crops, were developed by DBT starting with the Recombinant DNA Safety Guidelines in 1990, revised in 1994. These guidelines emphasized case-by-case biosafety evaluations, risk assessments for environmental impacts, and agronomic performance tailored to specific crops, traits, and agro-ecological systems [53]. They also addressed safety protocols for imported GM materials.

Concerns about GM crops encompass health risks, environmental hazards, and economic issues. These include potential toxicity from unexpected mutations, allergenicity risks, environmental impacts like non-target insect effects from Bt toxins, and economic disparities due to high seed costs. Despite potential benefits like enhanced agricultural efficiency and reduced pesticide use, the adoption of GM crops necessitates careful consideration of these complex and multifaceted issues.

7. CONCLUSION

Rising population in developing countries, and certainly in India, requires extraordinary steps to intensify agriculture production to meet the growing demand by substantially increasing crop yield for utilization of both humans and animals. It has now been recognized that neither the current agriculture production system nor the modern biotechnology alone can solve the complex challenges of feeding the world of tomorrow. GM crops have the potential to enhance the efficiency of animal agriculture by improving nutritional content, reducing pesticide use, and increasing crop yields. The use of genetically modified crops in animal feeding is hence a complex and multifaceted topic with both potential benefits and concerns.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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