THE EFFECTS OF SOME FEED ADDITIVES ON CHROMOSOME IN MAMMALS

EFECTELE UNOR ADITIVI FURAJERI ASUPRA CROMOZOMILOR LA MAMIFERE

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ABSTRACT

The undesirable influence of many substances on chromosomes has been long proven. Some feed additives are more recent, perhaps because of ignorance or exaggeration.Through our documentation, we hope to bring some clarity in these aspects. We have focused on the effects that some of the feed additives have on the genetic material, more precisely on the chromosomes - the easiest form of analysis.

We avoided considerations that referred to the consequences of chromosomes changes, numerical or structural being aware on the difficulty of these evaluations, and leaving room for punctual interventions related to the information contained in each of the chromosomes of a cellular complement and the cell type in which it was produced chromosomal alteration.

For a balanced perspective, we have also made references to those additives that are not attributed to unwanted effects on chromosomes.

Keywords: feed additives, chromosomes, mammals

REZUMAT

Influența indezirabilă a multor substanțe asupra cromozomilor a fost de mult dovedită. Cea a unor aditivi furajeri este de dată mai recentă, motivul, probabil, al unor ignoranțe sau exagerări. Efortul documentării noastre, sperăm să aducă unele lămuri în aceste privințe. Ea a vizat efectele pe care le au unii dintre aditivii furajeri asupra materialului genetic, mai precis asupra cromozomilor - forma mai facilă de analiză.

Am evitat considerațiile care au făcut trimiteri către consecințele modificărilor, numerice sau de structură a cromozomilor, conștienți de dificultatea acestor evaluări și lăsând loc pentru intervenții punctuale ce țin de informația conținută în fiecare dintre cromozomii unui complement celular și funcție de tipul celular în care s-a produs alterarea cromozomală.

Pentru echilibru, am făcut și referiri la acei aditivi cărora nu li se impută efecte nedorite asupra cromozomilor.

Cuvinte cheie: aditivi furajeri, cromozomi, mamifere

Heredity and variability have been established thanks to the great plasticity of the genetic material (in this case the DNA biomolecule), which has been permanently receptive to influences from the environment. Some of these inflexions have resulted in life benefits, not necessarily for the individual, and they have been preserved as new favorable arrangements in the structure of the genetic material, while others bringing injuries to life have been excluded, retained at most in the generation in which they appeared.

The influences of environmental factors on hereditary variability have been established by studying mutations at the chromosome level.All the recorded chromosomal changes are the result of complex physiological processes of the cell, based on chemical and physico-chemical reactions. Influences of fodder additives cannot be excluded from these laws.

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As such, long-term worries can be left behind. In the short term, they are problematic, because most of the animals are designated for an immediate use.

If we accept the opinion shared by Kan et all (1998), which suggests that certain additives considered harmless, can have negative effects but do not appear immediately and thus remain unnoticed for a long time, the issue of the influences on the integrity of the genetic material is complex [19].

Bibliographic assessments on additives with genetic risk

Undoubtedly, nutrition affects all cellular components, including genetic material. This dynamic relationship between nutrition and genetics generated a new branch called **nutritional epigenetics.**

From preservatives, **sodium benzoate and potassium sorbate** are most commonly used.

Their clastogenic, mutagenic and cytotoxic effects were demonstrated in vitro on lymphocytes [35].

Tartrazine is an additive from the category of synthetic dyes. The evaluation of the cytotoxic poten-

tial and the in vitro lymphocyte DNA restructuring by Soares et all (2015) showed that tartrazine does not have cytotoxic effects, on the contrary genotoxic effects are significant at all concentrations tested. These data demonstrate that tartrazine can be dangerous if its use is prolonged, triggering carcinogenicity, as the authors of the study reported [37].

Other authors, Kashanian and Zeidali [20], noted that tartrazine induces oxidative stress and DNA alteration.

The exchanges between sister chromatids (SCE) and chromosomal aberrations induced by **curcumin and tartrazine** were studied by Giry et al (1990) on mouse and rat`s bone marrow cells [13]. The authors noted a significant increase in shifts between sister chromatites at all concentrations of the tested dyes. Except for two large concentrations, curcumin-induced chromosomal aberrations were significantly reduced, while tartrazine induced a significant increase in these aberrations. The results indicate that tartrazine is more clastogenic than curcumin.

Azorubin, another additive in the coloring category, is incriminated by Poul et al (2009) for inducing a significant increase in the mRNA level of a gene involved in the metabolic activation of certain procarcinogenic substances in the mice`s liver [31]. According to the above authors, tartrazine would have the same effects. Amin et al., (2010) also obtained the same results following studies in rats [2].

Evaluating the genotoxic and cytotoxic potential of **Brilliant Blue** (E133) dye, Kus and Eroglu (2015), observed a decrease in the mitotic index and replication index, and an increase in micronucleus incidence [21]. These changes were significant, demonstrating the genotoxic and cytotoxic potential of this additive.

Proquin et all (2017) conducted ***titanium dioxide*** (E171) dye studies to determine whether exposure to this additive induces chromosomal changes [33]. The results showed that it induces single-strand breaks of DNA as well as various chromosomal aberrations.

Monosodium glutamate is one of the most commonly used flavorings. Following an in vitro study, Ataseven et al. (2016) noted that this additive significantly increased the frequency of chromosomal aberrations: exchanges between sister chromatids; and micronuclei formation without affecting the replication and nuclear divisions [4].

Furfur-alcohol, a flavoring agent, has been studied by Fields et al., (2012), in vivo and in vitro [12]. The authors observed the presence of its mutagenic activity in the reversion mutation assay and that the additive can induce exchanges between sister chromatids in lymphocytes.

Erexson et al., (1985) sought **phenol** derivatives (from the flavor position) suspected of inducing shifts between sister chromatids in human lymphocytes [9]. The results of the observations confirmed an increase in exchanges between sister chromatides, related to the increase of tested doses. In a later study performed on Chinese hamster cell cultures, Glatt [14] did not confirm the results obtained by Erexon et al.

Timol (flavoring agent) was tested in vitro to induce chromosomal aberrations in Chinese hamster lung cells, alone and in combination with an exogenous activation system. The test alone did not induced aberrations in the chromosome structure, but in the presence of bioactivity generated chromosomal aberrations in the proportion of 10-20% of the exposed cells [22].

Anthranilic derivatives (methylanthranilate and methyl N-methylanthranilate) are used as flavoring agents. The two compounds were tested for reverse mutation and unscheduled DNA synthesis on rat hepatocytes, the results being negative. In contrast, according to Pronk and Speijers (2006), methylantranilate showed evidence of clastogenicity in the chromosomal aberration assay on Chinese hamster cell lines with and without metabolic activation [32].

From the group of α and β unsaturated aliphatic aldehydes used as flavoring agents, as clastogens were confirmed two compounds: ethyl acrylate produced direct mutations in mouse lymphoma cells, also increased the number of exchanges between sister chromatids and chromosome aberrations in Chinese hamster ovary cells in the presence of metabolic activation and in mouse lymphoma cells in the absence of metabolic activation [39]; 2-Hexenal significantly increased the number of exchanges between sister chromatids in lymphocytes as well as the number of chromosomal aberrations in vitro, and induced micronucleus formation both in vitro and in vivo [39].

Maltol (flavoring agent) was tested in vitro for reverse mutation with negative results but it induced shifts between sister chromatids in Chinese hamster ovary cells and in human lymphocytes.

In vivo, administered intraperitoneally, Williams and Schlatter (2006) observed micronucleus formation in mouse`s bone marrow [40].

Aluminum can be found in the structure of feed additives, such as sodium aluminosilicate (E554).

Benford et al. (2007), by studying circulating lymphocytes from the human species with aluminum chlo-

ride, found micronucleus formation and apoptosis [5].

Arsenic is present in the composition of fodder dyes (riboflavin, tartrazine, azorubine). The results of a large number of studies have led to the conclusion that arsenic additives do not react directly with DNA. Inorganic arsenic does not covalently bind to DNA, induce point mutations in bacteria or mammals, and has been shown to be an insignificant mutagen in a single locus (EFESA1). Instead, as Okui and Fujiwara (1986) reported, inorganic arsenic increases the genotoxicity, mutagenicity and clastogenicity of other agents (such as UV radiation or alkylating agents) inducing DNA alterations [29].

Among the arsenic compounds used more often in the production of feed additives is **sodium arsenate**.

Following in vitro tests carried out by Petres and Berger (1972) on lymphocytes and fibroblasts treated with sodium arsenate and arsenite, the authors found changes in chromosomes, represented by chromatid rupture, even at subtoxic doses [30].

Mure, (2003) proved that chronic exposure to low, non-cytotoxic arsenic concentrations induces delayed mutagenesis at the hypoxanthine-guanine-phosphoribosyl transferase site and cell transformations after 20-30 generations in human bone sarcoma cell cultures [28].

Hei et al. (1998) observed that in large concentrations and in several mammalian species, arsenate induced large deletions, shifts between sister chromatids and aneuploidy [15].

Other in vivo observations [11, 41] confirm that oral administration of arsenate induced chromosomal aberrations in mice 'lymphocytes and bone marrow.

Bibliographic assessments of genetically engineered feed additives

Antioxidants: ascorbic acid (E300), sodium ascorbate (E301), calcium ascorbate (E302), ascorbypalmitate (E304), and tocopherols are considered safe [34], while propyl gallate (E310), butylhydroxyanisole - E320) and butylhydroxytoluene (BHT - E321) raise some safety concerns [34].

Lignosulfonates, binder additives, available as sodium and calcium salts. Evaluation of genotoxic potential was done by reversion mutation tests with and without metabolic activation and chromosome aberration identification on various cells. The bibliographic investigations and their own results, negative as evidence of clastogenicity, obtained by Munro and Baines (2009), led them to conclude that the lack of genotoxicity in vitro and in vivo testing would be unwarranted [26].

Taking into account many studies on **Cassia gum** (E499), a technological additive in the category of thickening agents and gelling agents, but especially those of Jeurissen-coordinated teams [17, 18], the OMS committee decided that this is not genotoxic.

The colorants E104 - quinoline yellow and E110 - orange yellow, tested in vitro for mutations, had negative results [24, 25].

In vitro and in vivo tests performed on **lutein** (E 161b) for chromosomal aberrations and micronucleus formation, were negative [3], as well as those involving Zeaxanthin (E161h), a xanthophyll pigment encountered in nature together with lutein [3].

Following their own studies, Munro et al. (2009), validates previous studies claiming that paprika extract used as a flavoring or coloring agent has no genotoxic potential [27].

Studying the genotoxic effects of aliphatic amines and amides such as butylamine, piperidine, pyrrolidine and trimethylamine used as flavorings in animal feed, Abbott et al. (2006) [1] did not detect structural chromosome deviations nor micronucleus formation.

Methyl eugenol, a flavoring agent, was tested in vitro for chromosome aberrations with negative and positive results for shifts between sister chromatids [40]. In vivo, the appearance of micronuclei with positive results was evaluated [40].

Aliphatic and alicyclic hydrocarbons are found naturally in some foods, most commonly d-limonene, and as additives these compounds are used as flavorings. Camphen, β-cariophenylene, d-limonene and mircen were tested. Results were negative for shifts between sister chromatids [32]. In vivo, micronuclei or chromosome aberrations in bone marrow cells in rats did not occur [32].

The results of the studies, made by Choudhuri et al. (2012) on 3 phytase - an enzyme preparation, were negative for chromosome aberrations [6].

Xylanase, an enzyme that catalyzes the xylan hydrolysis to mono- and oligosaccharides, tested in vitro, does not produce mutations or chromosomal aberrations, as reported by a group of researchers [38].

Chimotrysin-serine-protease catalyses peptide linkages in proteins, used as an additive to obtain hydrolyzed proteins of plant or animal origin, has no clastogenic activity, tested in vitro by Choudhuri et al. [7].

Lasalocid natrium did not displayed genotoxic potential as revealed by in vitro tests performed by Jaret et al. (2014) [16].

The safety of **probiotics** is discussed in general terms and does not refer strictly to those used in animal

nutrition. The possibility that probiotics used in animal feed may reach the human food chain is not excluded. However, there is little information available on the risk of contamination of probiotic foods used in animals. The only serious risk would be the transfer of antibiotic resistance due to bacteria used as probiotics with resistance genes.

Monensin had negative results in a series of genotoxicity assays (reversion mutation, chromosomal aberrations in Chinese hamster cells), and the OMS committee's conclusion was that this additive has no genotoxic potential [36].

Lasalocid sodium did not show genotoxic potential in a series of in vitro tests made by Jaret et al. [16].

CONCLUSIONS

Among feed additives, two groups constantly generated chromosomal lesions, as follows:

- **the colorants** (azorubin,curcumin, tartrazine, penceau 4R-E124, brilliant blue -E133, titanium dioxide -E171);

- **flavorings** (monosodium glutamate, furfur-alcohol, timol, 2-methyl furfuran, antrantilate methyl, acrilate ethyl, 2-hexenale, alyl0izocyanate and maltole.

The most frequent chromosomila abnormalities generated by feed additives are represented by exchanges between sister chromatides, micronuclei forming and nonbalanced changes (deletions, defficiencies).

Not all feed additives exert adverse effects on genetic material.

RECOMMENDATIONS

Due to all negative effects exerted on genetic material, at least above mentioned feed additives shloud be withdrawn from animales used in reproduction and carefully administered to all other categories.

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