BODY MASS INDEX DYNAMICS AND BEHAVIORAL CHANGES IN SWISS MICE RECEIVING BRILLIANT BLUE FCF (E 133) AND AZORUBINE (E 122) IN DRINKING WATER

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Abstract

Among the extensive list of approved additives in human food industry, colourings (dyes - either naturally originated or synthetic) are widely used, in almost all processed food. The latter ones are frequently associated in humans (mostly in children) with the onset of altered behavioural conditions, such as the Attention Deficit/Hyperactivity Disorder - ADHD. The aim of this study was to quantify the effects of two food colourings given 35 days to 120 Swiss mice: CON group – control, plain water; BLU group – 50 ppm E133 – brilliant blue FCF (mostly used in beverages and sweets); AZO group – 50 ppm E122 – azorubine (mostly used in concentrated soups and sweets). The starting hypothesis (the usage of experimental factors will not modify the reasoning criteria - body mass index BMI and behavioural pattern) was invalidated. The mice in the experimental groups were underweighted (-16.5% BMI in AZO group, and -20% BMI in BLU group) ($p<0.05$; $p<0.001$). The ethogram also changed (decrease of the sleep and rest duration: -10,6% AZO group; -13,4% BLU group; triple time lasting of active type behaviours – playing, running, aggressive stances on both groups). These findings suggest that the usage of synthetic food dyes are a possible trigger of the ADHD condition in studied biological material, inducing anorexia and behavioural changes, as other studies conducted until now on laboratory animals suggested. However, a straight and absolute correlation between the experimental factors and ADHD onset could not be yielded, as large scale studies of this kind are very difficult to organize on human subjects.

Key words: Swiss mice, brilliant blue FCF-E133, azorubine-E122, body mass index, behaviour

INTRODUCTION

Color represents one of the most important sensorial parameters of the food, alongside with flavor and texture. It is very often associated by the consumers with a special taste (flavor) as well as with a certain expected level of inner quality and safety [5]. In natural food (especially in raw one), color is a clear indicator of freshness and a warning related to the degree of consumption safety or toxicity. An antique tradition was speaking about the safe food to be eaten – colored in tints of yellow, orange, red, green and brown, as well as about the poisonous one to be avoided - colored in blue and black tints [4]. How about modern processed food? It is clear that the color is not anymore a safety indicator hence many aliments are purposely colored to serve marketing needs. Among the food colorings widely used in the industry, the natural originated ones (extracts from vegetal or animal tissue) are generally well accepted and tolerated by most consumers. Many of them (anthocyans, betacyans, lycopene etc.) are even praised for their therapeutic and prophylactic traits in humans (especially for the antioxidative properties) [3, 10, 13]. Apart from these, the synthetic food dyes also play an important role in rendering a specific desired color of a processed aliment. Despite the fact that their usage are clearly regulated through EU laws [7]and Codex Alimentarius recommendations [6], there are several studies indicating potential risks towards the health status of the consumers, when a certain food contains the synthetic pigments. The most incriminated

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ones in inducing carcinogenic processes, toxic syndromes and even behavioral alterations (such as ADHD) in animal or human subjects are: tartrasine, quinolone yellow, amaranth, red ponceau 4R, allura red AC, brilliant yellow FCF, brilliant blue FCF, brilliant green, tartrasine, azorubine (carmoisine), eritrosine [1, 2, 4, 8, 11, 12]. Within this context, we proposed to investigate the effect induced by the consumption of two artificial food colorings (azorubine and brilliant blue FCF) onto the growth dynamics and behavioral aspects of Swiss mice.

MATERIAL AND METHOD

Hypothesis: introduction of food dyes in the drinking water do not influence growth rate and behavior of the studied subjects.

Experimental factors: two food dyes – azorubine (E122) and brilliant blue FCF (E133) diluted in drinking water and provided throughout 4 weeks

Biological material:120 Swiss mice, aged 1-5 weeks, sex ratio 1:1

Treatments: CON group (control) 40 mice, receiving basal diet + common drinking water;

AZO group – 40 mice, receiving basal diet + 0.5 ‰ azorubine in drinking water

BLU group – 40 mice, receiving basal diet + 0.5 ‰ brilliant blue FCF in drinking water

Basal diet: 40% corn, 20% wheat, 30% soybeans, 10% sunflower seeds, 0.1% salt, 1% premix – grinded then boiled and served as semi-humid meal with 30% moisture level.

Reasoning criteria: body mass index (g/cm^2)(BMI=body weight/body length^2) [9, 14] – assessed weekly and behavioral dynamics (24 hours etogram – proportional occurrence of different behaviours, based upon recordings on experimental day 1 and 28).

***Data analysis*:** ANOVA single factor, one by one comparisons between the three treatments of 40 individual values each.

Study outcome: hypothesis validation/invalidation and extrapolation of findings.

RESULTS AND DISCUSSIONS

Data related to the growth rate and growth quality are presented apart per genders, hence the growing rate is different in males and females and the body proportions are also different.

Thus in male specimens (table 1), at the onset of the experiment (day 1, mice aged 7 days) the body mass index (BMI) was calculated within the normal weight range (18.5-24.9 g/cm^2) [14] for all studied groups: 19.05±0.34 g/cm^2 (CON), 18.99±0.32 g/cm^2 (AZO) and 18.95±0.32 g/cm^2(BLU), with no occurrence of statistical significance post ANOVA processing. Therefore, the studied mice started from the same level of development.

Giving the experimental factors to the mice induced differentiations in the growth rate and the groups averages began to split apart. One week after trial onset the mice receiving Brilliant Blue FCF scored the BMI below the threshold for normal weight and became slightly underweighted (18.43±0.42 g/cm^2) ($p<0.001$ vs. CON) while the ones receiving azorubine scored an average BMI of 18.62±0.27 g/cm^2($p<0.001$ vs. CON). The males in control group began to be better proportioned and reached 19.45±0.27 g/cm^2.

This trend was kept until the last day of the experiment and the gap between experimental groups and control groups became deeper (fig. 1). Thus, CON mice reached 21.29±0.36 g/cm^2, the ones in AZO group scored 17.46±0.31 g/cm^2, while those receiving brilliant blue FCF reached the lowest level (17.22±0.35 g/cm^2), indicating high probability ($p<0.001$) of experimental factors influence onto the normality of body development and on achieving underweight condition. Moreover, significant difference ($p<0.05$) occurred between the BMI values of groups AZO and BLU, consequently to 3 weeks of experimental factors ingestion. Therefore, the food dye E133-brilliant blue FCF induced more acute growing depression than E122-azorubine, in male mice.

Table 1 Body mass index values, calculated for the female mice, throughout the entire experiment

Days of Treatment	Group	$\bar{X}$	±StDev	V%	min	max	ANOVA
1	CON	19.05	0.34	1.78	18.60	19.60	LC x AZO = ns (p=0.29>p0.05)
	AZO	18.99	0.32	1.69	18.38	19.49	LC x BLU = ns (p=0.023>p0.05)
	BLU	18.95	0.32	1.70	18.57	19.68	AZO x BLU = ns (p=0.39>p0.05)
7	CON	19.45	0.37	1.93	18.90	20.20	LC x AZO = *** (p=0.00015<p0.001)
	AZO	18.62	0.27	1.47	18.10	19.00	LC x BLU = *** (p=0.0008<p0.001)
	BLU	18.43	0.42	2.25	17.80	19.00	AZO x BLU = ns (p=0.09>p0.05)
14	CON	19.85	0.39	1.94	19.30	20.60	LC x AZO = *** (p=0.0003<p0.001)
	AZO	18.20	0.33	1.79	17.80	18.70	LC x BLU = *** (p=0.0005<p0.001)
	BLU	18.00	0.37	2.06	17.50	18.50	AZO x BLU = ns (p=0.07>p0.05)
21	CON	20.60	0.31	1.50	20.00	21.10	LC x AZO = *** (p=0.0002<p0.001)
	AZO	17.89	0.36	2.04	17.00	18.40	LC x BLU = *** (p=0.0001<p0.001)
	BLU	17.62	0.40	2.24	17.10	18.30	AZO x BLU = * (p=0.03<p0.05)
28	CON	21.29	0.36	1.71	20.70	21.90	LC x AZO = *** (p=0.00005<p0.001)
	AZO	17.46	0.31	1.76	17.00	17.90	LC x BLU = *** (p=0.00003<p0.001)
	BLU	17.22	0.35	2.05	16.70	17.80	AZO x BLU = * (p=0.03<p0.05)

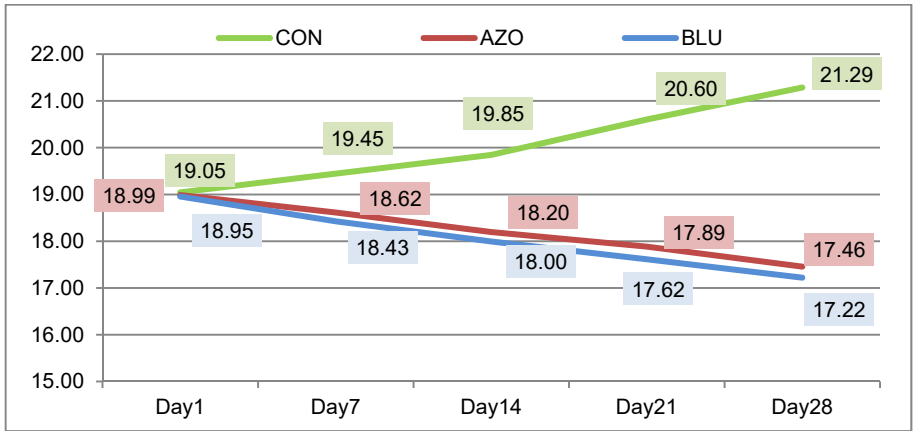

Fig. 1 Dynamics of the body mass index values in the male mice, throughout the entire experiment

From the facts presented in fig. 1, it could be seen that the experimental factors induced negative growth rate, hence the differences between experimental treatments ranged from quite null (0.5%, p>0.05) in the beginning of the trial to highly significant in the middle of it (-5.24%, p<0.001) as well as by its ending (-19.11%, p<0.001). This underweighted condition (BMI < 18.5 g/cm^2) negatively affects further development and retarded onset of the reproductive behavior, which normally should occur by the age of 50 days in this species.

Speaking of female specimens (tab. 2, fig. 2), the progressive trend was quite similar to that in males, while the calculated values were a bit higher. Thus, in the beginning, the lack of experimental factors influence was revealed in the close BMI values for all groups (CON=19.19±0.32 g/cm^2; AZO=19.10±0.33 g/cm^2; BLU=19.08±0.31 g/cm^2) (p>0.05). Progressively, the females in control group properly gained weight and increased the BMI value, which remained within the healthy weight area (21.82±0.53 g/cm^2), while the ones in the experimental groups stagnated in weight deposition and the BMI decreased till levels below the normality threshold and became under weighted (AZO group = 17.74±0.44 g/cm^2; BLU group = 17.53±0.46 g/cm^2). The differences versus control group were quite high to exceed the maximum likelihood threshold in ANOVA test (p<0.001), therefore it is a high

probability of this phenomena to repeat when the experimental factors will be provided under the same conditions. It is interesting to notice that, compared to males, the females did not differentiate significantly their growth performances in relation with a specific food dye we tested (azorubine vs. brilliant blue FCF) ($p>0.05$).

Table 2 Body mass index values, calculated for the male mice, throughout the entire experiment

Days of treatment	Group	$\bar{X}$	±StDev	V%	min	max	ANOVA
1	CON	19.19	0.32	1.64	18.70	19.60	LC x AZO = ns (p=0.38>p0.05)
	AZO	19.10	0.33	1.72	18.60	19.60	LC x BLU = ns (p=0.46>p0.05)
	BLU	19.08	0.31	1.65	18.60	19.60	AZO x BLU = ns (p=0.84>p0.05)
7	CON	19.71	0.33	1.65	19.20	20.30	LC x AZO = *** (p=0.0009<p0.001)
	AZO	18.72	0.38	2.05	18.30	19.30	LC x BLU = *** (p=0.0006<p0.001)
	BLU	18.61	0.41	2.23	18.10	19.30	AZO x BLU = ns (p=0.38>p0.05)
14	CON	20.35	0.40	1.98	19.70	21.10	LC x AZO = *** (p=0.0002<p0.001)
	AZO	18.54	0.38	2.04	18.00	19.00	LC x BLU = *** (p=0.0001<p0.001)
	BLU	18.29	0.43	2.33	17.60	18.80	AZO x BLU = ns (p=0.06>p0.05)
21	CON	21.10	0.46	2.16	20.40	21.90	LC x AZO = *** (p=0.00015<p0.001)
	AZO	18.10	0.41	2.24	17.50	18.60	LC x BLU = *** (p=0.00005<p0.001)
	BLU	17.91	0.49	2.73	17.20	18.50	AZO x BLU = ns (p=0.18>p0.05)
28	CON	21.82	0.53	2.45	21.30	22.60	LC x AZO = *** (p=0.00005<p0.001)
	AZO	17.74	0.44	2.50	17.10	18.60	LC x BLU = *** (p=0.00003<p0.001)
	BLU	17.53	0.46	2.65	16.80	18.30	AZO x BLU = ns (p=0.15>p0.05)

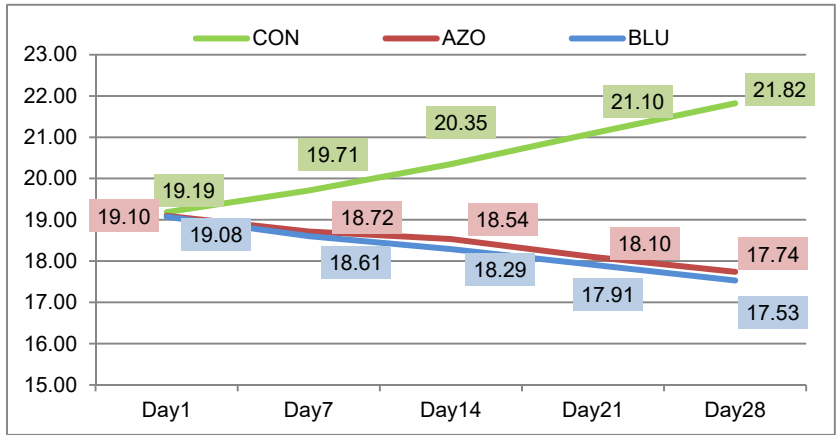

Fig. 2 Dynamics of the body mass index values in the female mice, throughout the entire experiment

The relative differences between groups ranged from -0.57% in the first day of study (CON vs. BLU, $p>0.05$) to -11.26% in the midway of the experiment (CON vs. BLU, $p<0.001$) and to -19.66% (CON vs. BLU, $p<0.001$).

These facts also suggest a negative influence of the food dyes E122 and E133 on the female normal development and a risk for the health of the upcoming mature and reproductive life.

From the data issued per genders, the results suggests that the growing rate depression (from experiment beginning to its end) follows a nonlinear, however an average negative trend of 1.83% per week in female mice receiving azorubin and of 2.09% per week in those receiving brilliant blue, 0.5 ‰ diluted in drinking water. In males, the decreasing trend was more critical (-2.07% per week in AZO group and -2.36% per week in BLU group).

Despite the evidences we found on the influence of the studied food colorings on Swiss mice development, these could not be categorically extrapolated towards humans and especially towards children, hence the laboratory animals we used had higher metabolism rates and a relatively short lifespan, compared to human consumers. Also, the inclusion rate was pushed to the upper limits admitted by EFSA and FAO. In the humans, a comparable study should rather take also into account the repetitive behavior in consuming artificially colored food and the cumulative effect from multiple sources.

What happened to mice behavior throughout the 4 experimental weeks? Fig. 3 shows not significant differentiations between the groups, relative to the main categories of studied behaviours: feed intake, exploring, rest, sleep, other activities (socializing, playing, aggressive stances etc.). Diagram in fig. 4 depicts the behavioral changes in the end of the experiment.

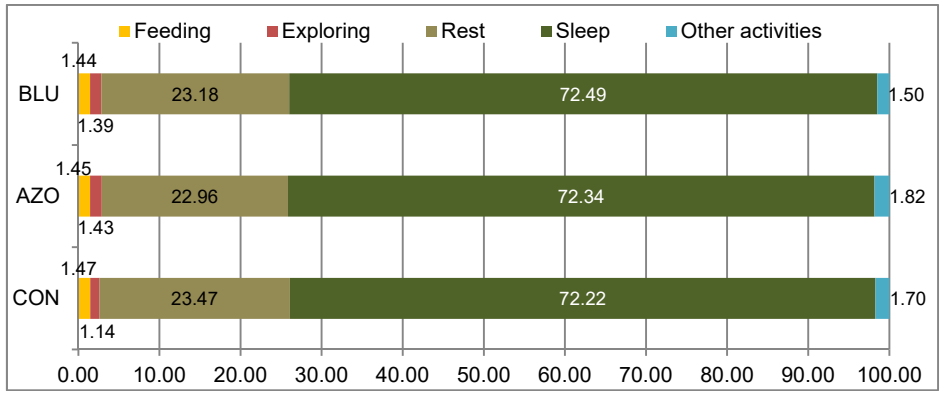

Fig. 3 Fractional ethogram(24 hrs cycle) of the 7 days old Swiss mice, comprising the main behavioral categories (%), at the beginning of the experiment

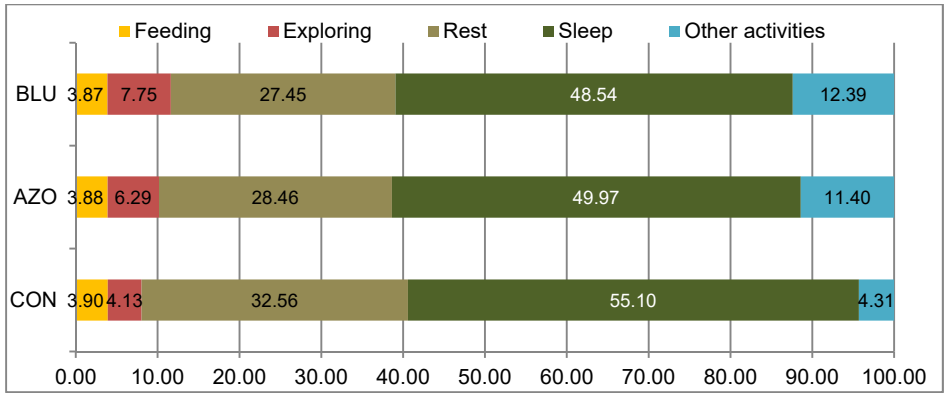

Fig. 4 Fractional ethogram (24 hrs cycle) of the 35 days old Swiss mice, comprising the main behavioral categories (%), at the end of the experiment

Thus, out of a 24 h observation cycle during experimental day 1, feeding reached 1.44-1.47%; exploratory behavior 1.14-1.43%, rest (not asleep) 22.96-23.47%, sleeping 72.22-72.49% and other kind of activities 1.50-1.82.

Providing the experimental factors throughout 28 days induced alterations of behavioral patterns, apart from those naturally occurring, observed in control group (decrease of sleeping period and increasing of the other behavioral categories proportion).

Thus, the quiet restful periods reached 87.66% from a whole day in CON group, while in experimental group were found at 78.43% (AZO, -10.53% vs. CON) and 75.99% (BLU, -13.31% vs. CON). Among the active behaviours, the timeframe allocated to feeding increased almost 3 folds (3.9%) compared to the experiment onset (1.47%) and remained quite close between groups (3.87-3.90% from a 24 hrs. cycle). Exploring activities also increased almost 3 folds (in CON from 1.14% to 4.13%) till 5 to 6 folds (in experimental groups). Thus, the mice receiving azorubine explored 34.34% more and those receiving brilliant blue explored 46.71% more than the mice in control group. Moreover, the other kinds of active behaviors (playing, socializing, aggressive stances) also increased significantly their proportion per 24 hrs. cycle, to reach by the end of experiment 4.31% in control group and almost triple (vs. CON) in AZO group (11.41%) as well as in BLU group (12.39%). Therefore, a hyperactive pattern could be noticed in the experimental groups and an apparent reversed correlation could be outlined between the behavioral changes and the decrease of the BMI in experimental treatments.

CONCLUSIONS

The hypothesis was invalidated, therefore the experimental factors (azorubine – E122 and brilliant blue – E133 included 0.5 ‰) in drinking water negatively affected growth rate and body mass index and induced hyperactive behavioral changes in studied mice.

The usage of synthetic food dyes is a possible trigger of the ADHD condition in studied biological material, inducing anorexia and behavioural changes.

A straight and absolute correlation between the experimental factors and ADHD onset in humans could not be tough yielded.

REFERENCES

[1] Amchova P., Kotolova H., Ruda-Kucerova J.: Health safety issues of synthetic food colorants, Regulatory Toxicology and Pharmacology, 2015, 73(3): 914-922.

[2] Amin K.A., Abdel Hameid H., Abd Elsttar A.H.: Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chemical Toxicology, 2010, 48(10):2994-2999.

[3] Antal D.S.: Medicinal Plants With Antioxidant Properties From Banat Region (Romania): A Rich Pool For The Discovery Of Multi-Target Phytochemicals Active In Free-Radical Related Disorders. Analele Universităţii din Oradea - Fascicula Biologie, 2010, XVII (1):14-22.

[4] Banu C., Stoica A., Bărăscu E., Buţu N., Resmeriţă D., Vizireanu C., Lungu C., Iordan M.:Aplicaţii ale aditivilor şi ingredientelor în industria alimentară, Editura ASAB, Bucureşti, 2010.

[5] Burrows J.D.A.: Palette of Our Palates: A Brief History of Food Coloring and Its Regulation. Comprehensive Reviews in Food Science and Food Safety, 2009, 8: 394–408.

[6] Codex General Standard for Food Additives - http://www.fao.org/fao-who-codexalimentarius/standards/gsfa/en/, 2016

[7] European Food Safety Association – Food additives,http://www.efsa.europa.eu/en/topics/topic/additives, 2016

[8] El-Wahab H.M., Moram G.S.: Toxic effects of some synthetic food colorants and/or flavor additives on male rats. Toxicol Ind. Health., 2013, 29(2):224-232

[9] Engelbregt M.J.T., Van Weissenbruch M., Popp-Snijders C., Lips P., Delemarre-van de Waal H.A.:Body Mass Index, Body Composition, and Leptin at Onset of Puberty in Male and Female Rats after Intrauterine Growth Retardation and after Early Postnatal Food Restriction. Pediatric Research, 2001,50, 474–478.

[10] He Y.K., Yao Y.Y., Chang Y.N.: Characterization of Anthocyanins in Perilla frutescens var. acuta Extract by Advanced UPLC-ESI-IT-TOF-MSn Method and Their Anticancer Bioactivity. Molecules, 2015, 20(5):9155-9169.

[11]McCann D., Barrett A., Cooper A., Crumpler D., Dalen L., Grimshaw K., Kitchin E., Lok K., Porteous L., Prince E., Sonuga-Barke E., Warner J.O., Stevenson J.:Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet,2007, 370 (9598):1560-1567.

[12] Schab D., Trinh N.: Do artificial food colors promote hyperactivity in children with hyperactive syndromes? A meta-analysis of double-blind placebo-controlled trials. Journal of Developmental and Behavioral Pediatrics, 2004, 25: 423-34.

[13] Sikora E., Cieslik E., Topolska K.: The sources of natural antioxidants. Acta Scientarum Polonicum, Technol. Aliment., 2008, 7(1):5-17.

[14] World Health Organization, Body mass index. http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi, 2016