MMSL Military Medical Science Letters Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2024, 93(4), 364-376
ISSN 2571-113X (Online)
DOI: 10.31482/mmsl.2023.039

REVIEW ARTICLE

JUVENIL, A NATURAL IMMUNE BOOSTER AFFECTING BIOLOGICAL RESPONSES THROUGH MODULATION OF GUT MICROBIOTA COMPOSITION

Vanda Bostik [1], Klára Kubelková [2] ✉, Aleš Macela [2]

[1] Department of Epidemiology, Military Faculty of Medicine, University of Defence, 500 01 Hradec Kralove, Czech Republic
[2] Department of Molecular Pathology, Military Faculty of Medicine, University of Defence, 500 01 Hradec Kralove, Czech Republic

Received 6th August 2023.
Accepted 24th October 2023.
Published 2nd December 2024.

Summary

Juvenil, a nontoxic extract of bovine blood, is registered as a dietary supplement having no side effects. It contains a broad spectrum of free amino acids, as well as small proteins and oligopeptides (molecular weights up to 10 kDa), various nucleotides, and small amounts of phospholipids. The complex of these exclusively natural components has been shown to support physiological responses of supplemented organisms.

Juvenil has been studied for several decades using a wide range of both experimental and clinical studies. Analyses have shown that it acts directly neither on individual functional systems of the body nor on individual metabolic processes. Current findings indicate that modulatory effects of Juvenil occur through modulation of gut microbiota composition, which is associated with the modulation of microbiota–gut–brain axis signaling. In murine model that modulatory effect is reflected in the expression of an early activation c-Fos marker in specific parts of the brain.

In this review we present a set of findings about Juvenil, which has a wide range of positive effects on the functional systems of organisms. These effects can be used to strenghten the resistance, immunity, and regeneration of human beings. According to its effects Juvenil can be classified as a psychobiotics.

Key words: dietary supplement; gut microbiota; Juvenil; biological response modifier; prebiotics; probiotics; psychobiotics

1. Rise of Juvenil as food supplements

Natural biological products were in ancient times the first drugs for improving the fitness of human beings. They were prepared in the forms of teas or pastes from plant tissues or as extracts prepared from plant or animal

✉ University of Defence, Military Faculty of Medicine, Department of Molecular Pathology, Třebešská 1575, 500 01 Hradec Kralove, Czech Republic
klara.kubelkova@unob.cz

tissues by extraction using various solvents or by fermentation processes. A considerable body of empirical experience was accumulated over the years and was reflected in technological improvements. Many historically developed natural biological products remain in use today in the forms of prebiotics (1-3), probiotics (4-6), symbiotics (7-9), and/or nutritional supplements (10-13) to support the effect of classical treatment. Over time, such products have contributed to changes in the approaches to some health problems and given rise to new prophylactic and therapeutic methods to support tissue regeneration or innate immune responsiveness during unspecific sicknesses or convalescence from infection or cancerous illnesses.

During the 1940s, a new technological procedure for the preparation of extracts of various plant and animal tissues was created. Bovine blood (Retisin, Sangitin for human use or Aminex and Imuvet for veterinary purposes), herring sperm and eggs (Silexil), honey RTN 119, RTN 134) and/or St. John's wort (Floristen) were used as starting materials (14). The method consisted in extraction from freshly dried tissues using various organic solvents. The resulting extract was then subjected to several sequential procedures to isolate low-molecular complexes of biologically active substances. A number of different preparations were obtained and subsequently evaluated by way of pharmacological analyses, preclinical testing, and clinical trials (14). Clinical testing was focused on malignancies in the terminal stage (15), on vascular (16, 17) and rheumatological diseases (18), and on chronic gynecological inflammations and gynecological oncological diseases (19-21).

One of the active preparations prepared in injectable form was Retisin (14, 15). Retisin has shown a polyvalent effect in the treatment of post-radiation complications such as proctitis and cystitis. Changes on the mucous membranes in the rectum or bladder disappeared quickly after the application of Retisin. In addition, Retisin significantly improved the general condition of patients with pneumonia, had a positive effect on the blood count, calmed the nervous system and eliminated depressive states, and alleviated pain. Retisin was produced and used in injectable form until 1975. In that year the production and utilization of Retisin as a supportive treatment for oncological malignancies was stopped in accordance with the regulation known as “Action Mars,” which had been intended to modernize the assortment of drugs in the Czechoslovak health care system (22).

The last two decades of the 20th century brought essentially a rebirth for this interesting preparation due to a general interest in alternative procedures for maintaining the physical and mental condition of employees burdened by many new stressors. A need arose for new dietary supplements based on natural materials. The original technology for the preparation of Retisin was reused for production of bovine blood extract under the copyright name Juvenil. The Juvenil is also the basis for products under the brand name Imuregen (23).

2. What does Juvenil actually contain?

Analyses of the Juvenil have been focused on those components that the extract has been assumed likely to contain, that is to say, amino acids, proteins, peptides, nucleotides, and lipids. According to the original analyses carried out with the extract, Juvenil contains the entire spectrum of amino acids, including both free amino acids as well as amino acids bound in peptides. Repeated analyses of amino acid content and their composition have shown Juvenil to contain the whole spectrum of amino acids, including all essential amino acids. The contents of methionine and cysteine in Juvenil seem to be understated, however, because both are partially decomposed during hydrolysis. An analysis carried out at the Medical Faculty of Palacky University, Olomouc, Czech Republic, revealed a presence of taurine, which is an amino acid derivative of cysteine (24).

One-dimensional mini electrophoresis of Juvenil has demonstrated the presence of only such proteins or peptides as have molecular weights not exceeding 10 kDa. According to repeted analyses, Juvenil contains several different peptides sequentially corresponding to alpha or beta casein, peptides corresponding to histones H2A, 62 peptides sequentially corresponding to the alpha hemoglobin subunit, and 10 peptides sequentially corresponding to the beta hemoglobin subunit. Bioinformatics analysis of tandem mass spectrometry data obtained from Juvenil’s testing identified seven peptide sequences corresponding to seven hemocidins. Not identifiable, however, were peptide sequences corresponding to bovine VV- or LVV-hemorphins and spinorphin or a bovine heptapeptide (LVVYPWT) belonging to the hemorphin family. Among the peptides corresponding sequentially to other biologically active peptides were also identified sequences corresponding to β defensin 13, β-defensin 2, β-defensin 4, β-defensin 4 precursor, and β-defensin 5 (24).

Inasmuch as the mass threshold of 10 kDa was not surpassed, it cannot be assumed that the original proteins of bovine blood occur at full length in Juvenil preparation. This indicates that the resulting peptides formed from the original proteins would no longer have the biological functions of the parent proteins. Such peptides, while inactive within the protein sequence, require enzymatic proteolysis to create bioactive fragments from the protein precursors. Peptide fragments from protein precursors are potential biological response modifiers influencing various regulatory processes in the living system or having direct effector functions. For example, buforins are histone H2A-derived peptides that enter into bacteria and fungi, bind to microbial nucleic acids, and thereby kill them (25); bovine lactoferricin derived from proteolytic cleavage of bovine lactoferrin is a multifunctional peptide that demonstrates antibacterial, antifungal, antiviral, antitumor, and immunomodulatory activities, including anti-inflammatory and anti-catabolic functions (26, 27); and there exists a whole range of hemocidins, which are antimicrobial peptides derived from mammalian alpha and beta hemoglobin chains that function as porins and thereby disrupt plasma membranes (28-30). Casein is a rich source of biologically active peptides having antihypertensive, anti-thrombic, and anti-aggregating effects or functioning as opioids (β casomorphins) (31). Casein is not the only protein having endogenous peptides with opioid function encoded in its amino acid sequence. Hemoglobins are source of hemorphins, which can be released from almost any of the hemoglobin chains (beta-, kappa-, delta-, or epsilon-chain) except the alpha chain (32).

Adenosine monophosphate dominates among the nucleotides in Juvenil. The second most abundant nucleotide was found to be inosine monophosphate, which is typically present in animal tissues and meat industry waste. Juvenil also contains small amounts of dinucleotides, of which cytidine diphosphate has a significant presence. Even repeated analyses could not identify thymidine monophosphate, which is utilized as a monomer in DNA (24). For this reason, it is possible to assume that the content of nucleotides in Juvenil does not come from nuclear DNA but that it represents the content of free nucleotides in bovine blood plasma.

The last molecular component of Juvenil upon which analyses were focused are lipids (phospholipids), which are essential components of cell membranes and could be the essential active component of Juvenil. Juvenil´s content of this component, however, appears to be entirely minor. The quantity of (phospho)lipids in Juvenil suggests that their content constitutes only a residuum of phospholipids that failed to be removed by the technology used in Juvenil's production (24).

Preparations prepared with the same technology as Juvenil also contain a colored complex, which differs from ordinary porphyrin derivatives in its absorption in the visible part of the spectrum (14). Polyphenols originating from the plant-based feedstuffs provided to cattle might be regarded as active ingredient candidates.

Organic solvent extracts from bovine blood constitute a group of functionally effective tissue preparations. In addition to the extract, the effects of which we summarize here, this group includes, for example, those products with trade names Solcoseryl (33-35) and Actovegin (36-39). Immunomodulation, tissue regeneration, and limitation of stress perception has been shown as general modulatory activity of all bovine tissue extracts.

3. Juvenil as a biological response modifier

Functional profile testing of commercial products based on the original "Retisin" technology is very extensive and has been carried out over several decades. It should be mentioned from the outset that, from a hygienic and toxicological point of view, Juvenil, as a food supplement, has repeatedly been proven completely safe and without any side effects both in experimental studies and in studies on volunteers (40). Preclinical testing was conducted based upon several experimental models and focused upon the modulation of biological responses *in vitro* as well as *in vivo* using the inbred mouse model (Table 1). Prolonged supplementation of mice with Juvenil through a 30-day preventive application in the form of a drinking regime modulated intercellular communication within cells of the immune system via regulatory cytokines and, from a functional point of view, demonstrably affected the differentiation and activity of cytotoxic T cells, the effectors of adaptive cell-mediated immunity, and the alertness of natural killer (NK) cells, which are powerful effector cells of the innate immunity. Moreover, testing of the humoral arm of immune responsiveness modulation showed increase in the absolute number of cells producing IgM and IgG antibodies in the spleens of treated animals (41, 42). Also lower doses of Juvenil have in an *in vitro* system significant cytostatic/cytotoxic effect on tumor cells while having only marginal effect on the nontumor cells (43).

Table 1. Modulating effects of Juvenil based on experimental (preclinical) models.

Modulating effect	Results	Experimental model	Reference(s)
Cytostatic/cytotoxic effect on tumor cells	Juvenil reduces viability and proliferation activity of the H1299 tumor cell line was significantly limited while leaving non-tumor NHLF cell line unaffected	*In-vitro* model	43
Bactericidal activity	Unsignificant effect on Gram-negative bacteria, no effect on Gram-positive one	*In vitro* model	44
Mitogenic activity	Juvenil don´t have mitogenic activity	Inbred strains of mice, *ex-vivo* studies (drinking mode)	41
Co-stimulatory aktivity	Down-regulation of spleen cell response to Noc-A, PHA, and LPS	Inbred strains of mice *ex-vivo* studies (drinking mode)	41
Lymphocyte proliferating activity	The response of spleen cells to ConA, PHA, as well as to LPS was significantly increased	Inbred strains of mice *ex-vivo* studies (drinking mode)	41, 42
Cytotoxic aktivity of macrophages	Significantly increases the cytotoxic activity of adherent spleen cells against P815 target cells	Inbred strains of mice *ex-vivo* studies (drinking mode)	41
Activity of NK cells	Juvenil significantly increases the cytotoxic activity of murine NK cells against YAC-1 target cells	Inbred strains of mice *ex-vivo* studies (drinking mode)	41, 42
Production of antibody	Production of IgM as well as IgG were significatly increased	Inbred strains of mice, *in vivo* studies	41, 42
Cytokine production	Production of IL-2, IL-4, IL-12, and IFN-γ was significantly increased, production of TNF was without changes	Tumorigenesis model using Balb/c and C57bl/6 inbred mice inoculated with B16F10 tumor cells (drinking mode – *in vivo*)	42
Regeneration of mucous membranes	Positive effect on the regeneration and growth of the epithelium of the terminal ileum	Murine imbred strain Babc/c, *in-vivo* study	42, 45

In the context of veterinary care for farm animals, Juvenil was tested as a supplement to feed rations (Table 2). It has been tested on broilers, piglets, calves, and even fur animals (silver foxes) and racehorses (48-50). All these studies demonstrated better utilization of feed rations by farm animals and improved health status as demonstrated by a reduced need for antibiotics use on farms.

Table 2. Testing of Juvenil on the models of farm animals.

Modulating effect	Results	Experimental model	Reference(s)
Fattening of farm animals	Improved health status, better utilization of feed nutrients and higher gains. Limited needs for antibiotic treatment	Swine - supplementation of feed rations	46, 48, 49
Fattening of farm animals	Improved health status, better utilization of feed nutrients and higher gains. Limited needs for antibiotic treatment	Calf - supplementation of feed rations	47, 51, 52
Influence on (super) ovulation of sows	Non-significant increase in the number of transferable embryos per donor	Sows – intramuscular application of Juvenil	50

Note: Juvenil for the veterinary used was produced under the commercial name Aminex or Imuvet.

As mentioned above, clinical testing of Juvenil was focused initially on terminal stage malignancies, vascular and rheumatological diseases, chronic gynecological inflammations, and gynecological oncological diseases (Table 3). After it became impossible to use in injectable form preparations not precisely defined molecularly and, moreover, originating from animal tissues, Juvenil was thereafter produced solely as a food supplement and, as such, was tested on human volunteers with various health problems (41, 53, 55, 56). In all these studies, the dominant effect of Juvenil was modulation of the test subjects' immune status. In malnourished children and children with chronic respiratory problems, supplementation with Juvenil contributed to improving their health status. The health status of workers employed in physically demanding conditions (glassworkers) was also improved, as documented by reduced morbidity.

Table 3. Clinical data from testing of Juvenil preparation.

Clinical status	Modulating effect	Results	Reference(s)
Healthy female university students (20.7 to 23.9 years old)	Blood pressure	Significant reduction in systolic and diastolic blood pressure, insignificantly increased heart rate	43
Healthy female university students (20.7 to 23.9 years old)	Psychological tests	Improving recent memory span, mental performance was not altered by Juvenile	43
Fatigue syndrome based on activated EBV infection	Laboratory and clinical status	Significant improvement of laboratory parameters and clinical manifestations by nutritional supplementation	42
Chronic respiratory problems	Six minutest walk test, levels of eNO, and IgA in saliva	Strong improvements in physical activity, strong decrease of eNO levels and maitaining of the levels of IgA	42, 53
Gynecological malignances	Actinotherapy	Reduction of postradiation complication (proctitis, cystitis)	15
Terminal stage of malignances	Health status	Appetite enhancement, insomnia management, alleviation of depressive states	4, 15
Degenerative joint disease	Arthritis	Improved movement activity and reduction of pain	54

A critical evaluation of bovine tissue extract's modulatory effects as presented in research reports and/or published in journal reveals a broad spectrum of beneficial effects for recipients of supportive remedies. How can we explain the multiple effects of the bovine blood extract Juvenil, and how should we interpret the results of the model studies?

In the original publications of Juvenil preparation technology, the authors had pointed out the possibility that modulating effects of Juvenil are effected by a color complex that is different from the porphyrin derivatives. This color complex remained unspecified. Considering the composition of cattle diets, the polyphenols can be examined as potentially constituting an effective component of Juvenil. Polyphenols are contained in different quantities and varying compositions in almost all plants and are components of many vegetable and herbal medicines. Later, opinion arose that the multiple effects of Juvenil are caused by the distribution of individual molecular components to relevant cell receptors, that after binding to the receptors there occur changes in the epigenetic functional profile of the corresponding cells, and the affected cells subsequently express their genetically preprogrammed functions (24). Bioactive peptides can interact with receptors on the luminal side of the intestinal tract and/or can be absorbed and through lymphatic and blood channels react with receptors of any cells within an organism's various functional systems. Finally, a working hypothesis can be stated that the molecular components of Juvenil change the composition of intestinal microbiota and thus modulate signaling within the microbiota–gut–brain axis.

4. Microbiota–gut–brain axis and Juvenil

The term microbiota encompasses a community of bacteria, fungi, parasites, and viruses that lives in a symbiotic homeostasis within a certain ecological niche of a eubacterial multicellular organism, more specifically in the *Deuterostomia*, meaning most predominantly the subphylum Vertebrata. Together with the host organism, the microbiota creates a so-called metaorganism (i.e., a dynamic ecosystem made up of a number of biological species). The gut microbiota plays a major role in maturation of the mammalian immune system, as has been demonstrated repeatedly using experiments in germ-free models. A balanced composition of intestinal microbiota also protects the intestinal epithelium by preventing pathogens from binding to mucosal cells. In relation to physical and mental health, the intestinal microbiota plays an irreplaceable role in the production of signals for the nervous system that is the production of neurotransmitters. Commensal intestinal bacteria from the families *Bacteroidaceae*, *Bifidobacteriaceae*, and *Lactobacillaceae** produce gamma-aminobutyric acid (GABA), which is the dominant inhibitory neurotransmitter of the central nervous system (30). GABA has the ability to influence a number of central nervous system disorders, including behavioral, pain, and sleep disorders (57). Another neurotransmitter whose levels are dependent on the intestinal microbiota is serotonin. Although it probably is not produced directly

by intestinal microorganisms, these bacteria constitute the main activators of serotonin production by enteroendocrine cells within the intestine. However, recent reports demonstrate the direct metabolism of tryptophan by some bacterial species (58). The same applies to melatonin, whose precursor is also the amino acid tryptophan (59). Moreover, short-chain fatty acids (SCFA) should be mentioned. These are formed through the fermentation of fiber by some bacteria from the phyla *Bacteroidetes* and/or *Firmicutes*; other producers include some *Actinobacteria* and *Verrucomicrobia* (60). SCFA, and especially butyrate, can utilize a defect in the intestinal barrier and after escaping from the intestine can cross the blood–brain barrier and thereby activate the vagus nerve. The signal terminates in that part of the brain that is the hypothalamus. The main backbone of the microbiota–gut–brain axis consists in the neurotransmitters produced by bacteria or their production by enteroendocrine cells activated by bacteria (61) (Figure 1). Experimental models of stress (62-64) and obesity (65-67), as well as in clinically significant situations, there has been demonstrated a diminished frequency of *Bacteroidetes* phyla in the gut and a proportional increase in the frequency of *Firmicutes*. Dysbiosis (i.e., disruption of the normal composition of microorganisms in the intestine and a reduction in the amounts of important bacteria) has been demonstrated in obese people (68, 69), in patients with inflammatory bowel disease (70, 71), in patients with colorectal cancer (72, 73), in children with autism spectrum disorder (74-76), and in people under chronic stress (77, 78).

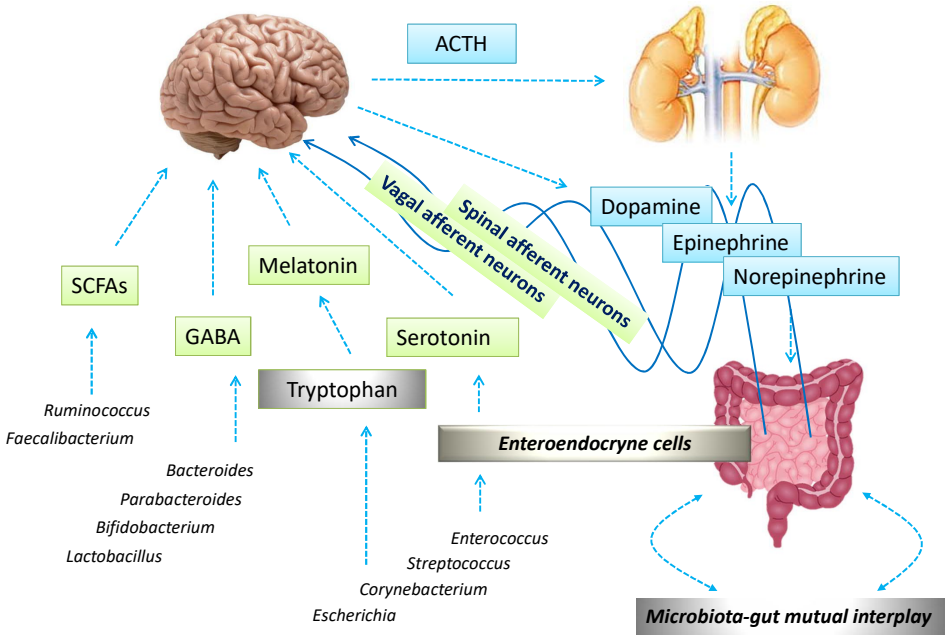

Figure 1. Schematic illustration of the microbiota-gut-brain axis. Intestinal microbiota and the gut have established equilibrium that protects the intestinal epithelium by preventing pathogens to populate mucosal cells and expressed their damaging effect. The disturbance of this equilibrium, dysbiosis, threatens both the physical and mental health. Dysbiosis threatens both physical and mental health by changing the production of neurotransmitters by bacteria or enteroendocrine cells. Neurotransmitters, as molecular signals, are the essence of the microbiota-gut-brain axis function. These are mainly short-chain fatty acids (SCFA), gamma-aminobutyric acid (GABA), melatonin or serotonin. The feedback is provided by the hypothalamus–pituitary–adrenal axis where the first signal is represented by secretion of adrenocorticotropic hormone (ACTH) produced by pituitary followed by catecholamines produced by the adrenal glands. The catecholamines that are produced also by the postganglionic fibers of the sympathetic nervous system can be the direct signals for microbiota-gut mutual interplay.

Juvenil that is the subject of this review, tested in a mouse model, showed exactly the opposite trend in the frequency of both bacterial strains dominant within the gut. Administration of Juvenil significantly reduced the abundance of the phyla *Firmicutes* relative to the phyla *Bacteroidetes*, both at the level of bacterial phyla as well as at the level of individual genera. A more detailed analysis concluded that Juvenil inhibited the growth of genera from the phylum *Firmicutes* rather than promoting the growth of genera belonging to the phylum *Bacteroidetes*. Given that Juvenil has a very weak bactericidal activity, it must be considered that its effect

on the intestinal microbiota results from internal modulation of intestinal physiology or the state of the host's natural immunity (44). The most frequent phyla in the murine gut are *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. From the phylum *Firmicutes* significantly downregulated are the genera *Faecalibacterium*, *Aerostipes*, and *Roseburia*, all producing SCFA butyrate, as well as *Phascolarctobacterium*, *Prevotella*, *Eubacterium*, and *Veillonella* producing propionate SCFA. Also downregulated is Fusobacterium from the phylum *Fusobacteria* producing acetate and butyrate. In contrast to the effect of Juvenil on SCFA-producing bacterial genera, the effect on GABA-producing bacterial genera is selective, the relative frequency of the *Bifidobacterium* and *Bacteroides* being downregulated and the frequency of *Escherichia/Shigella* and *Lactobacillus* genera from the phylum *Firmicutes* being upregulated. Juvenil has a completely positive effect, however, on the frequency of bacterial species involved in the production of serotonin. The frequencies of the genera *Corynebacterium*, *Streptococcus*, or *Enterococcus* participating in the activation of production by enteroendocrine cells are greatly increased among the genera of gut microbiota (44).

5. Juvenil as a modulator of neural activity

Testing of c-Fos expression in the brains of mice after their supplementation with Juvenil revealed selective expression of c-Fos in the different brain parts and its dependency on duration of Juvenil's supplementation. After stimulation of mice with Juvenil, changes in c-Fos expression were noted in the hippocampus (and specifically in the CA2 and gyrus dentatus areas), in the thalamus, hypothalamus, the area of the dorsomedial nuclei, and then in the amygdala (central body) and in the piriform cortex and the primary motor cortex (Figure 2). The dependency of c-Fos expression intensity at individual parts of the brain on the duration of supplementation with Juvenil was surprising. In some cases, prolonged supplementation had the opposite effect in comparison to brief exposure (79).

It should be noted that, due to the small number of experimental animals involved, no calculations were made to examine statistical significance of the results. Nevertheless, it is evident from the graph that the 30-day supplementation (Ext 30) had a general dampening effect on c-Fos expression, with the exception of in both parts of the cortex. Seven-day supplementation (Ext 7) had the opposite effect in both parts of the cortex, while in the hippocampus, thalamus, and amygdala, seven-day supplementation rather increased the expression of c-Fos. In the hypothalamus, by contrast, both supplementation scenarios had rather a dampening effect.

The protein c-Fos proto-oncogene encoded by the fos gene forms dimers with some proteins of the c-Jun group and occurs in the transcription factor AP-1 regulating gene expression in response to a variety of stimuli, including by cytokines, growth factors, stress, and bacterial and viral infections (80-84). AP-1 is a short-term impulse for changes in the expression of cellular functions corresponding to preprogrammed cellular functions that are epigenetically controlled. c-Fos protein is used as a marker of functional activity in neurons. The protein c-Fos is one of the most studied immediate early genes in the brain (85). Transcription of the c-Fos protein, both on positive and negative signals, is a very rapid process. Expression of c Fos mRNA occurs within minutes and lasts for as long as 60 minutes after the stimulus. These characteristics make c-Fos expression suitable as an early sign of neuronal stimulation (86).

4. Conclusion and Perspectives

Although Juvenil has been studied for decades as a food supplement using various experimental, veterinary, and clinical models, it turns out that Juvenil does not act directly on individual functional systems of the organism or on individual metabolic processes. A summary of veterinary reports regarding Juvenil suggests its stimulatory effect. At the recommended and tested dosage on large groups of farm animals, Juvenil does not directly affect metabolic processes, but, through the central nervous system, it affects the energy balance and the production of neurohormones, growth factors, and gonadotropins. It has been concluded definitively that Juvenil manifests itself only as an initiator of production capabilities and a regulator of "regulatory cycles" in farm animals (48). Since this report was presented in 1992, the overall conclusion has remained very close to the current opinion as to Juvenil's effect. This action can be described as impulse therapy "*therapia non specifica*, nonspecific therapy, impulse treatment". This is also how balneotherapy is characterized, as it achieves both local and global effects through the summation of several stimuli, both physical and chemical. Balneology induces immediate biological reactions to the natural sources and, by intervening in damaged life processes, induces long-term effects that cannot be achieved with other therapies. This characterization applies also to children with chronic respiratory problems treated by speleotherapy.

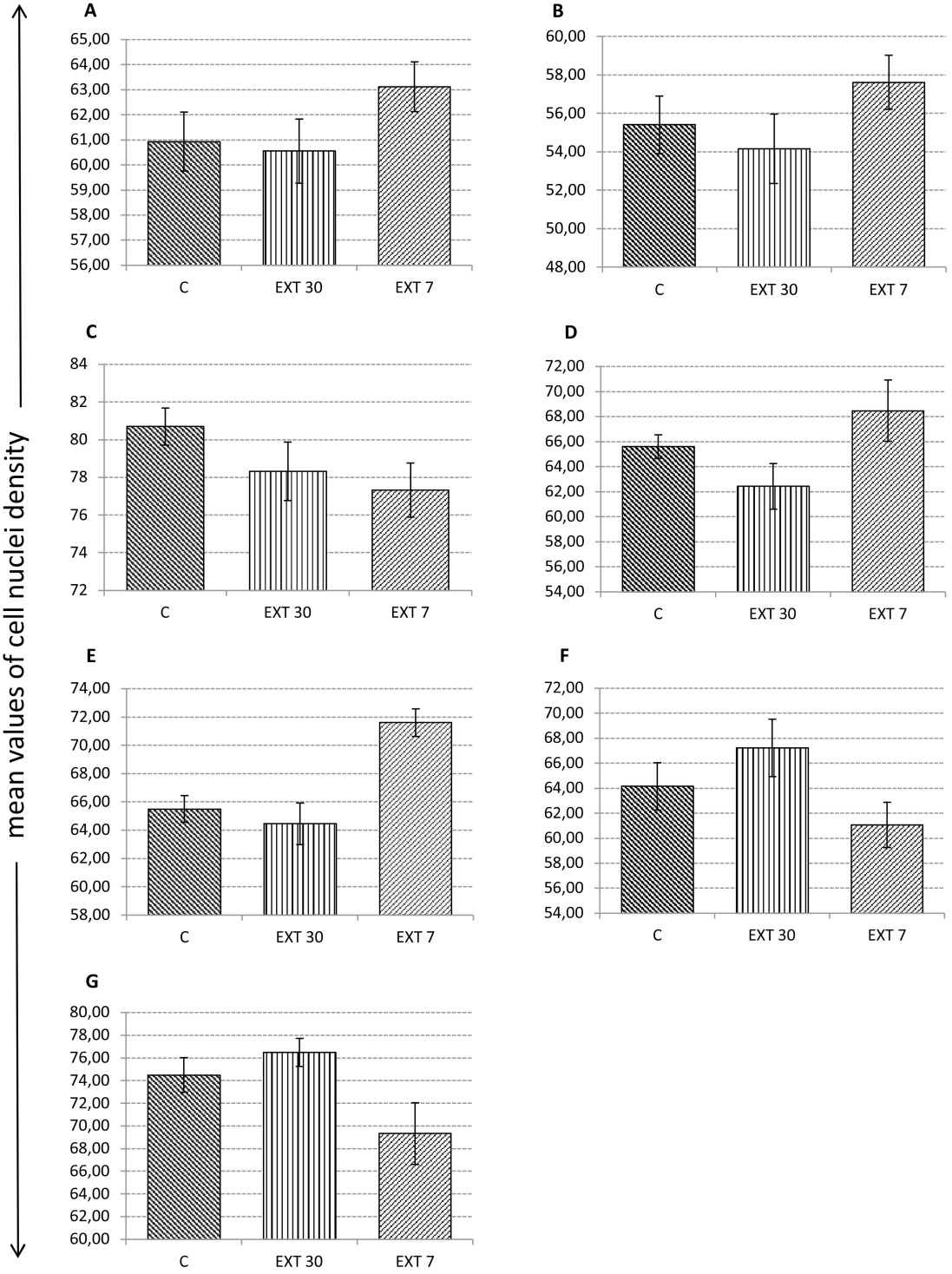

Figure 2. The result of a computer analysis of the image of the indicated brain areas focused on the expression of the early activation sign c-Fos. The supplementation of the inbred mice with Juvenil induces selective expression of the early activation marker c-Fos in the different brain compartments. The intensity of c-Fos expression in the brain of mice was dependent on the duration of Juvenil supplementation. Legend: **(A)** – hippocampus, CA2 area; **(B)** – hippocampus, *gyrus dentatus*; **(C)** – hypothalamus, dorsomedial nucleus; **(D)** – thalamus; **(E)** – amygdala, central body; **(F)** – piriform cortex; **(G)** – primary motor cortex. X-axis: **C** – control mice fed with standard diet; **EXT7** – mice fed with standard diet supplemented with Juvenil for seven days; **EXT30** – mice fed with standard diet supplemented with Juvenil for 30 days; Y-axis: mean values of cell nuclei density of the observed brain region ± SE. The experimetal data from reference (79) were used for construction of this figure.

By modulating composition of the gut microbiota, Juvenil can have a significant effect on signaling through the microbiota–gut–brain axis. Intestinal microbiota dysbiosis is reported to be involved in various physiological processes, including immunomodulation, energy balance, and activation of the enteric nerves. Given that Juvenil is involved in the regulation of dysbiosis and assuming that Juvenil causes changes in expression of the early marker of activation in different parts of the brain, it can be regarded as a substance with psychobiotic properties (87, 88). Originally, psychobiotics were defined as probiotics affecting functions and behavior of the organism related to modulation of the microbiota–gut–brain axis. Later, that definition was expanded to include prebiotics in the complex with probiotics (46). Nevertheless, Juvenil contains no probiotics and perhaps not even prebiotics (unless prebiotics are the components of Juvenil themselves), and, as such, may represent the first molecularly complex psychobiotics.

For a full understanding of the modulatory effects of Juvenil, and with some exceptions, the mapping of the functional profiles of the supplementation models will no longer be decisive. The key might consist in mapping of the centripetal signaling networks within the microbiota–gut–brain axis, and feedback regulation by activation of the hypothalamus–pituitary–adrenal axis with production of endogenous catecholamines.

Acknowledgement

The authors of this review would like to thank Anna Kadlecova and Igor Matena from Juvenil Products, a.s., Prezletice, Czech Republic, for making it possible to study original documents in the company's archives regarding studies conducted with bovine blood extracts during the second half of the 20th century.

Author Contributions

A.M., V.B., and K. K. wrote the manuscript. V.B. and A.M. edited the manuscript. A.M. supervised preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Ministry of Defence of the Czech Republic - DRO of the University of Defence, Faculty of Military Health Sciences Hradec Kralove, Czech Republic – Medical Issues of WMD II, (DZRO-FVZ22-ZHN II).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

Authors A.M., K.K., and V.B. have repeatedly attended seminars sponsored by Juvenil Products, a.s., Prezletice, Czech Republic, the manufacturer of Juvenil. At the workshops, the authors presented papers on testing biological response modifiers, including their own results from studies with Juvenil. A.M., K.K., and V.B. declare that they have no employment relationship with or sponsorship by Juvenil Products, a.s.

Adherence to Ethical Standards

Ethical standards were not applicable in this study.

References

1. Hijová E, Bertková I, Štofilová J. Dietary fibre as prebiotics in nutrition. Cent Eur J Public Health. 2019;(3):251-255. doi: 10.21101/cejph. a5313. PMID: 31580563.
2. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8(2):172-184. doi: 10.1080/19490976.2017.1290756. Epub 2017 Feb 6. PMID: 28165863; PMCID: PMC5390821.
3. Al-Ghazzewi FH, Tester RF. Impact of prebiotics and probiotics on skin health. Benef Microbes. 2014;5(2):99-107. doi: 10.3920/BM2013.0040. PMID: 24583611.
4. Gupta V, Garg R. Probiotics. Indian J Med Microbiol. 2009;27(3):202-209. doi: 10.4103/0255-0857.53201. PMID: 19584499.
5. Williams NT. Probiotics. Am J Health Syst Pharm. 2010;67(6):449-458. doi: 10.2146/ajhp090168. PMID: 20208051.
6. Ozen M, Dinleyici EC. The history of probiotics: the untold story. Benef Microbes. 2015;6(2):159-165. doi: 10.3920/BM2014.0103. PMID: 25576593.
7. Flesch AG, Poziomyck AK, Damin DC. The therapeutic use of symbiotics. Arq Bras Cir Dig. 2014;27(3):206-209. doi: 10.1590/s0102-67202014000300012. PMID: 25184774; PMCID: PMC4676381.
8. Olveira G, González-Molero I. An update on probiotics, prebiotics and symbiotics in clinical nutrition. Endocrinol Nutr. 2016;63(9):482-494. English, Spanish. doi: 10.1016/j.endonu.2016.07.006. Epub 2016 Sep 12. PMID: 27633133.
9. Cauli O. New Effects of Prebiotics, Probiotics, and Symbiotics. Curr Clin Pharmacol. 2020;15(3):172-173. doi: 10.2174/1574884715032011100934 35. PMID: 33327908.
10. Meeusen R, Decroix L. Nutritional Supplements and the Brain. Int J Sport Nutr Exerc Metab. 2018;28(2):200-211. doi: 10.1123/ijsnem.2017-0314. Epub 2018 Feb 23. PMID: 29252056.
11. Harvie M. Nutritional supplements and cancer: potential benefits and proven harms. Am Soc Clin Oncol Educ Book. 2014:e478-86. doi: 10.14694/EdBook_AM.2014.34. e478. PMID: 24857143.
12. Bloch MH, Mulqueen J. Nutritional supplements for the treatment of ADHD. Child Adolesc Psychiatr Clin N Am. 2014;23(4):883-897. doi: 10.1016/j.chc.2014.05.002. Epub 2014 Aug 12. PMID: 25220092; PMCID: PMC4170184.
13. Arentz S, Smith CA, Abbott J, et al. Nutritional supplements and herbal medicines for women with polycystic ovary syndrome; a systematic review and meta-analysis. BMC Complement Altern Med. 2017;17(1):500. doi: 10.1186/s12906-017-2011-x. PMID: 29178904; PMCID: PMC5702141.
14. Dolezal B, Rakusan B, Urbanek G, et al. [Existing experiences with tissue preparations RTN]. Vojenské zdravotnické listy, 1956 Suppl. 1, Part II.: 3-14 (Czech).
15. Dolezal B, Rakusan B, Urbanek G, et al. [Retisin, a new tissue preparation]. Cs. Farm. 1954;3(7):246-247 (Czech), PMID 13231128.
16. Urbanek G, Dolezal B, Rakusan B, et al. [Treatment of endarteritis with tissue preparations RTN]. Cas Lek Cesk. 1952;91(45-46):1375-1377 (Czech). PMID:14390438.
17. Pavlovsky P, Hanakova B, Petranova V. [The use of Floristen and Lyastin in arteriosclerotic affection of the brain]. Cesk Psychiatr. 1979;75(2):118-121.
18. Zícha K. Existing results of arthritis treatment with RTN tissue extracts. Military Medical Science Letters, 1950;XIX(3-4):63-65.
19. Dolezal B, Rakusan B, Urbanek G, et al. Floristen as a drug in the treatment of inflammation. Cesk Farm. 1954;3(7):247-248.
20. Kolarova O. Comparison of therapeutic results in inflammations of the adnexa obtained with Trypsin, Adnexbacterin, Floristen and Betalactin. Cesk Gynekol. 1973;38(8):589-590.
21. Urbanek G, Dolezel B, Rakusan B, et al. Therapy of chronic gynecological diseases with the tissue preparation Floristen mite (RTN 118). Cas Lek Cesk. 1954;93(49):1352-1354.
22. Jakoubkova J. Tissue preparation Retisin – the drug for patients with tumors? Prakt. Lek. (Praha) 1979;59(23):894-915 (Czech). ISSN>0032-6793`1805-4544.
23. Kubelkova K, Macela A. A short history of Imuregen –an original tissue extract. MMSL 2019;88(3):115-120.
24. Kubelkova K, Hubalek M, Rehulka P, et al. Molecular characterization of alcohol-ether extract from bovine tissue. MMSL 2021;90(3):120-136.
25. Cho JH, Sung BH, Kim SC. Buforins: histone H2A-derived antimicrobial peptides from toad stomach. Biochim Biophys Acta. 2009;1788(8):1564-1569.

26. Tomita M, Wakabayashi H, Yamauchi K, et al. Bovine lactoferrin and lactoferricin derived from milk: production and applications. Biochem Cell Biol. 2002;80(1):109-112.
27. Ahmadinia K, Yan D, Ellman M, et al. The anti-catabolic role of bovine lactoferricin in cartilage. Biomol Concepts. 2013;4(5):495-500.
28. Nedjar-Arroume N, Dubois-Delval V, Miloudi K. Isolation and characterization of four antibacterial peptides from bovine hemoglobin. Peptides. 2006;27(9):2082-2089.
29. Machado A, Sforça ML, Miranda A. Truncation of amidated fragment 33-61 of bovine alpha-hemoglobin: effects on the structure and anticandidal activity. Biopolymers. 2007;88(3):413-426.
30. Nedjar-Arroume N, Dubois-Delval V, Adje EY. Bovine hemoglobin: an attractive source of antibacterial peptides. Peptides. 2008;29(6):969-977.
31. Fiat AM, Migliore-Samour D, Jollès P, et al. Biologically active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. J Dairy Sci. 1993;76(1):301-310.
32. Nyberg F, Sanderson K, Glämsta EL. The hemorphins: A new class of opioid peptides derived from the blood protein hemoglobin. Biopolymers. 1997;43:147–156.
33. Ukraintseva SV, Arbeev KG, Michalsky AI, et al. Antiaging treatments have been legally prescribed for approximately thirty years. Ann N Y Acad Sci. 2004;1019:64-69.
34. Martí-Carvajal AJ, Knight-Madden JM, Martinez-Zapata MJ. Interventions for treating leg ulcers in people with sickle cell disease. Cochrane Database Syst Rev. 2012, 11. Update in: Cochrane Database Syst Rev. 2014, 12:CD008394.
35. Brzozowski T. Gastro-protection in vivo and in vitro. Patol Pol. 1992;43(1):1-9. (in Polish).
36. Ziegler D, Movsesyan L, Mankovsky B, et al. Treatment of symptomatic polyneuropathy with actovegin in type 2 diabetic patients. Diabetes Care. 2009;32(8):1479-1484.
37. Buchmayer F, Pleiner J, Elmlinger MW, et al. Actovegin®: a biological drug for more than 5 decades. Wien Med Wochenschr. 2011;161(3-4):80-88.
38. Lee P, Rattenberry A, Connelly S, et al. Our experience on Actovegin, is it cutting edge? Int J Sports Med. 2011;32(4):237-241.
39. Lee P, Kwan A, Nokes L. Actovegin--Cutting-edge sports medicine or "voodoo" remedy? Curr Sports Med Rep. 2011;10(4):186-190.
40. Wolf A. Health safety and biological activity of Juvenil. Research Report, Institute of Hygiene and Epidemiology, Prague 1987, 1-14 (Czech).
41. Macela A, Propper P. The results of preliminary Juvenil testing on immunomodulatory activity. Research Report 1989, 1-26 (Czech).
42. Richter J, Sima P, Pfeifer I, et al. Protective and immunomodulative influence of supplementing DNA: comparing of clinical testing and experimental model. Research Report 2004:1-61 (Czech).
43. Duskova K, Cechakova L, Plzakova L, et al. (2019). Natural immune booster Imuregen significantly affects the proliferation of tumor cells. MMSL. 2019;88(3):106-114.
44. Kubelkova K, Rychlik I, Crhanova M, et al. Gut microbiota alterations by nutritional supplement Imuregen. MMSL. 2020;89(3):114-125.
45. Slizova D, Sima P, Richter J, et al. Stimulation of ileal epithelium growth and regeneration by dietary nucleotide extracts. Acta Medica (Hradec Kralove), 2004;47(3):163-166.
46. Doležel B. Research report. Prague, April 5, 1989: 1-7 (Czech).
47. Brárt J. Use of Aminex in a trial test in Želetice calf farm, Znojmo district. Report Želetice, June 16, 1989: 1 (Czech).
48. Zahradníková V. Aminex - preparation for veterinary health prevention and increasing the utilization of nutrients in feed. Report Tochovice, January 7, 1992: 1-7 (Czech)
49. Orság M. Verification of the influence of Juvenil praparation on the health status and utilization of feed rations in pig fattening. Final report - verification of the effect of Juvenil, No. R-05-529-804-01. Inst. for the Scientific Management System, Bratislava, November, 1988: 3-32 (Slovak).
50. Burdych J. Preliminary information about the application of the preparation Imuregen to sows intended for super (poly)ovulation. Report Kramolna, Náchod, October 3, 1990: 1-2 (Czech)
51. Kohout L. Stimulating effect of Aminex on weight gain of calves by increased utilization of food components. Report Český Brod, November, 1989: 1-28 (Czech).
52. Burdych J, Fiala F. The use of tissue preparation Juvenil in the operating conditions of large calf farms during the stay in the dairy nutrition pavilion. Research report. District veterinary administration, Pardubice, May, 1988: 1-6 (Czech)

53. Richter J, Svozil V, Kral V, et al. Effects of dietary nucleotides on immune mechanisms and physical state in children with chronic resporatory problems. Am. J. Immunol. 2015;31(2):26-32.
54. Zícha K. Existing results of arthritis treatment with RTN tissue extracts. Military Medical Science Letters, 1950;XIX(3-4):63-65 (Czech)
55. Svozil V, Richter J, Vetvicka V. High exposure to pollution requires nutritional improvements in children. Arch Nutr Food Sci. 2020;1(2):30-34.
56. Imunoregen-reports of nucleotides and related contents (unknown authors). JNoracion, 2016-07-26, 1-289, (https://fliphtml/5.com/egwq/kdsm)
57. Sarasa SB, Mahendran R, Muthusamy G. A Brief Review on the Non-protein Amino Acid, Gamma-amino Butyric Acid (GABA): Its Production and Role in Microbes. Curr Microbiol. 2020;77:534–544.
58. Kaur H, Bose C, Mande SS. Tryptophan Metabolism by Gut Microbiome and Gut-Brain-Axis: An in silico Analysis. Front Neurosci. 2019;13:1365.
59. Zhao D, Yu Y, Shen Y, et al. Melatonin - Synthesis and Function: Evolutionary History in Animals and Plants. Front Endocrinol (Lausanne) 2019;10:249.
60. Martin-Gallausiaux C, Marinelli L, Blottière HM, et al. SCFA: mechanisms and functional importance in the gut. Proc Nutr Soc. 2021;80(1):37-49.
61. Kubelkova K, Macela A. Microbiota-gut-brain signaling: a minireview. MMSL. 2020;89(1):41-51.
62. Dumitrescu L, Popescu-Olaru I, Cozma L, et al. Oxidative Stress and the Microbiota-Gut-Brain Axis. Oxid Med Cell Longev. 2018; 2018:2406594. doi: 10.1155/2018/2406594. PMID: 30622664; PMCID: PMC6304899.
63. Molina G, Roman P, Sanchez-Labraca N, et al. Stress and the gut microbiota-brain axis. Behav Pharmacol. 2019;30(2 and 3-Spec Issue):187-200. doi: 10.1097/FBP.0000000000000478. PMID: 30844962.
64. Chang L, Wei Y, Hashimoto K. Brain-gut-microbiota axis in depression: A historical overview and future directions. Brain Res Bull. 2022;182:44-56. doi: 10.1016/j.brainresbull.2022.02.004. Epub 2022 Feb 11. PMID: 35151796.
65. Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: Metabolism and perspective in obesity. Gut Microbes. 2018;9(4):308-325. doi: 10.1080/19490976.2018.1465157. Epub 2018 May 24. PMID: 29667480; PMCID: PMC6219651.
66. Liu BN, Liu XT, Liang ZH, et al. Gut microbiota in obesity. World J Gastroenterol. 2021;27(25):3837-3850. doi: 10.3748/wjg. v27. i25.3837. PMID: 34321848; PMCID: PMC8291023.
67. Asadi A, Shadab Mehr N, Mohamadi MH, et al. Obesity and gut-microbiota-brain axis: A narrative review. J Clin Lab Anal. 2022;36(5): e24420. doi: 10.1002/jcla.24420. Epub 2022 Apr 14. PMID: 35421277; PMCID: PMC9102524.
68. Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, et al. Diet, Gut Microbiota, and Obesity: Links with Host Genetics and Epigenetics and Potential Applications. Adv Nutr. 2019;10(suppl_1):17-30. doi: 10.1093/advances/nmy078. PMID: 30721960; PMCID: PMC6363528.
69. Abenavoli L, Scarpellini E, Colica C, et al. Gut Microbiota and Obesity: A Role for Probiotics. Nutrients. 2019;11(11):2690. doi: 10.3390/nu11112690. PMID: 31703257; PMCID: PMC6893459.
70. Tomasello G, Mazzola M, Leone A, et al. Nutrition, oxidative stress and intestinal dysbiosis: Influence of diet on gut microbiota in inflammatory bowel diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2016;160(4):461-466. doi: 10.5507/bp.2016.052. Epub 2016 Oct 26. PMID: 27812084.
71. Lee M, Chang EB. Inflammatory Bowel Diseases (IBD) and the Microbiome-Searching the Crime Scene for Clues. Gastroenterology. 2021;160(2):524-537. doi: 10.1053/j.gastro.2020.09.056. Epub 2020 Nov 27. PMID: 33253681; PMCID: PMC8098834.
72. Cheng Y, Ling Z, Li L. The Intestinal Microbiota and Colorectal Cancer. Front Immunol. 2020; 11:615056. doi: 10.3389/fimmu.2020.615056. PMID: 33329610; PMCID: PMC7734048.
73. Koliarakis I, Messaritakis I, Nikolouzakis TK, et al. Oral Bacteria and Intestinal Dysbiosis in Colorectal Cancer. Int J Mol Sci. 2019;20(17):4146. doi: 10.3390/ijms20174146. PMID: 31450675; PMCID: PMC6747549.
74. Fattorusso A, Di Genova L, Dell'Isola GB, et al. Autism Spectrum Disorders and the Gut Microbiota. Nutrients. 2019;11(3):521. doi: 10.3390/nu11030521. PMID: 30823414; PMCID: PMC6471505.
75. Hu T, Dong Y, He C, et al. The Gut Microbiota and Oxidative Stress in Autism Spectrum Disorders (ASD). Oxid Med Cell Longev. 2020; 2020:8396708. doi: 10.1155/2020/8396708. PMID: 33062148; PMCID: PMC7547345.
76. Alharthi A, Alhazmi S, Alburae N, et al. The Human Gut Microbiome as a Potential Factor in Autism Spectrum Disorder. Int J Mol Sci. 2022;23(3):1363. doi: 10.3390/ijms23031363. PMID: 35163286; PMCID: PMC8835713.

77. Martel J, Chang SH, Ko YF, et al. Gut barrier disruption and chronic disease. Trends Endocrinol Metab. 2022;33(4):247-265. doi: 10.1016/j.tem.2022.01.002. Epub 2022 Feb 9. PMID: 35151560.
78. Hantsoo L, Zemel BS. Stress gets into the belly: Early life stress and the gut microbiome. Behav Brain Res. 2021;414:113474. doi: 10.1016/j.bbr.2021.113474. Epub 2021 Jul 16. PMID: 34280457; PMCID: PMC8380711.
79. Novakova T. Study of c-Fos expression in the brain of mice after the application of a modulator of biological reactions. Bachelor thesis. University of Hradec Kralove, Faculty of Science 2021,4-63 (Czech).
80. Piechaczyk M, Blanchard JM. c-fos proto-oncogene regulation and function. Crit Rev Oncol Hematol. 1994;17(2):93-131.
81. Dun NJ, Dun SL, Shen E, et al. C-fos expression as a marker of central cardiovascular neurons. Biol Signals. 1995;4(3):117-123.
82. Harris JA. Using c-fos as a neural marker of pain. Brain Res Bull. 1998;45(1):1-8.
83. Kovács KJ. c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int. 1998;33(4):287-297.
84. Pompeiano M, Cirelli C, Arrighi P, et al. C-Fos expression during wakefulness and sleep. Neurophysiol Clin. 1995;25(6):329-341.
85. Cirelli C, Tononi G. On the functional significance of c-fos induction during the sleep-waking cycle. Sleep. 2000;23(4):453-469.
86. Herrera DG, Robertson HA. Activation of c-fos in the brain. Prog Neurobiol. 1996;50(2-3):83-107.
87. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol Psychiatry. 2013;74(10):720-726.
88. Sarkar A, Lehto SM, Harty S, et al. Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals. Trends Neurosci. 2016;39(11):763-781.
89. Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. Int J Syst Evol Microbiol. 2021;71(10). doi: 10.1099/ijsem.0.005056. PMID: 34694987.