

REVIEW

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of the PhD dissertation by **Dr. Violeta Dimitrova Snegarova**

titled *"Effectiveness of a Low-Histamine Diet in Patients with Irritable Bowel Syndrome"*

submitted for the award of the educational and scientific degree "PhD"

in the field of higher education **7. Healthcare and Sports,**

professional field **7.1. Medicine**, scientific specialty: **Hygiene.**

In accordance with Order No. R-109-170/28.03.2025 of the Rector of the Medical University – Varna, Prof. Dr. Dimitar Raykov, DSc, I have been appointed a **member of the Scientific Jury**, and based on Protocol No. 1 of the Jury's meeting, I was assigned to prepare a review within the procedure for the defense of the PhD dissertation of Dr. Violeta Dimitrova Snegarova for the award of the degree "PhD"

All documents required for the procedure have been submitted in accordance with the regulations of MU-Varna. No procedural violations have been identified.

1. Brief Information on the Professional Development of Dr. Snegarova

Dr. Violeta Snegarova completed her secondary education in 2012 at the Foreign Language High School "Yoan Ekzarh" in Varna with a specialization in French. In 2018, she obtained her Master's degree in Medicine from the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna. During her studies, she demonstrated a pronounced interest in gastroenterology and dietetics and participated in teaching activities as a demonstrator in pharmacology at MU–Varna.

After graduation, she worked as a physician at the "Sanador M" Medical Center and later at the "Medicus" outpatient clinic, where she practiced actively as a dietitian. In 2020, she was admitted as a full-time PhD student in the scientific specialty "Hygiene" at the Department of Hygiene and Epidemiology of MU–Varna, under the scientific supervision of Prof. Dr. Darina Naydenova.

Concurrently with her PhD studies, Dr. Snegarova began a specialization in "Gastroenterology" at the Military Medical Academy – Varna, with intensive practical clinical training. She obtained additional qualifications through a number of certified courses,

including: “Precision and Personalized Medicine in Gastroenterology,” “Modern Therapy of Chronic Constipation,” “Nutrition in Type 2 Diabetes,” “Psychosomatic Medicine,” “Nutrition in Patients with Depression and Anxiety,” and training in cognitive behavioral therapy. She has also participated in continuing e-learning courses offered by United European Gastroenterology, such as “Neuroendocrine Tumors (NET).”

Her professional work also includes active engagement as a resident at the Clinical Laboratory “Laborespers 2000.”

Her scientific interests lie in the fields of functional gastrointestinal disorders, nutrigenomics, neurogastroenterology, and psychonutrition. She has authored 15 scientific publications, including 3 in impact factor journals and 5 in international peer-reviewed journals. She has presented posters and oral presentations at national and international scientific forums, including UEG Week, ESGE Days, and the Balkan Congress of Gastroenterology.

Dr. Snegarova is an active member of the Bulgarian Medical Association (BMA), the Bulgarian Society of Nutrition and Dietetics (BSND), the Bulgarian Society of Neurogastroenterology and Motility (BSNM), the Association of Young Hepatogastroenterologists in Bulgaria (AYHGB), the Bulgarian Association for the Study of Obesity and Related Diseases (BASORD), and the Bulgarian Society of Coloproctology.

Since 2022, she has been elected a member of the Management Board of AYHGB for Northeastern Bulgaria and serves as Vice President for Public Relations of BSNM. She was a finalist in the “Young and Energetic Scientist” (YES) competition in 2023 and a recipient of a PhD scholarship from the “Karoll Knowledge” Foundation.

Dr. Snegarova’s professional development is marked by consistency, dedication to scientific research, and a drive for innovative approaches in clinical and preventive gastroenterology and dietetics.

2. Relevance of the Dissertation

The topic of applying a low-histamine diet in patients with irritable bowel syndrome (IBS) is highly relevant and responds to current challenges in the fields of gastroenterology and nutrition science. IBS is a condition of considerable socio-economic significance—affecting between 10% and 20% of the global population—and leads to substantial deterioration in patients’ quality of life, reduced work capacity, and increased healthcare utilization.

To date, the therapeutic approach to IBS remains difficult due to the absence of a clearly defined organic cause, and treatment is often symptomatic and inconsistently effective. Consequently, interest in the role of dietary factors in the pathogenesis and symptom maintenance of IBS has increased significantly over the past decade.

In this context, histamine—as a mediator of inflammation, immune response, and gastrointestinal motility—has attracted attention as a potential pathophysiological factor in the development of IBS symptoms. Foods high in histamine, as well as those that trigger histamine release, may provoke or exacerbate gastrointestinal symptoms in susceptible individuals.

There is still a limited number of clinical studies in the scientific literature that systematically investigate the effect of a low-histamine diet in IBS patients. Most available data are fragmented or based on observational reports and case studies. This highlights the lack of strong scientific evidence for recommending this dietary regimen as a standard therapy, which makes the topic selected by Dr. Snegarova particularly significant.

Moreover, examining the effect of a low-histamine diet on objective markers—such as changes in symptoms, quality of life, and possibly serum levels of histamine or inflammatory mediators—aligns with the need for a personalized, evidence-based approach in the treatment of IBS.

Dr. Snegarova’s dissertation not only addresses an existing gap in the scientific literature but also offers new possibilities for developing individualized dietary strategies that are better tolerated, easier to follow, and potentially more effective than more restrictive diets such as the low-FODMAP diet.

3. General Characteristics of the Dissertation

The dissertation consists of 166 standard pages, including 28 figures and 5 appendices. The bibliography contains 135 references, over 70% of which are from the last five years. The dissertation follows the structural requirements, including: Introduction – 2 pages, Literature Review – 29 pages, Methodology – 10 pages, Results and Analysis – 28 pages, Discussion – 6 pages, Conclusions – 2 pages, Summary – 2 pages, Contributions – 1 page, Appendices – 18 pages, Bibliography – 11 pages

4. Evaluation of the Content of the Structural Components of the Dissertation

Evaluation of the Literature Review

The literature review prepared by Dr. Violeta Snegarova is impressively thorough and structured in a way that systematically introduces the reader to the key aspects of irritable bowel syndrome (IBS) and current concepts in the field.

First and foremost, the selection of thematic highlights stands out: the PhD candidate begins with an overview of the evolution of diagnostic criteria, tracing developments from Osler’s historical descriptions through the Manning criteria to the modern Rome IV consensus. The

detailed discussion of IBS subtypes and the use of the Bristol Stool Form Scale demonstrates not only erudition but also a critical approach to diagnostic challenges in clinical practice.

The pathophysiological section of the review is particularly strong, where the author explores the multifactorial etiology of the condition. Key hypotheses are systematized, including visceral hypersensitivity, disturbances in the gut-brain axis, the role of stress and psycho-emotional factors, and the importance of the gut microbiota. The integration of data from recent meta-analyses and large multicenter studies is impressive and reflects a deep and critical understanding of the subject matter.

Epidemiological data are presented comprehensively, with both global and Bulgaria-specific studies included, making the analysis particularly relevant to national healthcare practice. The PhD candidate correctly highlights the lack of sufficient national data, which underscores the need for local research in the field.

In the sections dedicated to the relationship between dietary factors and IBS, Dr. Snegarova demonstrates an excellent grasp of current trends. The literature review not only covers the role of low-FODMAP and gluten-free diets but also emphasizes the relatively new concept of histamine intolerance. It is precisely here that the author's innovative approach is evident, as she clearly identifies the lack of scientific data regarding the effectiveness of a low-histamine diet in IBS patients and frames this gap as a valid scientific problem justifying her own research.

The review also addresses modern understandings of neuroimmune interactions and the relationships between stress, anxiety, depression, and gastrointestinal symptoms. The coverage of the literature on these issues is in-depth and includes both classical and recent sources, attesting to the author's ability to integrate traditional knowledge with the latest scientific findings.

Strong emphasis is placed on the description of intestinal barrier function, the role of mast cells, visceral hypersensitivity, and intraepithelial mechanisms, which demonstrates solid knowledge in molecular gastroenterology and physiology. Modern concepts linking serotonin, intestinal motility, and IBS symptomatology are also presented, along with a critical interpretation of diverse findings in the literature.

A particularly strong impression is made by the critical analysis of the available therapeutic approaches—dietary, pharmacological, and psychotherapeutic—where the author evaluates not only their advantages but also their limitations. Dr. Snegarova convincingly argues for the need for a personalized approach and the exploration of new strategies for managing IBS. This

is especially important given the rapid development of gastroenterology and the constantly evolving knowledge in the fields of the microbiome and nutritional therapies.

The literature review is notable not only for its content but also for its clear, consistent, and logical presentation. The author successfully integrates diverse scientific data into a coherent conceptual framework, which naturally leads to the formulation of the research problem and the aims of the dissertation.

The literature review in Dr. Violeta Snegarova's dissertation is thorough, scientifically grounded, and critical. It provides a solid foundation for the development of the research component of the dissertation. The review reflects not only the author's excellent theoretical knowledge but also her capacity for analytical thinking and scientific argumentation.

Evaluation of the Methodology

1. Formulation of Objectives and Tasks

The objective of the dissertation is clearly defined and scientifically justified: to evaluate the effectiveness of a low-histamine diet in the context of IBS, compared with two established dietary regimens (low-FODMAP and Mediterranean diet). The eight research tasks are comprehensive and reflect both the main hypothesis and the intent to investigate the relationships among symptomatology, psycho-emotional factors, and sociodemographic characteristics.

Notably, the tasks are not only descriptive but also analytical in nature—they seek to explore interactions among variables, thereby increasing the scientific value of the research.

2. Characteristics of the Study Material (Population)

The choice of population (ambulatory IBS patients aged 18–50 from Varna) is logical and aligns with the research goals. An important strength is the exclusion of vulnerable groups, thus avoiding ethical risks. Strict adherence to international diagnostic criteria (Rome IV) enhances external validity and ensures comparability with other global studies. Strict inclusion and exclusion criteria minimize the risk of confounding factors, supporting the internal validity of the study. The male-to-female ratio (1:3) reflects the real-world epidemiological distribution of IBS, where women are more frequently affected—another strength of the study design.

3. Study Design

The study is prospective and controlled, with three time points (baseline, post-diet, one month after diet), allowing the tracking of dynamic effects rather than static snapshots. Randomization into three groups (low-FODMAP, low-histamine, Mediterranean diet) permits

direct comparisons between interventions—an important strength of the design. The use of active comparators (rather than a passive control, e.g., standard diet) is a more scientifically sound method for evaluating a novel dietary approach. The study was conducted with ethical approval and included individual instruction of participants, demonstrating adherence to ethical standards.

4. Methods Used

- **Documentary Method:** A comprehensive literature review was conducted to guide diet selection and validate the measurement methods, reflecting a high level of scientific preparation.
- **Survey Method:** The use of validated tools (PSS-4, GAD-7, IBS-SSS) is a major strength—ensuring data reliability and comparability. The three-time application of the surveys enables the assessment of symptom and psychological dynamics.
- **Clinical Method:** Detailed collection of medical history, sociodemographic data, and clinical characteristics was properly conducted. Providing written diet instructions and guidance from the lead researcher helped minimize errors in dietary adherence.
- **Statistical Methods:** A complex range of methods was employed—parametric (t-tests, ANOVA), non-parametric (Kruskal–Wallis, Wilcoxon), and regression analysis. The assumption of normal distribution was appropriately tested, indicating strong understanding of statistical principles. Repeated measures were effectively utilized. The inclusion of heteroskedasticity-consistent estimates (HC3) reflects high methodological literacy. The use of Python and modern libraries (Pandas, SciPy, Seaborn, Statsmodels) reflects the implementation of advanced tools, ensuring reliable and flexible data analysis.

Evaluation of Results

1. Participant Characteristics

Sex, age, ethnicity, education, and employment status are systematically and thoroughly presented. All key demographic characteristics are described. The gender and age distribution aligns with known IBS epidemiology, enhancing result validity. A predominance of university-educated participants is observed, which should be acknowledged as a potential limitation in generalizing findings to the broader population. The use of figures facilitates the understanding of participant characteristics.

2. Age and Symptom Severity Analysis

Linear regression was correctly used. Results show a statistically significant but weak correlation between age and severity of bloating and pain symptoms, with bloating more

strongly associated with age. Visualizations illustrate the trend clearly. Appropriate methodology and interpretation, with adequate explanation of the weak age-related influence.

3. Occupation and Symptom Severity Analysis

ANOVA was appropriately used to compare occupational groups. No statistically significant differences in symptom severity were found, and this is correctly interpreted.

4. Stress and Anxiety Impact on Diet Therapy Effectiveness

Stress (PSS-4) and anxiety (GAD-7) levels were analyzed via linear regression with HC3 correction—demonstrating rigorous data handling. In the low-histamine and low-FODMAP groups, stress had no significant effect. However, in the Mediterranean diet group, stress was a significant negative predictor. Anxiety levels showed a weak but borderline significant effect only in the low-histamine group.

5. Diet Effectiveness on IBS Symptoms

All three diets led to significant symptom improvement, with the low-histamine diet having the strongest effect. Appropriate statistical tests were used (paired t-test or Wilcoxon depending on data distribution). Results are clearly visualized.

6. Symptom Tracking One Month After Diet Therapy

Bloating and pain symptoms continued to decline four weeks after the end of dietary therapy. Stress levels also showed a significant decrease in the follow-up period—an important finding confirming the sustainability of the effect.

Interpretation of Results

1. **Participant Characteristics:** The distribution by sex, age, and ethnicity aligns with established epidemiological data for IBS. The population is appropriately selected and representative of an urban outpatient setting in Bulgaria.
2. **Age and Symptom Severity:** With increasing age, there is a slight increase in the severity of bloating and abdominal pain in IBS. While the correlation is statistically significant, it is weak and likely not clinically meaningful for this age group.
3. **Occupation and Symptom Severity:** No statistically significant association was found between type of employment and baseline symptom severity. Employment status alone does not appear to influence the basic clinical presentation of IBS.
4. **Stress and the Effect of Diet Therapy:** Higher levels of stress limited symptom improvement only in the Mediterranean diet group. In the low-histamine and low-FODMAP groups, stress levels did not significantly affect the therapeutic outcome.

5. **Anxiety and the Effect of Diet Therapy:** Anxiety (GAD-7) was associated with a modest, yet statistically significant reduction in symptom improvement following diet therapy, although this effect was weaker than that of stress.
6. **Diet Effectiveness:** The low-histamine diet produced the greatest improvement in bloating and abdominal pain symptoms in IBS patients, surpassing the effects of both the low-FODMAP and Mediterranean diets.
7. **Sustainability of the Effect:** The reduction in bloating and stress levels was maintained even four weeks after the completion of diet therapy, suggesting a potential long-term benefit of the low-histamine diet.
8. **Employment and Therapy Effectiveness:** In the low-histamine group, occupation had no impact on diet effectiveness. In the FODMAP and especially the Mediterranean diet groups, individuals engaged in intellectual work showed greater symptom improvement compared to unemployed participants and students.
9. **Overall Impact of Occupation:** Overall, occupation does not significantly influence the severity of pain and bloating symptoms but may modify the response to certain types of diet therapy in IBS.

Evaluation of the Discussion

1. **Logic and Coherence:** The discussion is well-structured and logically connects the study results with existing literature. The author demonstrates a solid understanding of the complexity of IBS as a functional disorder and interprets the findings skillfully within this context. Comparisons with international data are appropriately made.
2. **Adequacy of Literature Comparison:** Contemporary scientific sources are systematically cited to support the conclusions drawn. The author notes that the low-FODMAP diet has the strongest evidence base, while data for the low-histamine diet remain limited. This reflects a critical and balanced approach.
3. **Critical Analysis of Findings:** The author objectively considers possible explanations for discrepancies between the study results and existing literature—e.g., the age-related trend in bloating and pain, which differs from some earlier studies. This self-critical approach adds credibility to the analysis.
4. **Analysis of Limitations:** The author explicitly acknowledges that the methodology (self-reported surveys) cannot exclude placebo effects and that the lack of randomization and a control group limits the strength of conclusions. The absence of psychiatric evaluation (e.g., for depression) is also noted as a limitation.

5. **Adequacy of the Summary:** The conclusions are balanced. The positive effect of the low-histamine diet is emphasized, but the need for larger, well-controlled studies is also clearly stated. The author avoids overgeneralization, which is appropriate for a PhD dissertation.
6. **Perspective for Future Research:** The dissertation outlines future directions for research very well, including the need to develop guidelines for the low-histamine diet, investigate its long-term effects, and evaluate patients' psycho-emotional status using more comprehensive tools.
7. **Style and Clarity:** The language used in the discussion is scientific, coherent, and relatively clear. Despite slightly long paragraphs, logical flow is maintained. Citations are well-integrated and support the arguments convincingly.

Evaluation of Conclusions, Recommendations, and Contributions

The conclusions of the dissertation are logically derived and consistent with the findings of the conducted study. The author effectively demonstrates that IBS is more prevalent in women and that patients frequently report high levels of stress and anxiety. The study clearly establishes that the low-histamine diet yields the most pronounced positive impact on the clinical symptoms of IBS, surpassing both the low-FODMAP and Mediterranean diets in effectiveness. An important observation is that, although employment status does not significantly affect the severity of symptoms such as pain and bloating, age shows a weak but statistically significant correlation with symptom severity, with bloating increasing slightly with age. Additionally, in the context of psycho-emotional status, high levels of anxiety—but not stress—were found to influence symptom improvement, and this effect was statistically significant only in the low-histamine diet group.

Based on the results, it is confirmed that the low-histamine diet leads to a sustained reduction in gastrointestinal complaints, with the beneficial effect persisting during the follow-up period after the end of therapy.

The **conclusion** of the dissertation is well-reasoned and coherently aligned with the results obtained. The author emphasizes that the treatment of IBS requires a comprehensive and holistic approach that combines diet therapy, psycho-emotional regulation, and, where necessary, pharmacological interventions. The results of the study are placed within the context of existing scientific literature, recognizing that the low-histamine diet may represent a promising therapeutic alternative for IBS, though larger and well-controlled studies are needed to confirm its efficacy and long-term effect. The author appropriately notes that the

effectiveness of dietary therapy in IBS is individually variable, necessitating personalized treatment strategies.

Scientific and Practical Contributions

The dissertation includes both original and confirmatory contributions. For the first time in Bulgaria, a study has been conducted comparing the effectiveness of a low-histamine diet with a low-FODMAP and Mediterranean diet in IBS patients. For the first time, the dynamics of stress and anxiety in this patient population have been assessed in the context of nutritional interventions. Additionally, the work confirms existing data regarding the predominance of women among IBS patients and the high prevalence of psycho-emotional disorders in this group.

The practical significance of the dissertation is considerable, with findings supporting the recommendation of a low-histamine diet as a potentially effective strategy for symptom management in IBS, at least for an initial period of one month.

In summary, the conclusions, summary, and contributions of the dissertation are logically structured, well-argued, and contribute to the advancement of knowledge in the field of dietary therapy for functional gastrointestinal disorders.

The **Abstract book** consists of 54 pages and accurately reflects the content of the dissertation.

Critical Remarks

The dissertation does not devote sufficient attention to the limitations of the selected measurement tools and potential confounding factors such as subjective self-assessment, diet adherence (compliance), seasonal variations, and the influence of co-existing psycho-emotional states. While validated instruments such as IBS-SSS and HADS were used, the author does not discuss possible deviations from objectivity due to self-reporting or the need for clinical verification. A detailed rationale for the selected intervention period is also lacking, and it is not clear whether the observed effects would be maintained over a longer term. A more critical reflection on the methodology would have enhanced the scientific value and persuasiveness of the findings. Nevertheless, these limitations do not diminish the overall quality of the dissertation.

5. Conclusion

The dissertation titled “*Effectiveness of a Low-Histamine Diet in Patients with Irritable Bowel Syndrome*” stands out for its high relevance, originality of concept, scientific rigor, and comprehensive analysis. The objective and research tasks are clearly defined, logically justified, and successfully achieved. The methodology is well developed, with appropriately selected assessment tools and proper application of modern statistical methods. The results are

convincingly analyzed and presented, and the discussion meaningfully compares the findings with current international research in the field. The conclusions are clearly articulated and have practical applicability in clinical settings, offering realistic recommendations for integrating the low-histamine diet into the multidisciplinary treatment of IBS. The contributions of the dissertation highlight the significance of the research, and the author's recommendations for a multidisciplinary approach that considers patients' psycho-emotional status and the long-term monitoring of dietary therapy effects form a valuable basis for future scientific and practical developments in the field of functional gastrointestinal disorders.

I highly value the scientific work of the PhD candidate and consider that her dissertation fully meets the requirements for awarding the educational and scientific degree “PhD” as per the Law on the Development of the Academic Staff and the regulations of the Medical University – Varna.

I give a positive evaluation and recommend that the members of the Scientific Jury vote in favor of awarding Dr. Violeta Snegarova the educational and scientific degree “PhD” in the field of higher education 7. Healthcare and Sports, professional field 7.1. Medicine, scientific specialty: Hygiene.

May 8th, 2025

Member of the Scientific Jury:

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679
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