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## REVIEW

**Doctoral dissertation by Yulianri Rizki Yanza, MSc.  
titled “The effect of dietary polyphenols on methanogenesis and biohydrogenation  
in ruminants”**

**performed under the guidance of Prof. dr hab. Adam Cieślak,  
in the Department of Animal Nutrition, Faculty of Veterinary Medicine  
and Animal Science, Poznań University of Life Sciences**

### Legal and formal basis

The formal and legal basis for the review is the Resolution of the Scientific Council of the Discipline: Animal Science and Fisheries, Poznań University of Life Sciences (WWZ-4000-04/2022, 24.06.2022) appointing me as a reviewer in the doctoral proceedings for Yulianri Rizki Yanza, MSc. The dissertation was reviewed in accordance with the requirements specified in Article 187 of the Law on Higher Education and Science of 20.07.2018 (Journal of Laws 2021, item 478, 16.03.2021). The materials to be reviewed were prepared accurately, clearly presenting the research outcomes, which allowed adequate and complete evaluation thereof.

### Research problem and significance of the study

Feed generates the highest cost in ruminant husbandry. In the dynamically developing livestock production, securing the fodder base in the production of milk or meat cattle requires allocation of

huge areas of land. This may pose environmental problems, including climate change related to greenhouse gas emission.

Animal husbandry also contributes to this environmental burden through the production of methane and the processes of biohydrogenation of fatty acids in the animal rumen, which are regarded as the main limitations in the production of ruminants. Ruminants are responsible for 80% of the total emission from the livestock sector or about 56% of the total agricultural greenhouse gas emission. Rumen functions can be modulated by biologically active compounds of plant origin. Supplementation with plant material containing such bioactive substances as tannin, saponins, alkaloids, flavonoids, and phenolic acids may decrease the ruminant methane production. The dietary strategies that are implemented to modulate the rumen H<sub>2</sub> pathways leading to methane formation also affect the unsaturated fatty acid biohydrogenation processes. The determination of the mode of action of rechanneling the hydrogen pathways in the rumen as an effect of dietary manipulation could be the turning point in the effectiveness of methanogenesis and biohydrogenation modulation. Concurrently, the literature in this field confirms that the use of natural bioactive substances in animal nutrition facilitates dietary modification of animal products, such as milk or meat.

*Coleus amboinicus* Lour. is characterized by high content of bioactive substances. This plant is rich in polyphenols (phenolic acids and flavonoids), tannins, saponins, and alkaloids. Conventionally, the *Coleus amboinicus* cultivated as a garden plant has been used as a culinary ingredient or in herbal medicine. This plant has antimicrobial, antifungal, antioxidant, anti-inflammatory, and analgesic activities. Its effects are beneficial in treatment of asthma, constipation, headache, cough, fever, and skin diseases. However, references regarding the effects of this plant are still mainly limited to humans. Its properties encourage the search for potential applications of the plant also in animal production. The high concentration of polyphenols has the potential to reduce methane production and modulate the biohydrogenation of unsaturated fatty acids in the rumen.

The research problem addressed by the Author fits very well in the current trend of searching for a possibility of an effective use of local plants with high content of bioactive substances in cattle production. Therefore, the issue addressed in the doctoral dissertation by Yulianri Rizki Yanza, MSc. is relevant, up-to-date, and important for this area of science.



### Description and review of the study

The doctoral dissertation submitted for the review by Yulianri Rizka Yanza MSc. is a series of publications consisting of two original research studies published in peer-reviewed scientific journals indexed by the Journal Citation Report with Impact Factor (IF) values of 1.697 and 5.032 (IF in the year of publication) under the collective title “The effect of dietary polyphenols on methanogenesis and biohydrogenation in ruminants”.

The series consists of the following publications:

1. **Y.R. Yanza**, M. Szumacher-Strabel, M. Bryszak, M. Gao, P. Kolodziejcki, A. Stochmal, S. Ślusarczyk, A.K. Patra, A. Cieslak (2018). *Coleus amboinicus* (Lour.) leaves as a modulator of ruminal methanogenesis and biohydrogenation in vitro. *Journal of Animal Science*, 96 (11): 4868-4881.  
Impact Factor (2018) = 1.697; MNiSW = 45 pkt.
2. **Y.R. Yanza**, M. Szumacher-Strabel, S. Ślusarczyk, P. Kolodziejcki, A.K. Patra, Z. Varadyova, D. Lechniak, D. Lisiak, M. Vazirigohar, A. Cieslak (2022). Dietary *Coleus amboinicus* herb decreases ruminal methanogenesis and biohydrogenation, and improves meat quality and fatty acid composition in longissimus thoracis of lambs. *Journal of Animal Science and Biotechnology*, 13 (5): 1-19.  
Impact factor (2021) = 5.032; MEiN = 140 pkt.

The series of publications constitutes a coherent homogeneous set of innovative and valuable research papers. In both publications, Yulianri Rizka Yanza MSc. is the first author with a percentage contribution of 55%. This has been confirmed in the statements attached to the doctoral dissertation, in which the co-authors declare that the tasks performed by the doctoral student included devising the idea of the manuscript, participation in the creation of the research hypothesis, participation in the implementation of research, devising ideas for discussion of the results, writing some sections of the manuscript, and being responsible for responses to the journal reviewers. This indicates the extensive involvement on the part of the PhD candidate in the entire process of the creation and submission of the publications.

The doctoral dissertation, i.e. the two publications presented by the PhD student, has the following bibliometric indicators: total **IF = 6.729** and parametric assessment scores of the **Ministry of Education and Science (MEiN) = 185**. The bibliometric data of the publications are measurable and objective and clearly evidence the high quality and importance of the research



results presented in the doctoral dissertation.

The dissertation presented for the review is a 53-page study with the following sections characteristic for this type of work: title page, abstract in Polish and English, Abbreviations, Introduction, Hypotheses and aims, Materials and method, Results, Discussion, Conclusions, References, and Supplementary data. Additionally, it includes the publications and statements provided by the co-authors.

Based on the well-selected literature, the PhD candidate describes the research problem comprehensively on as many as 10 pages and provides the rationale for undertaking the research. The background of the research has been described in detail with its multifarious aspects ranging from food sustainability and climate changes through ruminant metabolism (rumen fermentation) to biologically active compounds in plants, particularly in *Coleus amboinicus* Lour. (CAL). This proves Author's excellent preparation for research work and commitment to studying literature in great detail. As many as 107 publications, adequately corresponding to the subject of the study and originating from the last 15 years in approximately 80%, were used in the dissertation. This confirms that the presented dissertation fits well in the scope of contemporary research.

### **Research Objective and Hypotheses**

The aim of the study is presented clearly and precisely. An important element of the doctoral dissertation is the formulation of research hypotheses common for the entire series of the 4 experiments. It underlines the consistency of the scientific concept implemented in the extensive experiments.

### **Research methodology**

In the "Material and research methods" section, the Author presents detailed methodological assumptions of the doctoral dissertation. The scope of the experiments presented in the reviewed dissertation is very extensive. The study was designed into four consecutive experiments:

1. the preliminary Hohenheim Gas Test to examine in vitro rumen fermentation in the dairy cow;
2. the RUSITEC test to examine in vitro rumen fermentation and digestibility in lambs;
3. the cannulated lamb test to investigate rumen fermentation in lambs;
4. the commercial experiment to investigate performance indices, rumen digestibility, rumen CH<sub>4</sub> production, and muscle quality in lambs.



**In experiment 1**, the Hohenheim gas test technique was employed to study the effect of *Coleus amboinicus* Lour. partially replacing total mixed ration (TMR). The TMR was composed of maize silage, lucerne silage, grass silage, beet pulp, brewer's grain, extracted rapeseed meal, and a commercial concentrate protein and mineral mixture. The control substrate consisted of 400 mg of TMR on DM basis, whereas in the experimental groups, TMR was replaced by 10, 20, 40, or 80 mg DM (2.5%, 5%, 10%, and 20% of *Coleus amboinicus* Lour.) of grounded *Coleus amboinicus* Lour. leaves. The study was performed in a 5×6 (treatment x glass syringe) experimental design. The ruminal fluid was obtained from nine Polish Holstein-Friesian dairy cows (three cows for each experiment) from the rumen's top, bottom, and middle.

**In experiment 2**, an *in vitro* experiment was carried out using the Rumen Simulation Technique (RUSITEC). The groups received a control diet (CON) and three experimental diets containing *Coleus amboinicus* Lour. leaves at 10%, 15%, and 20% of the total substrate. The *in vitro* CON diet was based on 30% DM of grass silage and 70% DM of concentrate, dried and prepared as a total mixed ration (TMR). Ruminal content was collected from the top, bottom, and middle of the rumen of each lamb separately.

**In experiment 3**, six lambs fitted with rumen cannulas were allocated into control and *Coleus amboinicus* Lour diet treatments. Lambs in the experimental group were fed with grass silage with two levels of DM (352 and 379 g DM/d). The lambs in the group were provided with 352 g DM/d concentrate, which was gradually replaced by *Coleus amboinicus* Lour. from 46 g DM/d to 173 g DM/d. From d 15, the experimental lambs received 176 g DM/d of concentrate. The experimental period lasted 24 d (a 21 d adaptation and a 3 d sampling period), and ruminal fluid samples were taken before morning feeding, 3 h, and 6 h after morning feeding.

**In experiment 4**, sixteen growing lambs ( $20 \pm 3$  kg live weight) were used. The lambs were allocated into two treatments (control and *Coleus amboinicus* Lour.) based on their live weight (n=8 per group). Two animals from each group were kept in a single cage from the beginning of the experiment. During both periods, the feeding strategy was analogous to that employed in experiment 3, and *Coleus amboinicus* Lour. gradually replaced the concentrate. Each cage was transferred into modified mobile respiratory chambers during the sampling period by daily rotation to determine the direct CH<sub>4</sub> emission for 24 h, consecutively.

It should be emphasized that the Author carried out numerous observations, measurements, and chemical analyses in an exceptionally wide range, e.g.:

- chemical analyses of the quality of the feed components and feed mixes, taking into account their basic chemical composition, fiber fraction, amino acid composition, and fatty acid profile,
- *in vitro* DM measurements of digestibility,
- analyses of the ruminal fermentation profile, CH<sub>4</sub> production, and FA composition in the rumen,
- analyses of protozoa, methanogens, and bacterial populations,
- analyses of such parameters as pH, ammonia content, and the volatile fatty acid (VFA) level,
- analyses of *longissimus thoracis* (LT) muscle quality: pH, meat color (lightness, redness, and yellowness), chemical composition, water holding capacity, marbling score, and sensory (aroma, juiciness, tenderness, and flavor) parameters,
- analyses of mRNA expression in meat samples.

The design of the experiments, the number of observations, and the analytical methods used do not differ from the adopted standards and have contributed to the achievement of the assumed goals. Adequate statistical methods were used in the study. In the doctoral dissertation, it would be worth providing information about the basis on which the levels of the experimental factor (*Coleus amboinicus* Lour.) were established in experiments 1-4. Other questions arise as well: Was the same batch of *Coleus amboinicus* Lour. used in all the experiments? Why were there such large differences in the content of bioactive substances in the plant material used in experiment 1 and in experiments 3-4? Was the level of bioactive substances, especially rosmarinic acid, in *Coleus amboinicus* Lour. standardized in experiments 2, 3, and 4?

### **Assessment of the results and discussion**

In the dissertation, the doctoral student discusses the research results in an extremely detailed and substantive way with reference to the well-chosen literature. The study results have been analyzed with great care and scientific maturity. In experiment 1 (Y.R. Yanza, M. Szumacher-Strabel, M. Bryszak, M. Gao, P. Kolodziejski, A. Stochmal, S. Ślusarczyk, A.K. Patra, A. Cieslak (2018). *Coleus amboinicus* (Lour.) leaves as a modulator of ruminal methanogenesis and biohydrogenation *in vitro*. *Journal of Animal Science*, 96 (11): 4868-4881), the candidate carried out a very detailed comparative analysis with the use of polynomial contrast (linear and quadratic), which facilitated tracking the stepwise changes in the rumen fermentation, and found an increase in the *in vitro* dry matter digestibility and the amount of ammonia accompanying the increase in the proportion of



CAL in the feed ration. CAL was found to linearly increase  $\alpha$ -linolenic acid and conjugated linoleic acid and elevate the stearic acid concentration in buffered ruminal fluid. The Author observed that CAL particularly increased total protozoa and bacterial populations during fermentation but inhibited methanogens.

In experiments 2, 3, and 4 (Y.R. Yanza, M. Szumacher-Strabel, S. Slusarczyk, P. Kolodziejski, A.K. Patra, Z. Varadyova, D. Lechniak, D. Lisiak, M. Vazirigohar, A. Cieslak (2022). *Dietary Coleus Amboinicus herb decreases ruminal methanogenesis and biohydrogenation, and improves meat quality and fatty acid composition in longissimus thoracis of lambs. Journal of Animal Science and Biotechnology, 13 (5): 1-19.*), the Author confirmed that CAL in the RUSITEC experiment reduced the methane production by 51% *in vitro* and by 20% *in vivo*, compared to the control. Simultaneously, the results confirmed a phenomenon that had already been observed in experiment 1, i.e. the reduction of total methanogen counts by approximately 28%, especially Metanobacteriales. In the experiment conducted on growing lambs, the Author observed increased populations of some rumen bacteria (*Ruminococcus albus*, *Megasphaera elsdenii*, *Butyrivibrio proteoclasticus*, and *Butyrivibrio fibrisolvens*). The CAL supplementation of the lamb diets increased the proportion of polyunsaturated fatty acids (PUFAs) in the rumen and reduced the mRNA expression of four out of five genes investigated in meat (fatty acid synthase, stearoyl-CoA desaturase, lipoprotein lipase, and fatty acid desaturase 1). A higher proportion of PUFAs was also found, including n-3 fatty acids, in the *longissimus thoracis* muscle in lambs receiving CAL. Were the technological properties of meat improved as well?

The “Conclusions” section presents a logical summary of the research results. They were adequately formulated, which proves the Author’s great capability of synthetic analysis of the investigation findings. The results of all 4 experiments have great practical importance and indicate a fairly simple dietary way to increase the production performance of both large and small ruminants. They also show the possibility of maintenance of the optimal level of rumen bacteria and animal health status, production of meat with improved dietary values, and minimization of the problem of environmental pollution through reduction of the amounts of gases produced by animals. However, there is no practical application-related conclusion that could be a recommendation for agricultural practice.

The doctoral dissertation submitted for the review is performed with extreme care, although this extensive study does have some inevitable minor inconsistencies and shortcomings. Here are some of these points:



- the legend to table 1 could specify the type of concentrate used in the study,
- page 22: table 14 is quoted as a source of information about the amount of diterpene in CAL, but it actually contains information about the ruminal microbial population,
- page 2: there is a citation of “Huang et al., 2021”, which is not included in the References section or in the publications
- page 5: there is a citation of “Dehorot et al., 2003”, which is not included in the References section or in the publications
- page 8: “Koba and Yanagita, 2013” is cited, which is “Koba K, Yanagita T. 2014” in the References section
- page 8: there is a citation of “Pietta, 2003”, which is not included in the References section or in the publications
- page 9: “Cobellis et al., 2016” is cited, while the References section comprises two publications “Cobellis et al., 2016a and b”
- page 9: there is a citation of “Kozłowska et al. (2021)”, which is not included in the References section but is cited in the publications

#### **Formal evaluation of the dissertation**

The descriptive part of the doctoral dissertation is an extensive research study on 53 pages, including 14 tables, 7 figures, and 107 references. The structure of the presentation of the contents follows the rules adopted in such theses and does not raise any objections. The dissertation is aesthetic, logical, and clear, and the consecutive sections make up a consistent dissertation.

The reviewed work is a reliable, up-to-date, and multi-faceted source of knowledge of the possibility of using plant sources of bioactive substances in the ruminant husbandry. It is a comprehensive and original approach to the research problem and a proof of the Author’s extensive and profound knowledge. It is characterized by a very high substantive level. **The dissertation is a significant contribution to the development of the Discipline: Animal Science and Fisheries and has great application-related importance.** The research required considerable commitment, work, and cooperation as well as expertise in research techniques and methods.

**To sum, I declare that the reviewed work fully meets the requirements for doctoral dissertations specified in Article 187 of the Act of July 20, 2018: Law on Higher Education and Science (Journal of Laws of 2021, item 478, 16.03.2021). Therefore, I recommend that the Scientific Council of the Discipline: Animal Science and Fisheries, Poznań University of Life Sciences, should admit Yulianri Rizki Yanza, MSc. for the subsequent stages of the doctoral proceedings.**





Given the high substantive value of the dissertation, the rarely encountered comprehensive approach to the problem, the huge amount of work devoted to the research, and the practical aspect of the research, I recommend that the PhD candidate should be awarded by the Scientific Council of the Discipline: Animal Science and Fisheries, Poznań University of Life Sciences.



Lublin, 18.06.2022

Prof. dr hab. Bożena Kiczorowska

