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Opinion on the doctoral dissertation

"The effect of dietary polyphenols on methanogenesis and biohydrogenation in ruminants"

(title in Polish: "Wpływ dodatku polifenoli w dawce pokarmowej na procesy metanogenezy i biouwodorowania u przeżuwaczy")

by Mr. Yulianri Rizki Yanza

PhD supervisor: prof. dr hab. Adam Cieślak

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Climate change, which results in the warming of the climate, causes that more and more often and loudly negative talk about animals, especially ruminants, which emit harmful greenhouse gases, primarily methane. In reducing the amount of methane secreted, the products of bacterial metabolism can also be used. Potential inhibitors of the metnogenesis process in rumen are currently considered to be bioactive compounds of plant origin such as: saponins, tannins, tannins, flavonoids, sulfur-border compounds and essential oils. The mechanism of their action consists in modulating rumen fermentation through changes in the proportion of individual types of microorganisms. Other additives whose use reduces methane emissions are, for example, yeast and fat used in both protected and unprotected form.

During 24h, an adult cow can produce 250-400 liters of methane, which represent 30%-40% of all rumens gases. Emission of methane from rumen is enormous and it is estimated, that cattle achieve up to 40% of the total production of this gas. In addition, other ruminants should be taken into account. Methane is highly energetic gas, which contribute a lot to global warming. We must also take into account, that apart from methane, ruminant emits into atmosphere also carbon dioxide, hydrogen, nitrogen and hydrogen sulfide.

The PhD student carried out a series of interesting studies, the task of which was usage appropriate nutrition to reduce methane emissions by ruminant. The cattle population on our planet is approximately 1.5 billion head. It is estimated, that one cow can emit to atmosphere over 100 kg methane per year, so it has, without a doubt an impact on the greenhouse effect. The existing problem forces farmers and nutritionists to use various dietary supplements (e.g. from seaweed), which have the properties of limiting the methanogenesis process in ruminants.

Research undertaken by the PhD student shows that also plant biologically active compounds (BACs) in Coleus amboinicus Lour. (CAL) reduced the production of methane (CH_4) and improved the quality of ruminant products.

This dissertation is written in English, consists of 2 published articles.

The doctoral dissertation begins with an abstract (in English and Polish). After the abstract, the Mr. Yulianri Rizki Yanza presents a list of the most important symbols and abbreviations (for the reader it would be more convenient to list abbreviations than like continuous text), followed by a theoretical introduction, presenting the issue climate change challenges of the livestock industry, ruminant metabolism, modulating ruminal FA biohydrogenation and its relation to human health and utilization of CAL as a potential dietary component in the daily ruminant ration.

Next, the PhD student posted the hypothesis and aims of the work, a description of materials and methods, and then the results. The discussion takes 11 pages of printout. The discussion is followed by a one page conclusion. I just wonder why the proposals were not separated as a separate chapter.

The dissertation ends with a bibliography, contained in 11 pages and covering 107 items of literature related to the topic and scope of the work, of which almost 64% comes from the last 10 years. The work is illustrated with figures and contains tables that are legible and significantly facilitate the reader to get acquainted with the large number of results obtained.

The materials have been prepared in a transparent manner and in a way that allows for proper and complete assessment and familiarization with the essence of the scientific achievement.

The issues discussed in the doctoral dissertation are very typical. The research topic taken is original, innovative and important, both for basic research, and has practical significance in relation to the influence of biological active compounds on the rumen microflora, the fermentation process

taking place in it, as well as the reduction of methane production and the regulation of the hydrogenation of unsaturated fatty acids.

The doctoral dissertation submitted for evaluation is a series of publications, consisting of two original creative works, published in peer-reviewed scientific journals Journal of Animal Sciences and Journal of Animal Science and Biotechnology, indexed by the Journal Citation Report with a total impact factor (IF) of 8.077, which the PhD student gave the collective title "The effect of dietary polyphenols on methanogenesis and biohydrogenation in ruminants ".

When assessing the series of publications, it should be stated that they constitute a coherent, homogeneous cycle of innovative and valuable research works. In both publications, the PhD student is the first author. The other co-authors of the papers provided statements, which show that the involvement of his in the preparation of these articles is 55 for each of them. I believe that this is a significant contribution and shows that it was the PhD student who was the main person in charge of the work contained in the attached publications. These papers have been published in recognized international journals and have already received positive opinions from independent experts related to the given issues, which proves their reliability and high scientific value. All 2 articles were cited (with self-citations) totally 9 i 1 times. The small number of citations may be due to the relatively recent publication date

The PhD thesis of Mr. Yulianri Rizki Yanza is thematically homogeneous and begins with the presentation of the current state of knowledge related usage appropriate nutrition to reduce methane emissions by ruminant. This dissertation provides important information on the impact of livestock nutrition on the environment. The introduction of a new feeding strategy (CAL applied) is a promising perspective that may contribute to the improvement of current climatic conditions but requires further research on effectiveness and animal welfare. It is worth praising the fact that in the description of the current state of knowledge, the PhD student cites many already published articles, the list of which fits well the subject of this thesis.

Reviewer comments:

No description of the result of experiment 3 in chapter 4 "Results".

In the description of the purpose of the work (experiment 3 and 4), the information of using two levels of CAL content - 0 vs. 190g/d) is given. However, in the description of the method of

Experiments 3 and 4, the doctoral student presents only the total feed rate, without taking into account the weight content of the test plant.

Why this plant was chosen, what guided the choice, and whether there are others with similar properties?

The selected plant contains thymol, which in high concentrations has a negative effect on internal organs. Were histopathological changes observed in the internal organs of the animals involved in the study? Is there a risk of the above changes in internal organs with long-term use of CAL in animal nutrition? Has the concentration of thymol in the ration used been estimated?

Was the effect of CAL addition in the ration, on the condition of rumen epithelium observed?

Will the use of this plant not affect the taste of meat?

Do bioactive compounds enter the milk of lactating cows and can they affect consumer health?

Out of the duty of the Reviewer, I must draw attention to some shortcomings, unfortunate wording, or so-called typos that appeared in the text, and which do not have a significant impact on my positive reception of the work.

Below are some of them:

Hypothesis - should be hypotheses

Food security - it should be food safety

PhD abbreviation of Various Forms of Ph.D. - need to be standardized

Materials and method - there should be "materials and methods", various methods are used in the text,

In vivo, in vitro - italics are missing

In Abstract - CH4 - the number should be written subscript

Chapter 4.4. it says "*Prevotella spp*" - spp. we do not write in italics

Item cited in the text "Yanza et al. 2022 "is not in the bibliography

4. Brief description and assessment of the PhD thesis

a) The title is fine and informative.

b) Structure of thesis is not the one of a classical PhD document, since the main part of the original research is reported in the journal articles, but it is fine in general

c) Bibliography is rich and adequately covers the state of the art.

d) Aims of the work is clearly specified in Sec. 2.2 of the thesis. As the main goal of the doctoral thesis, the PhD student identified the possibility of using *Coleus amboinicus Lour.*, as a dietary component in the nutrition of ruminants, improving rumen fermentation and modulating the microbial population in order to reduce methane production. The research hypothesis was verified in four experiments (both *in vitro* and *in vivo*).

e) Methodology is correct. When analyzing the methodologies in detail, it should be noted that the research was carried out using modern and well-selected methods that guarantee the reliability of the obtained results.

f) Assessment of results is provided in the first part of point 4 of this review. The scope and quality of results are appreciable for a PhD work. The chapter "Conclusion" is a logical effect of the results obtained in the research. They were aptly captured and they prove the author's great ability to synthetically analyze the obtained results against the background of previous research.

g) Applicability of findings. The doctoral dissertation concerns the global climate problem related to the education of methane emissions. Research is an important element in applying new CH4 emission strategies and increasing animal productivity or the quality of animal products. The obtained results may contribute to the reduction of environmental and economic costs, i.e., milk and meat. and to mitigate intestinal CH_4 production and to apply sustainable livestock production to address climate change issues.

h) Imperfections, suggestions for improvements, questions. Imperfections any not many (some are listed in point 4.2 above) – generally, the PhD document is carefully written and the Candidate has to be congratulated on the effort and its outcome. Concerning the questions related to the research work itself, some are formulated in point 4.1 above, mainly as an invitation for the Candidate to look beyond and as a support for discussion at the PhD defence.

i) General assessment about the solution of the research problem and its originality. As argued in point 1 of this review, the problem is clearly of actuality, the tasks for the PhD research have been correctly identified and solved. The originality of research has been confirmed through publications in scientific journals of international reputation.

j) General assessment of the Candidate's background and expertise in the subject area. The expertise in the area of usage appropriate nutrition to reduce methane emissions by ruminant is appreciated. My general assessment is undoubtedly positive, as reiterated just below.

5. Final conclusion

The doctoral dissertation presented by Mr. **Yulianri Rizki Yanza** provides a proof of his good knowledge of use different strategies to mitigate enteric CH_4 production and to use sustainable animal production regarding climate change issues The Candidate has demonstrated his capabilities to critically scrutinize the bibliography of the subject as well as various variants of computational approaches to turbulent flows. The thesis contains original analyses and novel findings beyond the state of the art. Judging by the PhD document, the Candidate has proven his good knowledge of the subject area, the professional skills, as well as the ability to think and work creatively. Given all the above, my final conclusion about Mr. **Yulianri Rizki Yanza** being a doctoral candidate is positive and I recommend that he orally defends the PhD dissertation with no reserve at all. Moreover, given (i) the degree of difficulty of the thesis subject, (ii) the quality findings reported in the PhD work, listed in this review and published in renowned research journals I propose that the PhD thesis of Mr. **Yulianri Rizki Yanza** be awarded distinction (summa cum laude).

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