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Opinion on the doctoral dissertation

“Fermented rapeseed cake mitigates enteric greenhouse gasses production
from broiler chickens and dairy cows”

(title in Polish: „Fermentowany makuch rzepakowy ogranicza produkcję gazów cieplarnianych w
przewodzie pokarmowym kurcząt rzeźnych i krów mlecznych”)

by Mr. Min Gao

PhD supervisor: prof. dr hab. Małgorzata Szumacher

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The livestock sector requires a significant amount of natural resources and has an important role in global greenhouse gas emissions. The most important greenhouse gases from animal agriculture are methane and nitrous oxide. Mitigation strategies aimed at reducing the emission intensity of this sector are needed to meet the increasing demand for livestock products driven by population growth. To increase the effectiveness of mitigation strategies, the complex interactions among the components of livestock production systems must be taken into account to avoid environmental trade-offs.

In animal agriculture, the greatest contributor to methane emissions is enteric fermentation and manure management. Enteric fermentation is the most important source of the methane in beef and dairy production, while most of the methane from poultry and swine production originates from manure. The main cause of agricultural nitrous oxide emissions is from the application of nitrogen fertilizers and animal manures. Application of nitrogenous fertilizers and cropping practices are estimated to cause 78% of total nitrous oxide emissions. Based on the life cycle

assessment of beef cattle, 86.15% of the GHGs are emitted during the production stage, while 68.51% of emissions take place during the production of pork and 47.82% of GHG emissions occur during the production stage of broiler chickens. The majority of the emissions from the beef cattle production comes from enteric fermentation while manure management is the major source during swine production and propane use during broiler poultry production.

The PhD student carried out a series of interesting studies, the task of which was to investigate the effect of different forms (raw or fermented) of rapeseed cake on the CH₄ mitigation ability of broiler chickens and dairy cows by replacing other sources of protein in the diet.

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

Gao, M., Cieślak, A., Kierończyk, B., Huang, H., Yanza, Y.R., Zaworska-Zakrzewska, A., Józefiak, D., and Szumacher-Strabel, M. 2020. Effects of Raw and Fermented Rapeseed Cake on Growth Performance, Methane Production, and Breast Meat Fatty Acid Composition in Broiler Chickens. *Animals*, 10(12), p.2250.

Gao, M., Cieślak, A., Huang, H., Gogulski, M., Petric, D., Ruska, D., Patra, A.K., ElSherbiny, M., and Szumacher-Strabel, M. 2023. Effects of raw and fermented rapeseed cake on ruminal fermentation, methane emission, and milk production in lactating dairy cows. *Animal Feed Science and Technology*, 300, p.115644.

The doctoral dissertation begins with an abstract (in English and Polish). After the abstract, the Mr. **Min Gao** 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 issues: protein source in animal diet, using rapeseed cake, fermentation, methane production from chickens and dairy cows, methanogenesis in the ceca and in the rumen, mitigation of methane productions.

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 16 pages of printout. The discussion is followed by a one page conclusion and future perspectives.

The dissertation ends with a references, contained in 20 pages and covering 239 items of literature related to the topic and scope of the work, of which almost 54% 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 effects of RRC and FRC on enteric methane production of broiler chickens and dairy cows.

The doctoral dissertation submitted for evaluation is a series of publications, consisting of two original creative works, published in peer-reviewed scientific journals *Animals* and *Animal Feed Science and Technology*, indexed by the Journal Citation Report with a total impact factor (IF) of 6.065, which the PhD student gave the collective title "Fermented rapeseed cake mitigates enteric greenhouse gasses production from broiler chickens and dairy cow"

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 6 . The small number of citations may be due to the relatively recent publication date.

The PhD thesis of Mr. **Min Gao** is thematically homogeneous and begins with the presentation of the current state of knowledge related usage rapeseed cake as a other sources of protein in the diet and fermentation as a promising way of re-processing of this protein source. This dissertation provides also important information on the impact of livestock nutrition on the environment. 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.

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. of the thesis.

The conducted studies aimed to assess the effect of RRC and FRC application in experimental diets for broiler chickens and dairy cows on the microbial population in their digestive tracts and enteric methane production.

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 increasing rapeseed production and its by-product (raw rapeseed cake: RRC) can play an essential role in addressing high-protein feed material deficiency. Meantime, it was subscribed to the belief that fermentation improves crude protein (CP) content from raw feed ingredients at the expense of fermentable sugars which are gas formers.

h) Imperfections, suggestions for improvements, questions. Imperfections any not many (some are listed below) – 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 below, mainly as an invitation for the Candidate to look beyond and as a support for discussion at the PhD defense.

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.

PhD research have been aimed to assess the effect of RRC and FRC application in experimental diets for broiler chickens and dairy cows on the microbial population in their digestive tracts and enteric methane production.

My general assessment is undoubtedly positive, as reiterated just below.

General comments:

The doctoral thesis raises very important and current issues related to the search for solutions to reduce environmental pollution. The author demonstrated the effectiveness of the proposed method of feeding farm animals in reducing the nuisance of animal production for the environment.

The work is written very carefully. The research undertaken in the work is described in a clear and understandable way.

The author repeatedly mentions the genus of bacteria *Lactobacillus* spp. (pp. 38, 51, 52, paper I (discussion), paper II (discussion, page 16). It should be remembered that this genus, in 2020, has been divided into many separate genera. Changes in the nomenclature resulted from the progress of knowledge on the relationship of individual microorganisms. When mentioning bacteria previously belonging to the genus *Lactobacillus*, it is worth using the name of the taxonomic level covering all the microorganisms discussed, e.g. of the *Lactobacillaceae* family.

In addition, the names of some microorganisms previously belonging to the genus *Lactobacillus* have changed. The author on page 6 mentions the following microorganisms: *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Lactobacillus plantarum*. The names of these microorganisms are currently: *Limosilactobacillus fermentum*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum* subsp. *plantarum*. Nevertheless, the literature on which the author relies comes from the period before the changes in nomenclature (2009-2019). I think it's worth only mentioning

these changes. The same situation is in paper I (introduction) where the author mentions *Lactobacillus fermentum*.

Whether the use of rapeseed by-products is entirely safe for animal health in terms of mycotoxicology is an important aspect that should be taken into consideration in such studies. The waste generated from rapeseed oil production is often stored under conditions favorable for the growth of mold fungi and the production of mycotoxins, such as ochratoxin A. Evaluating the potential presence of mycotoxins in these by-products is crucial to ensure the safety of animal consumption.

- Page 3 - "The literature data recognizes that fermentation can limit the ANFs contents and increase nutrient utilization. Consequently, the fermented rapeseed cake (FRC) application was also studied and examined." - For this fragment, it is worth providing a literature reference confirming the quoted facts.
- In the dissertation on page 4 the author writes; "Artuković et al. (2015) reported that rapeseed cake application at 5% and 10% of the broiler's diet enlarged both liver and thyroid size." Were these aspects taken into account during the conducted research, in the experiments where the animals involved were slaughtered? Were these changes observed?
- Page 19 there is a table (Table 1.) concerning the composition of the control diet and the diet containing the RRC/FRC addition. Please explain why the monocalcium phosphate content in the control diet is relatively higher (1.7 g·kg⁻¹) compared to the RRC/FRC diet (0.5 g·kg⁻¹).
- Page 10 Line 6 Are there any specific organic acids that are particularly dominant here?

Editorial comments:

Page 24 Line 25 diet at 0600 h and 1800 h per day

Page 28 Line 31 at 0600 AM and 0600 PM each day

It should be standardized

Page 29 Line 26 and Line 27 „gas-liquid chromatography“

In my opinion gas chromatography is sufficient. Adding „liquid“ can be misleading to the reader, as liquid chromatography also exists.

- in the bibliography there are literature items that do not exist in the main text:
 - Kellogg, D.W. 1969. Influence of sucrose on rumen fermentation pattern and milk fat content of cows fed a high-grain ration. *J. Dairy Sci.* 52:1601–1604.
 - Pimentel, M., Lin, H.C., Enayati, P., Van Den Burg, B., Lee, H.R., Chen, J.H., Park, S., Kong, Y., Conklin, J. 2006. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am. J. Physiol. - Gastrointest. Liver Physiol.* 290:1089–1095.
 - Pimentel, M., Mayer, A.G., Park, S., Chow, E.J., Hasan, A., Kong, Y. 2003. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig. Dis. Sci.* 48:86–92.
 - Shi, C., He, J., Wang, J., Yu, J., Yu, B., Mao, X., Zheng, P., Huang, Z., Chen, D. 2016a. Effects of *Aspergillus niger* fermented rapeseed meal on nutrient digestibility, growth performance and serum parameters in growing pigs. *Anim. Sci. J.* 87:557–563.
- Page 6, in the sentence: "Despite this, in contrast to the swine (Lu et al., 2019; Macasait et al., 2021; Fan et al., 2022), there is still limited information on feeding solid-state fermented feed ingredients to broiler chickens." it's worth moving the quote to the end of the sentence. Reading, you don't know what it's about yet and there are already citations.
- Page 18 author used full name „Glucosinolates“ instead of abbreviation GLS.
- Page 19 author used GHG abbreviation without explaining it (he explains it on page 25), abbreviation explanation should be given at first appearance in text.
- Page 31 author used CON abbreviation without explaining it (it is its first appearance in the text)
- Page 46 author writes "the such buffer...", in my opinion it should be without "the".
- Page 54 - stylistic note. It is worth moving the title of the subsection to the next page so that it is located together with the text it concerns.
- Page 60 of the bibliography, one of the authors' names is written in lower case: "Dosoretz, C., and lamed, R. 1987. Chicken manure methanogenesis. *Poult. Sci.* 66:576–585".
- The bibliography is arranged in alphabetical order, but only within individual letters. Items that start with the same letter are not always in alphabetical order. For example:

- "AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Whitlock, L.A. 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. *J. Dairy Sci.* 85:2266–2276.
- Azizi, A., Sharifi, A., Fazaeli, H., Azarfar, A., Jonker, A., Kiani, A. 2020. Effect of transferring lignocellulose-degrading bacteria from termite to rumen fluid of sheep on in vitro gas production, fermentation parameters, microbial populations and enzyme activity. *J. Integr. Agric.* 19:1323–1331.
- Ahlin, K., Emanuelson, M., Wiktorsson, H. 1994. Rapeseed products from double-low cultivars as feed for dairy cows: effects of long-term feeding on thyroid function, fertility and animal health. *Acta Vet. Scand.* 35:37–53."
- In the bibliography there is a literature item: "United Nations, Department of Economic and Social Affairs, Population Division (DESA). 2017. World population prospects: the 2017 revision, key findings and advance tables. Accessed Jun. 30, 2021. https://population.un.org/wpp/publications/files/wpp2017_keyfindings.pdf." - The year 2020 is given in the main text and 2017 in the bibliography.

Final conclusion

The doctoral dissertation presented by Mr. **Min Gao** provides a proof of his good knowledge of the effect of RRC and FRC application in experimental diets for broiler chickens and dairy cows on the microbial population in their digestive tracts and enteric methane production. 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. **Min Gao** 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. **Min Gao** be awarded distinction (*summa cum laude*).

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- “AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Whitlock, L.A. 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. *J. Dairy Sci.* 85:2266–2276.
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