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REVIEW

Doctoral dissertation of Min Gao, MSc.

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

performed under the guidance of prof. dr hab. Małgorzata Szumacher,

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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 (specific task contract No. 5/2/2023 of 5.06.2023), Poznań University of Life Sciences appointing me as a reviewer in the doctoral proceedings for Min Gao, 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

The greatest financial outlays in animal production are related to nutrition. The basis for proper animal nutrition is e.g. the optimal proportion of protein and energy provided by feed material rations. The demand for feed-related protein is still high in the animal production sector. It is partly covered by domestic sources of feed protein, e.g. post-extraction rapeseed meal. Although

rapeseed is a widely used and cultivated oil-bearing plant, and Poland is its third largest producer in the European Union, the deficit of this nutrient on the domestic market is not decreasing. The maintenance of animal production at the current high level requires securing the fodder base. This may be associated with the agricultural management of vast areas of land. Such activities can generate environmental problems, e.g. climate change caused by greenhouse gas emissions. Hence, there is a current search for methods for improvement of the quality of feed materials, including the availability of nutrients and reduction of their gas-forming properties. One such method is the fermentation process. The phenomenon of fermentation may exert a positive effect on the total protein content in feed materials at the expense of fermentable sugars, which may generate gases. Therefore, investigations of the effect of raw (RRC) and fermented rapeseed cake (FRC) on the production of methane (CH_4) in the cecum of broiler chickens and in the rumen of dairy cows are advisable. Detailed nutritional studies of broiler chickens targeted at determination of the optimal RRC dose ensuring high efficiency of rearing birds in terms of optimal weight gain and nutrient digestibility may reveal new nutritional solutions in poultry production. Additionally, the analysis of the impact of specific levels of RRC and FRC in milk production in dairy cows on the maintenance of high quality and optimal chemical composition of milk, low methane production, and the abundance of rumen fluid bacteria modulating the volatile fatty acid profile in the rumen may provide the basis for the development of new nutrition strategies in dairy farming. Concurrently, the literature in this field confirms that the use of fermented feed materials (including protein) in animal nutrition offers the possibility of dietary modifications of such animal products as milk or meat.

The research problem addressed by the Author fits very well in the current trend of searching for a possibility of an effective use of fermented rapeseed cake in broiler chicken and cattle nutrition in terms of limiting the production of greenhouse gases. Therefore, the issue addressed in the doctoral dissertation by Min Gao, 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 Min Gao 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 2,752 and 3,313 (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. **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.
Impact factor (2020) = 2.752
Ministry of Education and Science = 100 pts.
2. **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.
Impact factor (2021) = 3.313
Ministry of Education and Science = 200 pts.

The series of publications constitutes a coherent homogeneous set of innovative and valuable research papers. In both publications, Min Gao 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 the research, performance of the experiments and such basic analyses as determination of pH and ruminal pH, the concentration of ammonia in the cecum fermented fluid, ruminal ammonia and volatile fatty acids, total gas production, and methane concentration analysis, co-determination of the number of total bacteria and methanogens, determination of the number of rumen protozoa, co-analysis of the fatty acids profile in feed, chicken breast muscle, milk, and ruminal samples, performing the statistical analyses; devising ideas for discussion of the results, writing the original draft, 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 = 6.065** and the parametric assessment scores of the **Ministry of Education and Science (MEiN) = 300**. 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 76-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, and References. 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 16 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 worldwide patterns and challenges in production of protein for animal diets, taking into account the nutritional application of raw and fermented rapeseed cake and the detailed characteristics of methane production by chickens and dairy cows. This proves Author's excellent preparation for research work and commitment to studying literature in great detail. As many as 135 publications, adequately corresponding to the subject of the study and published in the last 15 years in approximately 73%, 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. Additionally, two specific aims were formulated. An important element of the doctoral dissertation is the formulation of research hypotheses common for the entire series of the three experiments on broiler chickens and five experiments on dairy cows. This 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 consecutive experiments:

I. Preparation of fermented rapeseed cake (FRC)



II. Broiler chicken study:

1. growth performance results;
2. cecal content fermentation characteristic (*in vitro*);
3. analysis of fatty acid profiles in the breast muscle.

III. Dairy cow study:

1. Hohenheim Gas Test to examine *in vitro* rumen fermentation in dairy cows;
2. Batch Culture Test to examine *in vitro* rumen fermentation in dairy cows;
3. cannulated dairy cow test to investigate rumen fermentation;
4. *in vivo* experiment on dairy cows kept in commercial farm conditions;
5. *in situ* incubations – *in sacco* test.

The fermented rapeseed cake (FRC) was prepared following patent no. 237575 procedure. Rapeseed cake was milled and mixed with water at a ratio of 1:2. The 6-phytase enzyme preparation was mixed with raw cake at a ratio of 1:1000 toward FRC preparation. The mass was fermented in anaerobic conditions for 24 h at a stable temp. of 30 °C. The fermented biomass was dried at 55 °C.

Broiler chicken study

The three experiments were conducted on 420 one-day-old female Ross 308 chicks (300 birds in **experiment 1**, 60 birds in **experiment 2**, and 60 birds in **experiment 3**) were used in the broiler chicken study. Experiment 1 lasted from day 7 to day 35 of birds' age to examine the effect of the partial replacement of SBM with 150 g/kg RRC or FRC addition in experimental diets on the growth performance and apparent total tract digestibility coefficient of ether extract and nitrogen retention. All diets were given *ad libitum* for two weeks in experiment 2 and four weeks in experiment 3, respectively. Experiments 2 and 3 examined CH₄ emission in the broiler chickens and the composition of breast muscle fatty acids.

Dairy cow study

The Hohenheim gas (**experiment 1**) and batch culture tests (**experiment 2**) were conducted on the rumen inoculum obtained from three ruminal cannulated Polish Holstein-Friesian dairy cows (620 ± 25 kg; 2nd month of lactation) before morning feeding. The rumen fluid donors were offered a total of 21 kg DM of a TMR diet. The ruminal content from each dairy cow was



collected from 3 different sites (top, middle, and bottom) of the rumen and filtered by four layers of cheesecloth to a Schott Duran bottle and immediately transported to the water bath at 39 °C.

In **experiment 3**, *in vivo* cannulated dairy cows were used. The experiment was established and designed by two dietary groups (RRC and FRC; n = 4) in a replicated (2 (groups) × 2 (periods)) crossover design (21-d adaptation and then 3-d ruminal fluid sampling and 6-d gas analysis/period) with four Polish Holstein-Friesian cows (multiparous, 630 ± 30 kg of BW; 33.5 ± 1.29 kg/d milk production; 5-6-month of lactation) fitted with rumen cannulas.

Experiment 4 was based on the *in vivo* use of dairy cows kept in commercial farm conditions. Twenty multiparous Polish Holstein-Friesian dairy cows at their 5-6th month of lactation (640 ± 31 kg body weight; parity: 2.6 ± 0.32; 148 ± 26 d in milk, and 35 ± 2.4 kg/d milk production) in a completely randomized design were assigned into two groups (10 dairy cows/group). The animals from both groups received the same PMR at 06.00 AM and 06.00 PM each day, and a computer controlled feeder station supplied the RRC/FRC 2.65 kg/d/cow. The experiment was conducted for 27 days, including 21 days of adaptation and six days of sampling.

Experiment 5 was conducted with *in situ* incubations – an *in sacco* test. *In situ* rumen kinetics of RRC or FRC degradation was tested using two cannulated multiparous Polish Holstein-Friesian cows (620 ± 15 kg body weight) with a 21-d feed adaptation period. The timing of 0, 2, 4, 8, 16, 24, 48, and 72 h was settled as the incubation time for RRC/FRC.

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

- observations and measurements of chicken's production parameters and dairy cow production traits: milk production, yield, and composition
- chemical analyses of the quality of the feed components and feed mixes, taking into account their basic chemical composition and fatty acid profile;
- *in vitro* DM measurements of digestibility;
- fatty acid profile in breast tissue of broiler chickens and milk;
- analyses of the cecal and ruminal fermentation profile, CH₄ production, and FA composition in the rumen,
- analyses of methanogens and bacterial populations in broiler's ceca and ruminal fluid, protozoa,
- analyses of such parameters as pH, ammonia content, volatile fatty acid (VFA) level, and



ruminal degradation kinetics,

- gene expression analysis and microbial quantification and analysis

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. It would be worth supplementing the information in the doctoral dissertation on how the desaturation index (DI) was calculated as well as the explanation of the DI RA abbreviation in table 10 (publication II, pp.14).

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 the study conducted on broiler chickens (*paper I - 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*), the candidate confirmed that soybean meal in the feed rations for broiler chickens can be partially replaced with 15% of raw (RRC) and fermented (FRC) rapeseed meal. The introduction of RRC and FRC contributed to the reduction of CH₄ production and the number of methanogens in the fermentation of cecal contents, without negative effects on the birds' growth performance and nutrient utilization. Concurrently, a favorable modification of the FA profile was observed in the 28-day-old birds, i.e. a decrease in the SFA share as well as an increase in the n-6 to n-3 FA ratio and the share of MUFAs in the breast muscle of the birds in the FRC treatment compared with the control.

In the study conducted on dairy cows (*paper II - 2. 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.1156*), the doctoral student conducted a very detailed comparative analysis using polynomial contrast (linear and quadratic), which allowed detailed tracking of step-by-step changes in rumen fermentation in the batch culture system and confirmed that the introduction of 11.5% of FCR to



the diet had a favorable effect on changes in the fermentation pattern in the rumen. A favorable phenomenon of reduction of the CH₄ production and limitation of the amount of methanogens was noted both *in vitro* and *in vivo*. Beneficial modifications were also noted in the milk fatty acid profile: the proportion of C18:2 cis-9, trans-11, C18:2 trans-10, cis-12, and MUFA increased. In contrast, the lowest n6 to n-3 FA ratio was noted in the FRC100 treatment.

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 experiments (conducted on broiler chickens and dairy cows) have great practical importance and indicate a fairly simple dietary way to increase the production performance of ruminants and poultry. They also show the possibility of maintenance of the optimal level of cecal and rumen fermentation, bacteria and animal health status, production of meat and milk with improved dietary values, and minimization of the problem of environmental pollution through reduction of the amounts of gases produced by animals.

During the evaluation of the doctoral thesis, I had a few questions to the doctoral student:

1. What are the methods for reduction of the level of anti-nutritive substances in feed in addition to fermentation?
2. There were interesting changes in the proportion of eicosadienoic acid in the fatty acid profile of chicken breast muscles. What could be the reason for its lower level in the experimental chickens at the age of 14 days compared to the control and the higher amount versus the control group already at the age of 28 days? What is its role in the human diet?
3. The type of feed can increase methane production in cows. Which feeds are these? What does it depend on?
4. How, besides the use of rapeseed meal in the nutrition of dairy cows, can the profile of fatty acids in milk be improved?

Formal evaluation of the dissertation

The descriptive part of the doctoral dissertation is an extensive research study on 76 pages, including 5 tables, 8 figures, and 239 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 protein in poultry and 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. Simultaneously, it should be emphasized that the indicated inaccuracies or the questions asked are comments to the discussion and do not affect the substantive value of the entire study.

To sum up, 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 Min Gao, 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, 26.07.2023

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