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**OPTIMIZATION OF THE FERMENTATION PROCESS OF RICE AND ADLAI WASH
FOR LACTIC ACID BACTERIA CULTURE USING
VARIOUS DILUTION RATES**

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Ybañez, Novelito B. 1MS Animal Science

Integrated liver proteomics and metabolomics identify metabolic pathways affected by pantothenic acid deficiency in Pekin ducks

The research article entitled "Integrated liver proteomics and metabolomics identify metabolic pathways affected by pantothenic acid deficiency in Pekin ducks" (Tang et al., 2022) was published in the *Animal Nutrition* journal in the year 2022. This article focused on the effects of Pantothenic Acid Deficiency (PAD) on the energy metabolism of Pekin ducks (*Anas platyrhynchos*), specifically on liver proteins and metabolites in relation to changes in hepatic lipid profiles. The study, conducted with a total of 128 male white Pekin ducks, revealed several responses of PAD on the physiological processes and liver of ducks, including fasting hypoglycemia, liver damage, increased liver glycogen levels, and decreased liver triglycerides (TG) or Total Fatty Acids (TFA). Furthermore, PAD was found to be responsible for the downregulation of proteins and metabolites involved in the synthesis and degradation of glycogen, as well as other metabolic processes such as glycolysis and gluconeogenesis in ducks. The researchers noted that these effects led to fasting hypoglycemia, a lack of hepatic ATP production, and growth depression. Additionally, the researchers presumed that fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation counteracted ATP production.

Pantothenic Acid, a water-soluble vitamin, serves as a component of coenzyme A (CoA) and acyl carrier protein (Tang et al., 2022). This vitamin plays a crucial role in the metabolism of proteins, carbohydrates, and lipids (Miller and Rucker, 2012; Smith and Song, 1996). The deficiencies of this vitamin have been documented in mammals and birds, including Pekin ducks, as outlined in the paper. Overall, the results of this study enhance understanding of the importance of Pantothenic Acid (Vitamin B5) in Pekin ducks and its physiological response when deficient in the diet. This research holds significant value for advancing knowledge, particularly in detailing the effects of PAD at the molecular level, an aspect the researchers claim has not been adequately addressed in existing literature. Moreover, as I dig into my specific responses to the paper, please allow me to just focus on three key aspects: the methodology employed in the paper, the choice of words, and the experimental results. As we progress through the remaining part of this paper, these key aspects will be chunked for further explanation.

Firstly, I'd like to address one of the methodologies employed in the paper. As an animal enthusiast, prioritizing the ethical treatment of animals is of utmost importance to me. I commend the researchers for adhering to ethical regulations approved by the Animal Welfare Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. I am so curious by the possibility of relating these ethical considerations to the Philippine context, I conducted a Google search to identify the authorities responsible for permitting the use of animals in research and education. I discovered that the Institutional Animal Care and Use Committee (IACUC) under the Department of Agriculture Bureau of Animal Industry holds the mandate for such approvals. Additionally, the Republic Act No. 8485, known as the Animal Welfare Act of the Philippines, serves as the legal foundation for ensuring humane treatment of animals. Thus, it is also essential to emphasize the need for obtaining permits for the use of animals in research or education at USM, if not already in practice.

Secondly, based on my prior knowledge in agricultural research, I appreciate the researchers' attention to the uniformity of the tested animals by using male ducks of the same age and ensuring the reliability of the source of chicks. This is a crucial aspect that contributes to the validity of the study. However, regarding on the data and sample collection, specifically blood samples (p. 2), may I suggest considering an increase in the number of samples per group. Given that there are at least eight birds per replication, utilizing only two samples per group may be insufficient. Half of the number of birds per replication could be more enough sampling unit.

Moreover, regarding the laboratory analysis, software used, and procedures conducted, I must admit that these aspects are beyond my current understanding. Nevertheless, I find it fascinating to learn about these processes, and they could serve as valuable references for any potential future research with similar procedures. However, I will give my personal belief on the validation for metabolite identification. I believe that there should be other validations made aside from the laboratory company mentioned in the acknowledgement part. The metabolites were identified based on mass spectrometry data without confirmation by authentic standards or second party, which could have led to false positives or negatives result.

To add further, I would like to address the terminology and language utilized by the researchers. While reading the entire paper, I must admit that some of the terms and biological processes mentioned, such as gluconeogenesis, glycogenesis, and glycogenolysis, as well as certain codes and abbreviations, still pose some ambiguity for me. Although I've encountered these terms before, the paper prompted me to delve

Ybañez, Novelito B. 1MS Animal Science

Metabolomic and transcriptomic study to understand changes in metabolic and immune responses in steers under heat stress

The research article entitled "Metabolomic and transcriptomic study to understand changes in metabolic and immune responses in steers under heat stress" was published in *Animal Nutrition* journal in the year 2022. This article focused on the understanding of the changes in metabolic and immunological responses in Jersey steers under heat stress (HS) condition. Three body fluids such as the rumen fluid, serum, and urine were analyzed with the used of $^1\text{H-NMR}$ spectroscopy and the immunological responses were determined with the RNA-seq in blood performed to understand the complexity of the processes involved. The results of the study revealed that there were several metabolite biomarkers can be used to diagnose HS in steers, specifically the rumen fluid and the serum. A meta-analysis showed that heat stress (HS) has a negative impact on riboflavin metabolism and the expression of genes related to glyoxylate and dicarboxylate metabolism. Additionally, the study found out downregulation of metabolic pathways, including the hypoxia-inducible factor-1 signaling pathway, in immune cells under heat stress conditions. The observed effects were statistically significant ($P < 0.05$). It is very important to note the commendable results and methods shown in this paper. However, it is also equally important to figure out some possible error. The next part of this paper are my insights regarding the above-mentioned study.

My first reaction on this article is on the statistical analysis part. I would like to point out the experimental units or the number of steers used in the study. The methods section provided a thorough numbers of the experimental setup involving eight Jersey steers within a 3x3 Latin square design. However, it was inefficient considering that conventional Latin square design typically requires a number of experimental units that is a multiple of the Latin square order, such as 9 or 12. The used of eight steers in this design introduced an imbalance in the allocation of treatments across rows and columns. This is far from the standard design raises concerns about the correct statistical procedure and the potential for confounding factors. Given this limitation, it may be advisable to consider alternative experimental designs that better align with the available resources, such as a 2x2 Latin

square or other factorial designs just to cater the eight steers or add more steers to fit on the desired number.

Secondly, I am so much concern on the collection of rumen fluid, which was one of the three biological fluid samples collected, with the used of stomach tube. While I commend the authors for citing the study of Shen et al. (2012) on the use of a stomach tube, which showed no significant difference compared to the use of a rumen cannula for rumen fluid collection, the authors overlooked another crucial consideration when using an oral stomach tube. The authors should specify from which site of the rumen the samples were collected. Accordingly, Shen et al. (2012) mentioned that rumen indicators such as the pH levels, ammonia, etc., vary significantly among the rumen sites. This consideration is essential to ensure consistency in the results of samples collected via rumen fluid from each steer.

Thirdly, upon reading the procedure for collecting blood samples from the jugular vein of the Jersey steer and placing them in Vacutainer tubes, my interest was awakened regarding the scientific basis of this procedure and the subsequent laboratory analysis to be conducted. This led me to delve into Google Scholar to search for scientific answers on this matter. While I came across several interesting studies, they were quite broad, involving a large number of test animals, and I did not read a review specifically focused on livestock. This has sparked a question in my mind: to conduct a thorough literature review that focuses on the various procedures for collecting blood samples in livestock currently practiced in the scientific world. Through this review, I hope to gain a deeper understanding and uncover possibilities for exploring studies involving this particular procedure. Apparently, my plan may not be limited only on livestock, but also in all farm animals. I am willing to seek advice for this matter if time permits.

Furthermore, since the study focused on the effects of heat stress in steers, I understand that the Temperature Humidity Index (THI) was considered a crucial factor in relation to heat stress in cattle research. However, I believe that other environmental factors should also be taken into consideration. In my opinion, this study lacks proper environmental control measurement and methods. It did not measure the other environmental factors that may impact the metabolic and immune responses of steers. These factors include: the solar radiation, wind speed, and air quality in the study area. Besides, these factors are fundamental aspects covered in animal environment and behavior courses in the undergrad.