Bioeksperimen: Jurnal Penelitian Biologi
Volume 12, Issue 1, March 2026, pp. 65 – 71
p-ISSN: 2460-1365, e-ISSN: 2527-2799
https://journals2.ums.ac.id/bioeksperimen

Preliminary study of acute febrile response to crude LPS extract from *Salmonella typhimurium* in domestic chicken (*Gallus gallus domesticus*) AKY strain

Rizky Surya Wijaya, Asmoro Lelono*, Rike Oktarianti
Master's Program in Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember, Jember, Indonesia

*Corresponding e-mail: lelono.fmipa@unej.ac.id

How to cite:
Wijaya, R. S., Lelono, A., & Oktarianti, R. (2026). Preliminary study of acute febrile response to crude LPS extract from *Salmonella typhimurium* in domestic chicken (*Gallus gallus domesticus*) AKY strain. *Bioeksperimen: Jurnal Penelitian Biologi*, *12*(1), 65–71. https://doi.org/10.23917/bioeksperimen.v12i1.16139.

Article info

Article History:
Received: 14 February 2026, Revised: 2 March 2026, Available Online: 31 March 2026

Keywords:
Fever, Febrile response, *Gallus gallus domesticus*, Innate immune response, *Salmonella typhimurium*

Abstract

Salmonella is a genus that commonly attacks the health of poultry, especially chickens. One type of poultry that is widely attacked by *S. typhimurium* is chickens. *S. typhimurium* infects the host with lipopolysaccharides (LPS). This study aims to determine the innate immune response of domestic chickens to LPS-based vaccines *S. typhimurium* as the first step in vaccine development. The sample was in the form of female domestic chickens of the AKY strain which was divided into 3 groups, the control group was injected using 0.1ml of Phosphate Buffer Saline (PBS) treatment group 1 was injected with LPS with an adjuvant with a concentration of 0.5mg/kg as much as 0.1ml. Treatment group 2 was injected with LPS with an adjuvant with a concentration of 1mg/kg of 0.1ml. Injection treatment group using Incomplete Freund Adjuvant (IFA). The results obtained were that the treatment groups had an increase in temperature at the 1st hour to the highest in the 3rd hour with a temperature range of 42.5°C to 42.6°C, contrast to the control group which has a static temperature with a range of 41°C to 41.8°C. The comparison of chicken body temperature among treatment groups using a Liner Mixed Model revealed a significant difference ($p < 0.001$) between the treatment groups and the control group. These results indicate that subcutaneous LPS administration induces a controlled acute febrile response, which is consistent with involvement of innate inflammatory pathways.

Introduction

Salmonella is a genus that commonly attacks the health of poultry, especially chickens. This genus can live in the digestive tract specifically in the intestines (Chen et al., 2025). *Salmonella typhimurium* is a species that attacks many birds with symptoms that are almost undetectable but can still reduce the immunity and health of poultry. *S. typhimurium* has a wide range of hosts with invasive but non-adaptive infectious biological activity to the host (Shaji et al., 2023).

One type of poultry that is widely attacked by *S. typhimurium* is chickens. Chicken farms in Indonesia are the sectors most affected by *S. typhimurium* infection, including domestic chicken farms (Daryono et al., 2017). One of the domestic chickens that is quite commonly known in the community is Yudhistira Kampung Chicken. The AKY domestic chicken strain represents a locally adapted genetic line with limited immunophysiological characterization. Domestic chickens basically have a stronger character than broilers, namely being adaptable and more resistant to stress and disease (Badaruddin et al., 2025; Daryono et al., 2017). This character does not rule out the possibility that domestic chickens can also be infected by *S. typhimurium*.

Salmonella typhimurium is an intracellular pathogenic bacterium capable of surviving and replicating within the host through various virulence mechanisms. It infects the host via multiple pathways, including directly into cells, receptor recognition, *Salmonella Pathogenecity Island* (SPI), and Lipopolysaccharide (LPS) (Barbosa et al., 2017). Lipopolysaccharides are molecules found in the cell membrane structure of *S. typhimurium* that are responsible for pathogenicity and virulence to hosts called endotoxins (Hassan et al., 2020).

Lipopolysaccharide *S. typhimurium* can infect the host through binding to *Toll-Like Receptor 4* (TLR4) found on the host cell membrane. This bond will give rise to an acute, local, and even systemic immune response of the host (Hassan et al., 2020). The initial or congenital immune response that arises is usually the occurrence of fever because cells will produce a considerable amount of cytokines, one of which is IL-6. Increased cytokines are directly proportional to the occurrence of fever in the host with a time span of one to 24 hours from the beginning of infection (Petes et al., 2018).

Handling *Salmonella* infections has actually been carried out by many farmers and the government. Antibiotics have been routinely administered by farmers, but infections caused by *Salmonella* can still occur, reducing the quality of meat and production quantity. Vaccination is considered an effective preventive strategy to reduce Salmonella infection in poultry by stimulating protective immune responses. The vaccines currently available on the market are salmonellosis vaccines with inactivated vaccine manufacturing methods and attenuated vaccines (Khan et al., 2024; Khan et al., 2025). These two vaccines are actually enough to reduce the problem *of Salmonella infection*, but the weakness of these two vaccines is the potential for cross-contamination, is not suitable for DOC chickens, and there is a fear of reversal (Khan et al., 2025).

LPS derived from *Salmonella typhimurium* has been explored as a potential immunostimulatory component due to its ability to trigger host inflammatory responses without introducing live bacteria (Loor-Giler et al., 2025). An LPS-based approach may offer practical advantages, including relatively simple production and lower cost compared to conventional vaccine platforms. Therefore, investigating the physiological and early immune-related responses of domestic chickens to *S. typhimurium*-derived LPS represents an initial step toward understanding its potential relevance in future vaccine-oriented research.

Materials and methods

This research was conducted in the biology laboratory, Faculty of Mathematics and Natural Sciences, University of Jember, from August to November 2025

1. Research subject

The study used six-week-old AKY domestic laying hens obtained from Sysga Farm, Jember, Indonesia. The AKY strain is a locally developed domestic chicken strain. All experimental animals were clinically healthy and confirmed to be free from visible signs of infectious disease prior to the experiment. The domestic chicken used is a hen with a weight of 700gram to 1000gram. A total of 24 hens were divided equally into three groups. The first group was injected with PBS (Phosphate Buffer Saline). Treatment group 1 was injected with LPS with an adjuvant with a concentration of 0.5mg/kg and treatment group 2 was injected with LPS with an adjuvant with a concentration of 1mg/kg.

2. Method and research design

a. LPS *S. typhimurium* extraction

S. typhimurium cultures were obtained from the biology laboratory in the field of microbiology, 6-hour-old cultures were transferred in an eppendorf tube and centrifuged for 30 minutes at 2500 rpm. Supernatant is discarded until deposits remain in the form of bacterial pellets. Added 2 ml of 96% alcohol to the eppendorf pellet contains and disvorcizes until the alcohol and pellets are perfectly mixed. Centrifuged for 10 minutes at a speed of 2000 rpm. This step is repeated three times. The supernatant is discarded and the pellets are dried by letting it sit until the alcohol evaporates. The bacterial pellets are suspended with 1 ml of 10% EDTA and then sonicated. Added 1 ml of saturated methanol:chloroform solution (1:2 ratio) to the bacteria-EDTA solution. The tube was closed using parafilm and dishaker for

2 hours, then centrifuged for 10 minutes at a speed of 2000 rpm. Three layers are formed; namely chloroform, methanol, and biomass residues including lysate cells. The chloroform and methanol layers are removed and allowed to evaporate completely. Pellets dried at room temperature are LPS *S. typhimurium*. Crude LPS extract was obtained using [metode ekstraksi], without further purification steps (Mirzaei et al., 2011; Xie et al., 2000).

b. Animal Treatment

A total of 24 hens were divided into three groups. The sample size was determined based on the exploratory nature of this preliminary study, aiming to detect biologically relevant differences while minimizing animal use in accordance with ethical considerations. The control group was injected using 0.1ml of PBS. Treatment group 1 was injected with LPS with an adjuvant with a concentration of 0.5mg/kg as much as 0.1ml. Treatment group 2 was injected with LPS with an adjuvant with a concentration of 1mg/kg of 0.1ml. Injection treatment group using *Incomplete Freund Adjuvant* (IFA). All injections are done once every two weeks for six weeks. Injections are carried out subcutaneously by inserting into the space between muscle tissue and skin in the upper neck of chickens (Groves *et al*., 2015).

c. Chicken Body Temperature Measurement

The chicken's body temperature was measured once every 1 hour for 10 hours after injection. Chicken body temperature measurement in the cloaca using *a Thermo One digital thermometer*. Temperature measurements are carried out by inserting the end of the thermometer into the cloaca until the number on the thermometer is stable. LPS is known to induce pyrogenic responses through activation of innate immune pathways and pro-inflammatory cytokine release; therefore, body temperature was included as one of the physiological response parameters (Luo etal., 2025).

3. Data Analysis

Analysis of post-injection chicken body temperature data using *Linear Mixed Model* (LMM) with a confidence level of 95%. The analysis was carried out using SPSS software (Murphy et al., 2022).

4. Ethical Clearence

All experimental procedures involving animals were reviewed and approved by the Health Research Ethics Committee (KEPK), Faculty of Medicine, Universitas Jember, Indonesia (Approval No. 119/UN25.1.10.2/KE/2026).

Results and discussion

Chicken body temperature measurements are carried out every hour for ten hours post-injection. There was a difference between temperatures in each group. The results of the temperature measurement of chicken breasts can be seen in figure 1.

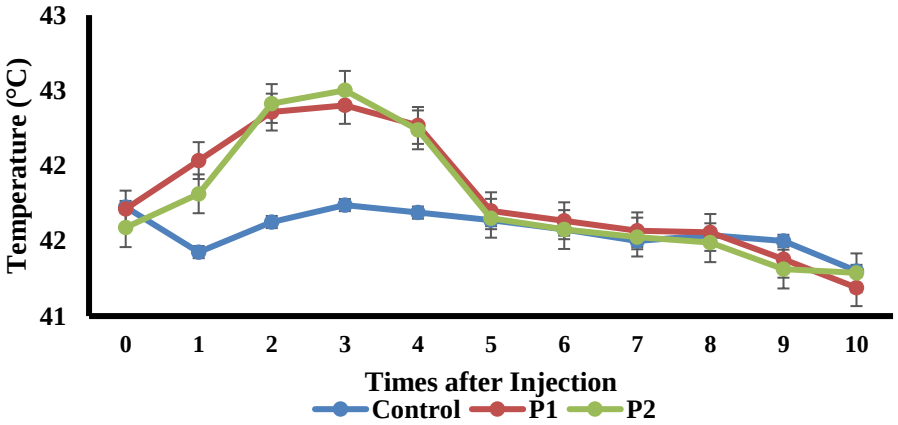

Figure 1. Chicken body temperature for 10 hours post-injection

The results of the chicken body temperature measurement showed that treatment groups 1 and treatment 2 had different results from the control group. Treatment groups 1 and 2 had an increase in temperature at the 1st hour to the highest in the 3rd hour with a temperature range of 42.5°C to 42.6°C. In contrast to the control group, which has a static temperature with a range of 41°C to 41.8°C. These results can illustrate in simple terms that LPS+adjuvant injection as a vaccine model can increase the body temperature of chickens. The increase in the body temperature of chickens here means that chickens are able to respond to the presence of LPS in their bodies, triggering fever (Groves et al., 2015). The fever that occurs is still in a normal stage and does not cause severe effects on the health of chickens or even death. Broadly speaking, LPS+adjuvant is able to trigger an early innate immune response in chickens that reflects the activation of early defense mechanisms and potentially supports the formation of subsequent immune responses (Zhang et al., 2019). The results of the statistical analysis of chicken body temperature can be seen in table 1 and table 2.

Table 1. Effect of treatment and time on chicken body temperature based on *Linear Mixed Model*

Source of Variation	df	F	*p-value*
Treatment	2	19.07	0.000
Time	10	33.41	0.000
Treatment*Time	20	4.95	0.000

Note : *An asterisk (**) denotes a statistically significant interaction effect between treatment group and observation time ($p < 0.05$).*

The results of the LMM analysis showed that the treatment, time, and interaction between treatment and time had a significant impact on the body temperature of chickens. The treatment given in the form of LPS + adjuvant was able to trigger a real chicken immune response in the treatment group compared to the control group. This shows that the fever that occurs reflects the activity of the initial innate immune response in chickens that is active to overcome the presence of non-specific pathogens that enter from outside the body (Zhang et al., 2019).

The significant influence of time shows that the temperature changes that occur at any given time are dynamic, which means that they change at any time. This characteristic is typical of the innate immune response that changes over time according to the physiological conditions that occur (Groves et al., 2015; Zhang et al., 2019). The significant interaction of treatment and time reinforced that each group had different temperatures throughout the observation time. These differences include the speed of the increase in temperature, the time of the highest fever peak, etc. This difference illustrates that the injection of the LPS vaccine model is influential in triggering an innate immune response (Zhang et al., 2019).

Table 2. Comparison of chicken body temperature between treatment groups based on post-hoc test (LMM)

Group comparison	Average temperature difference (°C)	OR	p-value
Control (PBS) vs LPS 0.5 mg/kg	−0.48	0.09	0.000
Control (PBS) vs LPS 1 mg/kg	−0.72	0.10	0.000
LPS 0.5 mg/kg vs LPS 1 mg/kg	−0.24	0.08	0.012

The results of the above statistics show that in the control group when compared to the two treatment groups the treatment differs significantly. The two treatment groups had higher temperatures significantly different from the control group. This shows that the LPS-based vaccine model can trigger an innate immune response in the form of fever, while in the control group that was only injected with PBS, the temperature tended to be normal without fever. This study is in line with the research of Xie et al. (2000), that broiler chickens injected with LPS had a higher temperature than the control group that only injected NaCl.

In both treatment groups, there was also a significant difference with temperatures at a concentration of 1mg/kg having higher temperatures and fever peaks. The increase in body temperature observed following LPS administration is associated with the activation of innate immune responses. LPS is

recognized by Toll-like receptor 4 (TLR4), leading to the release of pro-inflammatory cytokines such as IL-1β and IL-6, which act on the hypothalamic thermoregulatory center and induce fever as part of the systemic inflammatory response (Santacroce et al., 2023). Therefore, body temperature in this study was interpreted as a physiological indicator of systemic inflammatory response rather than direct evidence of pathogen-specific immunity. In this case, the administration of LPS with a higher concentration triggers a higher fever than a lower concentration.

Fever is the body's normal response in overcoming the presence of external pathogens that enter the body. LPS binds to TLR4 receptors which will then trigger the formation of cytokines including IL-10, IL-6, INF, etc. (Santacroce et al., 2023; Windoloski et al., 2022). The formation of these cytokines is a stage of the innate immune response in response to nonspecific pathogens. In responding to this pathogen, the body will emit the same response, namely the occurrence of fever.

The occurrence of fever in a normal way shows a good indicator because it means that the body is able to respond to the presence of pathogens. Fever can also occur due to a response from immune cells, namely macrophages that induce the production of cytokine IL-1, mast cells that also secrete histamine (Santacroce et al., 2023). This histamine production by the brain will cause an increase in body temperature until a fever occurs (Blomqvist, 2024).

The research conducted also had the same results as the research of Xie et al. (2000) and Groves et al. (2015), namely chickens injected with LPS had a higher body temperature than chickens in the control group. The occurrence of fever in treatment groups 1 and 2 showed positive results that the vaccine model can trigger a chicken-innate immune response. The response caused was not severe, which shows that the LPS vaccine model has the potential to be developed in the future (Groves et al., 2015). The response of the chicken's body also looks good by showing a regular fever with a time span that tends to be stable.

Although a significant febrile response was observed following crude LPS administration, fever alone does not fully represent protective innate immune activation. The present study did not evaluate cytokine expression, leukocyte dynamics, or molecular inflammatory markers. Therefore, further studies are required to confirm the immunostimulatory profile of the LPS preparation at the cellular and molecular levels.

Conclusion

This study confirms the pyrogenic effect of crude LPS derived from *Salmonella typhimurium* in domestic chickens, as reflected by a dose-dependent increase in body temperature. While these findings indicate systemic inflammatory activation, additional immunological assessments are necessary to determine its relevance for vaccine-related applications.

Author Statements

Acknowledgements and funding statements: The authors gratefully acknowledge to BIMA 2026 grant via Dr. Asmoro Lelono for providing financial support for this research. The authors also thank the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, University of Jember, for technical assistance and facilities provided during the experiment.

Competing of interest: The authors declare that there are no financial, professional, or personal relationships that could be perceived as influencing the research outcomes. The authors declare no competing interests.

Author's contributions: Conceptualization: Rizky Surya Wijaya, Dr. Asmoro Lelono, Dr. Rike Oktarianti; Methodology: Rizky Surya Wijaya, Dr. Asmoro Lelono, Dr. Rike Oktarianti; Investigation and Data Collection: Rizky Surya Wijaya; Formal Analysis: Rizky Surya Wijaya; Funding Acquisition: Dr. Asmoro Lelono; Supervision: Dr. Asmoro Lelono; Writing – Original Draft Preparation: Rizky Surya Wijaya; Writing – Review & Editing: Dr. Asmoro Lelono, Dr. Rike Oktarianti.

Generative AI: No generative artificial intelligence tools were used in the preparation of this manuscript.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

Barbosa, F. D. O., Freitas, O. C. D., Batista, D. F. A., Almeida, A. M. D., Rubio, M. D. S., Alves, L. B. R., ... & Berchieri, A. (2017). Contribution of flagella and motility to gut colonisation and pathogenicity of *Salmonella Enteritidis* in the chicken. *Brazilian Journal of Microbiology*, *48*(4), 754-759. https://doi.org/10.1016/j.bjm.2017.01.012

Blomqvist, A. (2024). Inflammation-induced fever depends on prostaglandin E2 production by brain endothelial cells and EP3 receptors in the median preoptic nucleus of the hypothalamus. *Acta Physiologica*, *240*(12). https://doi.org/10.1111/apha.14238

Chen, S., Xie, Y., Guo, D., Li, T., Tan, Z., Ran, X., & Wen, X. (2025). Effects of *Salmonella typhimurium* infection on intestinal flora and intestinal tissue arachidonic acid metabolism in Wenchang chickens. *Frontiers in Microbiology*, *16*, 1514115. https://doi.org/10.3389/fmicb.2025.1514115

Daryono, B. S., & Puspita, U. E. (2017). Pattern of inheritance of chicken crest (*Gallus gallus domesticus,* Linnaeus 1758) backcross result of crossing crown chickens with free-range chickens. *Journal of Veterinary Science*, *33*(2):164-172. https://doi.org/10.22146/jsv.17884

Groves, P. J., Sharpe, S. M., & Cox, J. M. (2015). Response of layer and broiler strain chickens to parenteral administration of a live *Salmonella typhimurium* vaccine. *Poultry Science*, *94*(7), 1512-1520. https://doi.org/10.3382/ps/pev127

Hassan, H. M., M.A. Fadel, & M.A. Soliman. (2020). Evaluation of a modified method of extraction, purification, and characterization of lipopolysaccharide (O antigen) from *Salmonella typhimurium*. *Veterinary World.* 13(11):2338. 10.14202/vetworld.2020.2338-2345

Khan, S., McWhorter, A. R., Andrews, D. M., Underwood, G. J., Moore, R. J., Van, T. T. H., & Chousalkar, K. K. (2024). A live attenuated *Salmonella typhimurium* vaccine dose and diluent have minimal effects on the caecal microbiota of layer chickens. *Frontiers in Veterinary Science*,*11*, 1364731. https://doi.org/10.3389/fvets.2024.1364731

Khan, S., McWhorter, A. R., Willson, N. L., Andrews, D. M., Underwood, G. J., Moore, R. J., & Chousalkar, K. K. (2025). Vaccine protection of broilers against various doses of wild-type *Salmonella typhimurium* and changes in gut microbiota. Veterinary Quarterly,*45*(1), 1-14. https://doi.org/10.1080/01652176.2024.2440428

Loor-Giler, A., Sanchez-Castro, C., Robayo-Chico, M., Puga-Torres, B., Santander-Parra, S., & Nuñez, L. (2025). High contamination of *Salmonella spp* in raw milk in Ecuador: Molecular identification of Salmonella enterica serovars Typhi, Paratyphi, Enteritidis and *Typhimurium*. Frontiers in Sustainable Food Systems,*9*, 1593266. https://doi.org/10.3389/fsufs.2025.1593266

Luo, R., Yao, Y., Chen, Z., & Sun, X. (2025). An examination of the LPS-TLR4 immune response through the analysis of molecular structures and protein–protein interactions. *Cell Communication and Signaling*, *23*(1), 142. 10.1186/s12964-025-02149-4

Mirzaei, A., Hedayati, M., Ashtiani, H., Rahbar, M. H., & Rastegar, H. (2011). A simple method for non phenolic extraction of lipopolysaccharide from *Salmonella typhimurium* and *Salmonella enteritidis* with high purity and pyrogenicity in rat. Sci. Res. Essays, *6*(5), 1101-1105. DOI: 10.5897/SRE10.1015

Murphy, J. I., Weaver, N. E., & Hendricks, A. E. (2022). Accessible analysis of longitudinal data with linear mixed effects models. Disease Models & Mechanisms, *15*(5), dmm048025. https://doi.org/10.1242/dmm.048025

Petes, C., Odoardi, N., Plater, S. M., Martin, N. L., & Gee, K. (2018). IL-27 amplifies cytokine responses to Gram-negative bacterial products and *Salmonella typhimurium* infection. Scientific Reports, *8*(1), 13704. 10.1038/s41598-018-32007-y

Santacroce, L., Colella, M., Charitos, I. A., Di Domenico, M., Palmirotta, R., & Jirillo, E. (2023). Microbial and host metabolites at the backstage of fever: current knowledge about the co-ordinate action of receptors and molecules underlying pathophysiology and clinical implications. Metabolites, *13*(3), 461. https://doi.org/10.3390/metabo13030461

Shaji, S., Selvaraj, R. K., & Shanmugasundaram, R. (2023). *Salmonella* infection in poultry: A review on the pathogen and control strategies. Microorganisms 11:2814. https://doi.org/10.3390/microorganisms11112814

Windoloski, K. A., Bansgaard, E. O., Dobreva, A., Ottesen, J. T., & Olufsen, M. S. (2022). A unified model for the human response to lipopolysaccharide-induced inflammation. arXiv preprint arXiv:2210.05712. https://doi.org/10.48550/arXiv.2210.05712

Xie, H., Rath, N. C., Huff, G. R., Huff, W. E., & Balog, J. M. (2000). Effects of *Salmonella typhimurium* lipopolysaccharide on broiler chickens. Poultry Science, *79*(1), 33-40. https://doi.org/10.1093/ps/79.1.33

Zhang, Z., La Placa, D., Nguyen, T., Kujawski, M., Le, K., Li, L., & Shively, J. E. (2019). CEACAM1 regulates the IL-6 mediated fever response to LPS through the RP105 receptor in murine monocytes. BMC immunology, *20*(1), 7. https://doi.org/10.1186/s12865-019-0287-y