Malaysian Applied Biology (2023) 52(3): 23-28
https://doi.org/10.55230/mabjournal.v52i3.2676

Research

Change in Immune Biomarkers of Laboratory Mice Responding to Experimental Inoculation of Different Drinking Water Quality from Selected Dairy Farms

Faez Firdaus Abdullah Jesse[1,2]*, Nagachandra Rao Gopi Naidu[1], Bura Thlama Paul[3,4], Eric Lim Teik Chung[2,5], Wan Lutfi Wan Johari[6], Yusuf Abba[7], Mohd Azmi Mohd Lila[8] and Mohd Jefri Norsidin[1]

1. Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2. Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3. Department of Animal Science and Fisheries, Faculty of Agriculture and Forestry Science, Universiti Putra Malaysia Campus Bintulu Sarawak, Malaysia
4. Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, 600230, Borno State, Nigeria
5. Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, UPM, Serdang, Selangor, Malaysia
6. Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
7. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069 Bama Road, Maiduguri, Nigeria
8. Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author: jesse@upm.edu.my

ABSTRACT

Current knowledge of abnormal physiological responses in livestock due to consumption of substandard water is limited. This study was designed to explore the host cell responses in mice orally inoculated with different drinking water qualities from selected dairy cattle farms. A total of 28 female mice used in this study were divided into Group 1- negative control (treated with sterile deionized distilled water), Group 2 - treated with good quality water sample, Group 3 - treated with moderate quality water sample, and Group 4 - treated with unsatisfactory quality water sample. All the mice were given 0.25 mL of water samples three times daily for 30 days. Blood samples were collected from all mice before euthanasia at 30 days post-inoculation for reproductive hormones and biomarkers analyses. All treatment groups showed significant (p<0.05) weight loss compared to the control group. There was a significant difference in the serum immunoglobulin-G (IgG), immunoglobulin-M (IgM), interleukin-12 (IL-12), haptoglobin (Hp), and serum amyloid A (SAA) profiles of mice among the different water quality treatments as compared to the control group (p<0.05). In conclusion, the host cell responses exhibited by the mice in the treatment groups indicates a high risk of potential negative effect on the production and health of the livestock due to long-term consumption of drinking water with subpar quality.

Key words: Biomarkers, dairy cattle farm, drinking water, host cell responses, mice, water quality

Article History
Accepted: 29 July 2023
First version online: 30 September 2023

Cite This Article:
Jesse, F.F.A., Naidu, N.R.G., Paul, B.T., Chung, E.L.T., Johari, W.L.W., Abba, Y., Lila, M.A.M. & Norsidin, M.J. 2023. Change in immune biomarkers of laboratory mice responding to experimental inoculation of different drinking water quality from selected dairy farms. Malaysian Applied Biology, 52(3): 23-28. https://doi.org/10.55230/mabjournal.v52i3.2676

INTRODUCTION

According to the Department of Veterinary Services Malaysia, Malaysia has a total cattle population of 721,341 in 2014 (DVS Malaysia, 2022). Most cattle in Malaysia occur throughout the country in smallholder farms with more primitive management and infrastructure without good pasture (Tong *et al*., 2018). However, small fractions of large-scale farms with better management systems are available in the government sector. Most of the large-scale dairy farms in Malaysia practice an intensive management system that provides good-quality of feed and water, to ensure optimum production (Moran, 2009). In small-scale production, dairy cattle graze extensively or semi-intensively in grass paddocks or open fields (Tong *et al*., 2018).

The United States Geological Survey (USGS)

reported that groundwater makes up about 60 % of the livestock freshwater source (USGS, 2005). Animal feeds with 50 to 70 % moisture content such as green chop, pasture, and silage can also fulfill their daily water requirements (Landefeld & Bettinger, 2000). Water pollution with sludge, chemicals, drugs, and other elements raises water safety concerns in farms (FAO, 2019). Previous reports have shown that 98% of the total water used in Malaysia originates from the rivers and 70% of the water is for agricultural use (Department of Environment, 2010). Despite this, only 3% of total water usage is accounted for by groundwater due to the lack of data on their quality and the high cost of groundwater extraction (Department of Environment, 2010). According to a survey by the Malaysian Department of Environment in 2010, all 107 groundwater monitoring wells showed excessive amounts of arsenic, iron, manganese, total coliform, and phenol. The quality of tap water is consistent and safe for consumption due to proper treatment before distribution, but other sources are questionable due to lack of treatment (MOH Malaysia, 2004).

A previous study by Su *et al*. (2017) showed that if present, the consumption of high concentrations of copper, zinc, manganese, and chromium produced degenerative necrotic changes in the liver and renal parenchyma of albino rats. Moreover, the consumption of high amounts of cadmium can cause lipidosis, focal necrosis, and proliferation of Kupffer cells in the liver tissue, degeneration of the renal tubules, edema, aggregation of the inflammatory cell, and thickening of interalveolar septa of the lung tissue (El-Refaiy & Eissa, 2013). Additionally, Nakade *et al*. (2015) have reported that if present, lead can induce vacuolar degeneration in the uterine tissue while Nasiadek *et al*. (2018) recorded uterine edema in rats because of cadmium toxicity. The consumption of excessive amounts of nitrate induced inflammatory cell infiltration and necrotic fibrosis in the liver tissue (Bouaziz-Ketata *et al*., 2014). Exposure to excessive amounts of phosphate caused renal tubular degenerative changes and peritubular infiltration of inflammatory cells (Eller *et al*., 2011). Also, certain water contaminants known as endocrine disruptors such as atrazine, phenols, and phthalates can reduce reproductive efficiency in humans and animals by interfering with the reproductive cycle (Rattan *et al*., 2017). The risk of important bacterial (coliforms, Salmonella spp., typhoid, leptospirosis, melioidosis), viral (including dengue, hepatitis, enterovirus), and parasitic (amoebiasis, giardiasis and cryptosporidiosis, helminth infections, Blastocystis infections and sarcocystosis) infections of livestock are increased by poor drinking water quality (Rahman *et al*., 2021; Ho *et al*., 2022). However, there is no data on the abnormal physiological and pathological effects in host cells due to consuming substandard water quality in Malaysia. The responses of the host cells mediated by proinflammatory cytokines, acute-phase proteins, antibodies, and reproductive hormones due to challenges from water contaminants are not fully understood in animals. The study of host cell responses due to the effects of different water qualities is an essential indicator of the possible impact that the animals might suffer from the consumption of these water samples. This study investigated the host cell responses of mice receiving different qualities of drinking water.

MATERIALS AND METHODS

Collection and assessment of water samples

Seven dairy cattle farms from the state of Selangor in West Malaysia were selected randomly and visited to collect livestock drinking water samples in acid-washed containers and transported at 4 °C to the laboratory for further analysis. The water samples were initially examined onsite using probes to determine the dissolved oxygen, temperature, pH, turbidity, conductivity, and salinity. In the laboratory, water samples were further tested to determine the biological oxygen demand (BOD), chemical oxygen demand (COD), total coliform count, total soluble solids (TSS), total dissolved solids (TDS), magnesium and iron, nitrate, phosphate, and ammoniacal nitrogen using standard methods (Jesse *et al*., 2022). Drinking water samples were further categorized as good (Class I and Class II), moderate (Class III), or unsatisfactory (Class IV and Class V) according to the National Water Quality Standard of Malaysia (MOH Malaysia, 2004).

Experimental design

The female mice were acclimatized for 14 days in the Livestock Research Facility, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Estrous synchronization was done by the intraperitoneal injection of 0.5 µg of cloprostenol twice given 3 days apart in combination with a single dose of 3µg subcutaneous of progesterone together with the first cloprostenol injection (Pallares & Gonzales-Bulnes, 2009). The mice (n=28) were divided into 4 groups, with 7 mice in each group. All the mice were fed 0.25 mL of the water sample three times daily by oral gavage and the same water sample was given *ad libitum* in feeding bottles for 30 days. Group 1 (negative control) was given sterile deionized distilled water, Group 2 was given good water quality, Group 3 was given moderate water quality and Group 4 was given unsatisfactory water quality. The mice were weighed and observed daily for any clinical signs during the 30-day experiment. At the end of 30 days, all the mice were euthanized by anesthesia, and blood was quickly withdrawn from the heart before death.

Radioimmunoassay for quantification of serum progesterone and estrogen

Oestradiol and progesterone radioimmunoassay (RIA) kits (Beckman Coulter Diagnostics) were used to determine the serum oestradiol (ng/mL) and progesterone (ng/mL) concentrations. All the serum samples were diluted in a 1:4 ratio using distilled water. 500 µl of ^{125}Iodine labeled tracer solution was first diluted in the tracer buffer by gently and carefully pouring it inside the tracer buffer vial. 100 µL of the calibrator, control, or sample was added with 500 µl of the tracer solution into the anti-hormonal antibody-coated tubes. The test tubes were then incubated for 3 h at 18 – 25 °C with shaking (350 r.p.m). The content of the test tubes was then aspirated carefully after incubation. The test tubes were then run through the Packard 2100TR gamma counter to determine the radioactivity of the test tubes. The gamma counter results were then run through the ARIA II automated radioimmunoassay analyzer to determine the serum oestradiol and progesterone concentrations.

Enzyme-linked immunosorbent assays for acute-phase response

Commercial enzyme-linked immunosorbent assay (ELISA) was used to quantify serum immunoglobulins (Ig) G (µg/mL), IgM (µg/mL), interleukin (IL)-12 (µg/mL), Haptoglobin (Hp) (µg/mL), and serum amyloid A (SAA) levels (ng/mL). Briefly, the test sera were diluted to 1:10000 using the diluent solution. The standards (100 µL) and the test samples (100 µL) were pipetted in duplicates into the microtiter plate and incubated at room temperature for 60 min. This step was followed by washing before adding 100 µL of the Enzyme-Antibody conjugate and further incubation at room temperature in the dark for 30 min. The plates were then rewashed before adding 100 µL of the ready-to-use peroxidase substrate containing TMB in a mildly acidic buffer in each well. The microtiter plate was then left at room temperature in the dark for another 10 min, followed by the addition of 100 µL of stop solution. The absorbance of the plate at 450 nm was recorded using a Labomed EMR-500 ELISA microplate reader. The optical densities obtained from the ELISA microplate reader were quantitatively analyzed using a Four Parameter Logistic curve fit to calculate the concentrations of immunoglobulins, cytokine, and acute-phase proteins, in the serum samples (www.myassays.com).

Statistical analysis

The analysis of experimental data was done using the IBM statistical package for social sciences software version 25.0. A one-way analysis of variance (ANOVA) and Dunnett's posthoc test were used to compare means between treatment groups at a 5% level of significance (p<0.05).

RESULTS

Changes in serum immune biomarkers and reproductive hormones

In this study, no significant clinical signs were observed in the mice from Groups 1, 2, and 3. For Group 5, all the mice showed severe ruffled fur, dehydration, and severe emaciation while most of the mice in Group 4 exhibited moderate emaciation and mild ruffled fur at the end of the experiment period. The results of ELISA assays for various serum biomarkers are summarised in Table 1. It was hypothesized that there would be no difference in the mean serum immune and reproductive biomarkers of mice inoculated with different drinking water quality. As expected, the results of statistical analysis on experimental data using one-way ANOVA and Dunnett's post-hoc test revealed no difference in the serum concentration of estrogen (F (3, 50) = 0.57, p=0.475) and progesterone (F (3, 50) = 0.57, p=0.638) hormones among the different water quality treatments. However, contrary to our null hypothesis, there was a significant difference in the serum IL-12 concentration (F (3, 50) = 43.86, p=0.0001), SAA (F (3, 50) = 256.19, p=0.0001), Haptoglobin (F (3, 50) = 6.367, p=0.001), IgG (F (3, 50) = 8.38, p=0.0001), and IgM (F (3, 50) = 77.71, p=0.0001) among the different treatments. The mean serum concentration of IL-12 was increased by 4.9% in Group 3 (318.14±16.23 µg/mL) and 27.4% in Group 4 (389.60±25.82 µg/mL) compared with the control group. The mean SAA was increased by 24% in Group 3 (281.91±8.26 ng/mL) and 25% in Group 4 (297.16±7.32 ng/mL) compared to Group 1 (224.62±7.86 ng/mL). The mean serum Hp was increased by 3.7% in Group 3 (68.21±1.54 µg/mL) and 3.6% in Group 4 (68.20±1.20 µg/mL) compared to the control Group 1 (65.74±2.94 µg/mL). The mean serum concentration of IgG was increased by 1.7% in Group 3 (919.23±11.12 µg/mL) and 2.4% in Group 4 (926.22±9.03 µg/mL) compared with the control. The mean serum concentration of IgM was increased by 10.6% in Group 3 (567.13±38.11 µg/mL) and 40.8% in Group 4 (742.70±35.85 µg/mL) compared with the control. But there is no difference between Group 2 (525.60±51.11 µg/mL) and the control (Table 1).

Table 1. Results of assays for immune and reproductive biomarkers of laboratory mice inoculated with different qualities of livestock drinking water expressed as Mean±SD

Variables	Group 1	Group 2	Group 3	Group 4
SAA (ng/mL)	224.62±7.86^{a}	231.22±9.22^{a}	281.91±8.26^{b}	297.16±7.32^{b}
Haptoglobin (µg/mL)	65.74±2.94^{a}	65.99±1.66^{a}	68.21±1.54^{b}	68.20±1.20^{b}
Iinterleukin-12 (µg/mL)	302.52±22.72^{a}	316.31±18.57^{a}	318.14±16.23^{b}	389.60±25.82^{b}
Immunoglobulin-G (µg/mL)	904.01±14.57^{a}	909.85±13.22^{a}	919.23±11.12^{b}	926.22±9.03^{b}
Immunoglobulin-M (µg/mL)	511.55±49^{a}	525.60±51.11^{a}	567.13±38.11^{b}	742.70±35.85^{b}
Oestrogen (ng/mL)	4.24±0.09^{a}	4.28±0.08^{a}	4.00±0.48^{a}	4.27±0.10^{a}
Progesterone (ng/mL)	1.37±0.26^{a}	1.13±0.35^{a}	1.10±0.71^{a}	1.08±0.42^{a}

Row means represented by different superscripts (^{a,b}) were significantly different (p<0.05). Progesterone and estrogen data were log-transformed LG10(SAA) in SPSS.

DISCUSSION

The result of this study has revealed significant changes in host immune biomarkers with different degrees of severity observed depending on the treatment groups. Findings of weight loss, dehydration, emaciation, and ruffled fur observed during the experiment may be due to decreased water intake as a direct consequence of poor palatability of the inferior quality of the drinking water provided to the mice (Maharjan *et al*., 2016). The turbidity and TSS levels of water cause decreased palatability and less consumption (Bilotta *et al*., 2008). Decreased water intake in animals causes a reduction in their daily food consumption and slows down the metabolic process (Fortun-Lamothe & Boullier, 2007). The high levels of heavy metals in the drinking water can also damage the internal organs such as the liver and kidneys, leading to reduced appetite and weight loss over time (Su *et al*., 2017). In this study, the treated mice showed moderate to severe emaciation with pale and dry muscle and atrophy of the fat tissues. These changes are usually associated with prolonged starvation in animals due to the breakdown of fat and muscle to produce energy for bodily functions (Patel *et al*., 2017; Jesse *et al*., 2019).

However, there was a significant difference in the serum IL-12 concentration, SAA, Haptoglobin, IgG, and IgM profiles of mice among the different water quality treatments. The IgM antibody concentration for treatment groups 3 and 4 showed an increment in concentration. The elevation of these immune parameters indicates that the animals are having active infections ongoing, which may be potentially caused by coliform bacteria in the untreated drinking water samples evaluated (Jesse *et al*., 2020). Exposure to high levels of heavy metals over a prolonged period as seen in this study can also affect the immunological parameters. A study done by Marth *et al*. (2001) reported that exposure to high levels of heavy metals over a prolonged time could affect the immunological parameters in this study it was observed that all the treatment groups had a significantly elevated serum IL-12 concentration and this can be indicative of regulation of T-helper cells responses due to toxicity caused by contaminants (Colosio *et al*., 1998). Moreover, the significant increase in serum Hp and SAA proteins in the present study suggests the presence of an active inflammatory reaction. Both Hp and SAA are positive acute-phase proteins that will increase in episodes of acute inflammation which indicates that the animals from all the treatment groups were having bouts of inflammation of different severity (Eckersall *et al*., 2010; Takata *et al*., 2011; Shih *et al*., 2013; Chung *et al*., 2019).

As stated in our null hypothesis, the results of statistical analysis revealed no difference in the serum concentration of estrogen and progesterone hormones among the different water quality treatments. The absence of significant differences in the serum progesterone and estrogen hormone profiles of mice for the different livestock drinking water quality samples evaluated in this study suggests that the present water quality used in various livestock farms is not detrimental to the reproductive physiology of the animals. However, the numerical reduction of estrogen hormone concentration might be due to the presence of contaminants such as heavy metals (Ryzmski *et al*., 2016), because the water samples were not treated. Previously, Kenny *et al*. (2002) reported that ingestion of excessive amounts of ammonia could cause changes in the uterine hormones and the oviductal fluids of animals. Other chemicals such as atrazine, phenols, and phthalates, which are collectively known as endocrine disruptors can also affect hormonal dynamics in animals (Rattan *et al*., 2017). Furthermore, Ferguson *et al*. (2013) have shown that prolonged exposure to heavy metals could cause irregularities in hormone levels and immunological parameters.

CONCLUSION

This study has established that drinking unsafe water can cause detrimental effects on the host cell evidenced by increased immune activity in mice. The knowledge of host cell responses of acute-phase proteins, antibodies, proinflammatory cytokines, and reproductive hormones due to water contaminants

in this study is new in ruminant medicine. Therefore, constant monitoring is required to ensure that the drinking water provided to livestock in the dairy farms meets the required standard.

ACKNOWLEDGEMENTS
The Universiti Putra Malaysia, Research and Innovation Unit funded the cost of travel, sampling, data curation, analysis, and laboratory consumables. The authors are grateful to the Faculty of Veterinary Medicine staff and the Faculty of Environmental Studies for lending tremendous support to the successful conduct of this project.

ETHICAL STATEMENT
The Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia approved all the protocols used in this study (UPM/IACUC/AUP-R004/2017).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Aksoy, A.N., Toker, A., Celık, M., Aksoy, M., Halıcı, Z. & Aksoy, H. 2014. The effect of progesterone on systemic inflammation and oxidative stress in the rat model of sepsis. Indian Journal of Pharmacology, 46(6): 622-626. https://doi.org/10.4103/0253-7613.144922

Bilotta, G.S. & Brazier, R.E. 2008. Understanding the influence of suspended solids on water quality and aquatic biota. American Journal International Water Association: Water Resources, 42: 2849-2861. https://doi.org/10.1016/j.watres.2008.03.018

Bouaziz-Ketata, H., Salah, G.B., Salah, H.B., Marrekchi, R., Jamoussi, K., Boudawara, T., Fakhfekh, F. & Zeghal, N. 2014. Nitrate-induced biochemical and histopathological changes in the liver of rats: Ameliorative effect of *Hyparrhenia hirta*. Biomedical and Environmental Sciences, 27(9): 695-706.

Chung, E.L.T., Jesse, F.F.A., Marza, A.D., Ibrahim, H.H., Abba, Y., Zamri-Saad, M., Haron, A.W., Mohd Lila, M. A., Saharee, A.A., Omar, A.R., Bakar, M.Z.A. & Norsidin, M.J. 2019. Responses of proinflammatory cytokines, acute phase proteins and cytological analysis in serum and cerebrospinal fluid during haemorrhagic septicaemia infection in buffaloes. Tropical Animal Health and Production, 51: 1773-1782. https://doi.org/10.1007/s11250-019-01870-w

Colosio, C., Barcellini & W., Corsini, E. 1998. Immunotoxicology in occupational and environmental medicine: prospectives, limitations, and research objectives. Medicina del Lavoro, 89(3): 203-25.

Department of Environment (DOE) Malaysia. 2010. Environmental Requirements: A Guide For Investors. Ministry of Natural Resources and Environment, Malaysia. pp. 1-82.

DVS Malaysia. 2022. Livestock Statistics. Department of Veterinary Services. Malaysia. pp. 1-25

Eckersall, P.D. & Bell, R. 2010. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal, 185: 23-27. https://doi.org/10.1016/j.tvjl.2010.04.009

El-Refaiy, A.I. & Eissa, F.I. 2013. Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi Journal of Biological Sciences, 20(3): 265-280. https://doi.org/10.1016/j.sjbs.2013.02.004

Eller, P., Eller, K., Kirsch, A.H., Patsch, J.J., Wolf, A.M., Tagwerker, A., Stanzl, U., Kaindl, R., Kahlenberg, V., Mayer, G., Patsch, J.R. & Rosenkranz, A.R. 2011. A murine model of phosphate nephropathy. The American Journal of Pathology, 178(5): 1999-2006. https://doi.org/10.1016/j.ajpath.2011.01.024

FAO. 2019. Water use in livestock production system and supply chain. Food and Agriculture Organization of the United Nations, Rome.

Ferguson, K.K., O'Neill, M.S. & Meeker, J.D. 2013. Environmental contaminant exposures and preterm birth: a comprehensive review. Journal of Toxicology and Environmental Health Part B: Critical Reviews, 16: 69-113. https://doi.org/10.1080/10937404.2013.775048

Fortun-Lamothe, L. & Boullier, S. 2007. A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. Livestock Science, 107: 1-18. https://doi.org/10.1016/j.livsci.2006.09.005

Ho, J.Y., Lavinya, A.A., Kay, D.S.W., Lee, C.I.S., Razmi, A.H., Walsh, C.L., Goodson, M.L. & Eswaran, J., 2022. Towards an integrated approach to improve the understanding of the relationships between water-borne infections and health outcomes: Using Malaysia as a detailed case study. Frontiers in Water, 4: 779860. https://doi.org/10.3389/frwa.2022.779860

Jesse, F.F.A., Bitrus, A.A., Chung, E.L.T., Peter, I.D., Mohd Lila, M.A. & Salleh, A. 2019. Severe parasitic gastroenteritis (PGE) in a goat: a veterinary case report and way forward. The Thai Journal of Veterinary Medicine, 49(3): 295-299.

Jesse, F.F.A., Hashi, H.A., Chung, E.L.T. & Mohd Lila, M.A. 2020. Review on current seroprevalence status of common small ruminants' bacterial and viral diseases in tropics focusing in Malaysia: a holistic of current status essential to be study and known. Advances in Animal and Veterinary Science, 8(2): 140-146. https://doi.org/10.17582/journal.aavs/2020/8.2.140.146

Jesse, F.F.A., Naidu, N.R.G., Johari, W.L.W., Chung, E.L.T., Paul, B.T., & Lila, M.A.M., 2022. Assessment of drinking water and wastewater quality in selected dairy cattle farms from Malaysia. Journal of Advanced Veterinary Research, 12(2): 128-134.

Kenny, D.A., Humpherson, P.G., Leese, H.J., Tomos, A.D., Diskin, M.G. & Sreenan, J.M. 2002. Effect of elevated systemic concentrations of ammonia and urea on the metabolite and ionic composition of oviductal fluid in cattle. Biology of Reproduction, 66: 1797-1804. https://doi.org/10.1095/biolreprod66.6.1797

Landefeld, M. & Bettinger, J., 2002. Water effects on livestock performance. Ohio State University Agriculture and Natural Resources. Columbus, Ohio.

Maharjan, P., Ingmanson, S. & Watkins, S. 2016. Animal drinking water sanitation with AOP technology. International Journal of Agricultural Science, 6(2): 931-937.

Marth, E., Jelovcan, S., Kleinhappl, B. & Gutschi, B.S. 2001. The effect of heavy metals on the immune system at low concentrations. International Journal of Occupational Medicine and Environmental Health, 14(4): 375-86.

MOH Malaysia. 2004. National Standard for Drinking Water Quality. Engineering Services Division. Ministry of Health. Malaysia. pp. 1-11.

Moran, J.B. 2009. Key Performance Indicators to diagnose poor farm performance and profitability of smallholder dairy farmers in Asia. Asian-Australasian Journal of Animal Sciences, 22(12): 1709-1717. https://doi.org/10.5713/ajas.2009.90201

Nakade, U.P., Garg, S.K., Sharma, A., Choudhury, S., Yadav, R.S., Gupta, K. & Sood, N. 2015. Lead-induced adverse effects on the reproductive system of rats with particular reference to histopathological changes in uterus. Indian Journal of Pharmacology, 47(1): 22-26. https://doi.org/10.4103/0253-7613.150317

Nasiadek, M., Danilewicz, M., Sitarek, K., Świątkowska, E., Daragó, A., Stragierowicz, J. & Kilanowicz, A. 2018. The effect of repeated cadmium oral exposure on the level of sex hormones, estrous cyclicity, and endometrium morphometry in female rats. Environmental Science and Pollution Research, 25(28): 28025-28038. https://doi.org/10.1007/s11356-018-2821-5

Pallares, P. & Gonzalez-Bulnes, A. 2009. A new method for induction and synchronization of oestrus and fertile ovulations in mice by using exogenous hormones. Laboratory Animals, 43(3): 295-299. https://doi.org/10.1258/la.2008.008056

Patel, B.G., Rudnicki, M., Yu, J., Shu, J. & Taylor, R.N. 2017. Progesterone resistance in endometriosis: origins, consequence and interventions. Acta Obstetricia et Gynecologica Scandinavia, 96: 623-632. https://doi.org/10.1111/aogs.13156

Rahman, M.S.A., Bejo, S.K., Zakaria, Z., Hassan, L. & Roslan, M.A., 2021. Seroprevalence and distribution of leptospiral serovars in livestock (cattle, goats, and sheep) in flood-prone Kelantan, Malaysia. Journal of Veterinary Research, 65(1): 53. https://doi.org/10.2478/jvetres-2021-0003

Rattan, S., Zhou, C., Chiang, C., Mahalingam, S. & Brehm, E.F. 2017. Exposure to endocrine disruptors during adulthood: Consequences for female fertility. Journal of Endocrinology, 233(3): R109-R129. https://doi.org/10.1530/JOE-17-0023

Rzymski, P., Niedzielski, P., Rzymski, P., Tomczyk, K., Kozak, L. & Poniedzialek, B. 2016. Metal accumulation in the human uterus varies by pathology and smoking status. Fertility and Sterility, 105(6): 1511-1518. https://doi.org/10.1016/j.fertnstert.2016.02.006

Shih, A.W.Y., Mc Farlane, A. & Verhovsek, M. 2013. Haptoglobin testing in hemolysis: Measurement and interpretation. American Journal of Hematology, 89(4): 443-447. https://doi.org/10.1002/ajh.23623

Su, H., Li, Z., Kenston, S.S.F., Shi, H., Wang, Y., Song, X., Gu, Y., Barber, T., Aldinger, J., Zou, B., Ding, M., Zhao, J. & Lin, X. 2017. Joint toxicity of different heavy metal mixtures after a short-term oral repeated administration in rats. International Journal of Environmental Research and Public Health, 14(10): 1164. https://doi.org/10.3390/ijerph14101164

Takata, S., Wada, H., Tamura, M., Koide, T., Higaki, M., Mikura, S.I. & Yasutake, T. 2011. Kinetics of c-reactive protein (CRP) and serum amyloid A (SAA) in patients with community-acquired pneumonia (CAP), as presented with biologic half-life times. Biomarkers, 16(6): 530-535. https://doi.org/10.3109/1354750X.2011.607189

Tong, A.X., Nor, N.M., Indrawan, D. & Suhaimi, N.A.M. 2018. A Review: Should small holder dairy farms rear own young stock? Malaysian Journal of Animal Science, 21(2): 1-6.

Usgs.gov.edu. 2005. Livestock water use. US Department of the Interior | US Geological Survey.