

INSIGHTS INTO HEMATOLOGICAL AND BIOCHEMICAL RESPONSES IN RABBITS: A COMPARATIVE STUDY IN EVALUATING THE SAFETY AND EFFICACY OF LASER ACUPUNCTURE

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ABSTRACT

This study evaluated the hematological profile, total blood cholesterol, and total blood protein in Hycole rabbits after laser acupuncture treatment at specific and non-specific acupuncture points. This randomized controlled trial was conducted using 24 healthy Hycole rabbits aged 30 days with an average body weight of 1046 ± 79 g. The animals were randomly allocated into three equal treatment groups ($n = 8$ per group). Group 1 served as the control and received placebo laser treatment, Group 2 received laser acupuncture at specific acupoints (Bladder 21, Bladder 15, and Bladder 13), while Group 3 underwent laser stimulation at non-acupoint locations. All treatments were administered once weekly for a total of six sessions over six weeks. Data were analysed descriptively. The study findings demonstrated that all measured parameters including hematological profiles, total cholesterol, and plasma protein levels, remained within established physiological ranges without clinically significant variations. These results confirm the safety of laser acupuncture as a welfare-enhancing intervention, suggesting its potential utility in commercial livestock production systems.

Key words: hematological profile, hycole rabbits, laser acupuncture, livestock health management, total blood cholesterol

ABSTRAK

Penelitian ini mengevaluasi profil hematologi, kadar kolesterol darah total, dan kadar protein darah total pada kelinci Hycole setelah dilakukan induksi laser akupunktur pada titik akupunktur spesifik dan non-spesifik. Dalam penelitian ini digunakan 24 ekor kelinci Hycole yang berumur 30 hari dengan rata-rata berat badan 1046 ± 79 g. Kelinci dibagi secara acak ke dalam tiga kelompok, masing-masing terdiri atas delapan ekor per kelompok. Kelompok satu berperan sebagai kontrol/plasebo, kelompok dua diberi induksi laser akupunktur pada titik akupunktur spesifik (Bladder 21, Bladder 15, dan Bladder 13), sedangkan kelompok tiga menerima induksi laser akupunktur tidak tepat pada titik-titik tersebut. Perlakuan diberikan sebanyak enam kali selama enam minggu. Data disajikan sebagai rata-rata per kelompok dan dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa profil hematologi, kadar kolesterol darah total, dan kadar protein darah total kelinci berada dalam rentang nilai normal tanpa perubahan signifikan antar kelompok. Temuan ini mengindikasikan bahwa induksi laser akupunktur merupakan metode yang aman jika diaplikasikan pada ternak dan memiliki potensi dalam peningkatan manajemen kesehatan ternak.

Kata kunci: profil hematologi, Hycole, laser akupunktur, manajemen kesehatan ternak, kolesterol darah total

INTRODUCTION

Animal protein is a protein with higher quality than other types of protein for humans because animal protein has an excellent amino acid pattern and digestibility. Animal protein is also essential for maintaining health in vulnerable groups such as young children, the elderly, pregnant and lactating mothers. Animal protein has high-quality protein for optimal human growth and development, including stimulating bone growth and maintenance (Wu 2016; Vieux *et al.* 2022). Besides that, about half of the total adult protein intake must come from animals to meet nonprotein nutrient-based recommendations (Vieux *et al.* 2022). Adequate consumption of high-quality protein from animal products (e.g. lean meat and dairy) has been proven to affect consumer health and influence many physiological responses (Wu 2016; Albenzio *et al.* 2017).

The demand for animal protein is expected to be high in the future, so new initiatives are needed to produce high-quality protein in sufficient quantities. Increasing rabbit meat production can be an alternative to meet animal protein needs. Rabbit meat has high nutritional and dietetic characteristics, but its consumption rate is relatively low compared to other

types of meat. The nutritional profile of rabbit meat, compared to beef, pork, and poultry, is due to its relatively higher proportion of n-3 fatty acids and low amounts of intramuscular fat, cholesterol, and sodium, suggesting that its consumption may provide health benefits to consumers (Kumar *et al.* 2023). Rabbit meat has high nutritional value, meeting better dietary values than other wild poultry and mammal species, and rabbit meat is healthier (Frunzä *et al.* 2023).

Three simultaneous changes that are being made to meet future demand for animal protein are shifting protein sources within the supply chain, using plant-based substitutes, and using new sources (Boland *et al.* 2013). Several studies to increase rabbit production have been carried out as increased rabbit meat production has been carried out through feed supplementation with Azolla leaves (Abdelatty *et al.* 2021), rosemary and ginger essential oils (Elazab *et al.* 2022), and extract supplementation from plants (Vizzarri *et al.* 2017). The technology applied to realize the advanced and efficient rabbit farming sector is still limited to feed technology or feed substitution. For this reason, a method is needed to increase rabbit livestock production to maximize the potential of producing meat rabbits.

The method required is straightforward, suitable technology that can collaborate effectively with rabbit farmers, enabling the goal of improving their welfare through the combined use of knowledge and innovation. A practical and effective technological innovation that can complement rabbit farming to boost livestock production is the use of laser acupuncture technology. Acupuncture is an ancient Chinese medical practice that has become the subject of increasing scientific interest in recent decades, especially for its potential therapeutic effects. Studies on rabbit acupuncture commonly explore its benefits for health, such as pain management, alleviating corneal inflammation, and enhancing post-surgical healing (Ding *et al.* 2022). There are several studies on the effect of acupuncture on livestock production, such as improving the quality of quail eggs (Warsito *et al.* 2021), anti-inflammatory using the ST36 acupuncture point (Oh and Kim 2022), regulation of the immune system (Lai *et al.* 2019) and equine rehabilitation (le Jeune *et al.* 2016).

To date, no research has assessed the hematological impact of laser acupuncture on livestock. Many also remain doubtful about its use in enhancing production, seeing it as limited to medical or experimental applications. Addressing this gap is essential, as understanding the impact of acupuncture on rabbits' hematological profiles could provide valuable insights into its application in livestock and its potential use in human treatments (Díaz-Hernández *et al.* 2022). The objective of this research is to investigate the hematological effects of laser acupuncture in rabbits by monitoring blood parameters, with the anticipation that no clinically significant changes will result from the treatment. The findings are also considered for potential applications in livestock management.

MATERIALS AND METHODS

The research concept has received an ethical clearance certificate from the Animal Ethics Commission, Faculty of Veterinary Medicine, Airlangga University. The research was carried out in the experimental animal laboratory of the Faculty of Veterinary Medicine, Airlangga University. This study employed a completely randomized design, utilizing 24 Hycole rabbits which were equally distributed across three treatment groups (eight animals per group). The study used healthy, 30-day-old weaned rabbits with an average body weight of 1046 ± 79 g. prior to study, the rabbits were allowed a one-week acclimatization period to adjust to the research environment. During acclimatization, rabbits were kept individually in pathogen-free cage (45.5 x 37.5 x 30 cm) with free access to water provided through nipple drinkers. The rabbits were maintained under natural light cycles (12-hour light/12-hour dark) at an ambient temperature of approximately 27.5 °C. Feed was given at 8.00 am in the morning and 4.00 pm in the afternoon. The physical condition of the rabbits was observed every day during the study.

The acupuncture points used were Bladder 13, Bladder 15, and Bladder 21. The first treatment was a control (placebo). The rabbits received simulated acupuncture at the exact predetermined points. The placebo acupuncture serving vital research functions by reducing physiological variables, establishing clear response baselines, and remaining biologically neutral (Kaptchuk 2020). All laser acupuncture treatments were performed on the right and left sides of the rabbits. This study utilized the VLP-100 veterinary laser device, a specialized tool designed specifically for animal treatment applications. This tool uses a 20 mW semiconductor acupuncture laser from the Biophysics Laboratory of Airlangga University. During the laser acupuncture procedure, rabbits were carefully positioned to ensure both comfort and minimal movement throughout the treatment. The rabbits were given laser acupuncture every seven days for six weeks.

Three millilitres of blood were taken via the auricular vein when the rabbit was 75 days old. The blood was placed in a vacuum tube containing ethylene diamine tetra-acetate EDTA. Blood was examined on a Hematology Analyzer ABX Micros 60 (made in France). Blood analysis data generated by the hematology analyzer were tabulated, transferred to Microsoft Excel for organization, and statistically evaluated using SPSS descriptive analysis.

RESULTS AND DISCUSSION

Hematological profiles serve as valuable indicators of overall physiological status in both humans and animals, reflecting systemic health through the vital interconnection between blood composition and tissue metabolism (Pearce 2009). Blood components are greatly influenced by the quality and quantity of food absorbed. Blood components significantly affect productivity and life and the harmony of the function of each animal organ. Through blood tests, metabolic and hematological profiles can be determined (Zahirovic *et al.* 2024). This blood test is essential to evaluate and analyze the effects of laser acupuncture treatment on rabbit health. Knowledge of reference values for blood metabolites in rabbits is very beneficial for information on animal health status, especially hematological parameters, which can provide information on whether the rabbit is experiencing infection, inflammation and necrosis in the rabbit's body (Díaz Cano *et al.* 2021; Sierra-Galicia *et al.* 2022).

Hematological profiles of rabbits treated with laser acupuncture were presented in Table 1. The hematological analysis revealed that all parameters remained within established normal ranges, with no clinically significant deviations observed. As evidenced by the tabulated data, laser acupuncture administration demonstrated no adverse effects on rabbit health. These findings confirm that the treatment protocol maintains the animals' physiological well-being. Average hemoglobin concentration and red blood cell counts, proving they were not anemic. The concentration of

Table 1. Hematological profile of rabbits treated with laser acupuncture comparison with standart hematology of rabbits (n= 24)

Parameter	Group A	Group B	Group C	Normal*
RBC ($\times 10^3 \mu\text{L}^{-1}$)	5.7 $\pm$ 0.114 (5.531-5.902)	5.56 $\pm$ 0.050 (5.484-0.042)	5.82 $\pm$ 0.106 (5.366-6.001)	5.46-7.94
WBC ($\times 10^3 \mu\text{L}^{-1}$)	4.48 $\pm$ 0.235 (4.046-4.821)	4.56 $\pm$ 0.206 (4.089-4.844)	4.42 $\pm$ 0.200 (4.053-4.713)	3-12.5
Hemoglobin (g/dL ⁻¹)	10.76 $\pm$ 0.136 (10.452-10.948)	10.7 $\pm$ 0.126 (10.521-10.946)	10.68 $\pm$ 0.152 (10.462-10.972)	10.4-17.4
Hematocrit (%)	35.5 $\pm$ 0.353 (34.773-35.994)	34.38 $\pm$ 0.310 (33.881-35.586)	33.76 $\pm$ 0.102 (33.516-34.451)	33-50
Thrombocytes ($\times 10^3 \mu\text{L}^{-1}$)	311.4 $\pm$ 3.893 (304.919-317.414)	302.5 $\pm$ 3.679 (296.582-308.418)	343 $\pm$ 14.821 (313-951-372-049)	304-656
Lymphocytes (%)	64.2 $\pm$ 037 (63.564-64.769)	61.8 $\pm$ 0.374 (61.013-62.320)	61.8 $\pm$ 0.489 (61.123-62.877)	30-85
Monocytes (%)	13 $\pm$ 0.447 (12.380-13.953)	12.2 $\pm$ 0.374 (11.661-13.339)	13.4 $\pm$ 0.244 (13.062-13.938)	4-13.4
Granulocytes (%)	23.4 $\pm$ 0.748 (22.360-24.973)	26 $\pm$ 0.447 (25.047-26.620)	24.8 $\pm$ 0.374 (23.661-25.339)	21-73
Cholesterol (mg/dL)	63 $\pm$ 1.945 (57.889-68.111)	59.25 $\pm$ 0.796 (57.760-60.907)	61.25 $\pm$ 1.517 (58.152-64.098)	50-110
Total Protein (g/dL)	6.26 $\pm$ 0.160 (5.857-6.677)	5.65 $\pm$ 0.088 (5.454-5.879)	5.6 $\pm$ 0.053 (5.495-5.705)	5.4-7.7

*= Values in this table are presented as means $\pm$ standard deviations, 95%CI: 95% confidence interval

cholesterol and blood protein total values in rabbits also showed no change in an abnormal direction.

Rabbit hematology is not drastically different from other mammals (Marshall 2008). Average hemoglobin (HGB) concentration and red blood cell (RBC) counts, proving they were not anemic. Hemoglobin has a directly proportional relationship with erythrocytes. If the erythropoiesis process is disturbed, hemoglobin synthesis will also be disturbed because hemoglobin synthesis occurs from the beginning of erythrocyte formation (Díaz-Hernández *et al.* 2022). The primary function of erythrocytes is to transport hemoglobin, which contains a lot of oxygen, from the lungs to all body tissues (Helms *et al.* 2018). RBC concentration and HGB values were still within the normal range and similar.

In the present study, cholesterol and total protein concentrations were similar between treatments and were within the ranges reported in the rabbit health literature. In rabbits, the range of cholesterol levels is 50-110 mg/dl, and total protein is 5.4-7.7 g/dL (Table 1). The average cholesterol levels vary depending on age, sex and time of day (Washington and van Hoosier 2012). According to several significant studies, it is stated that blood cholesterol levels in rabbits can be reduced with a mixture of herbs (Liaqat *et al.* 2023), using a combination of selenium and inactive yeast (Al-Sagheer *et al.* 2023), Legume stovers (Andrade *et al.* 2021) or using humic acid (HA) supplementation (Abu Hafsa *et al.* 2021). Acupuncture methods have also been explored for their potential in treating various health conditions, including regulating total blood cholesterol. Acupuncture caused more significant reductions in total cholesterol and low-density lipoprotein cholesterol (Chen *et al.* 2020), hyperlipidemic rats (Jin *et al.* 2023), obese women (Cabioğlu and Ergene 2005), and dyslipidemic patients (Rerksupphaphol and Rerksupphaphol 2014).

High blood cholesterol levels can be called hypercholesterolemia, generally diagnosed as

hyperlipidemia (Pirillo *et al.* 2021). Several diseases are closely related to high blood cholesterol levels, such as diabetes, obesity and cardiovascular disease (Kihara 2013; Powell *et al.* 2021). According to Jin *et al.* (2023), electroacupuncture at the Feng long point (ST40) can reduce total blood cholesterol by regulating lipids in plasma and inhibiting lipid deposition in the liver. Clinical evidence shows that lowering total blood cholesterol and serum triglyceride levels can be achieved by applying ST36 points and giving electroacupuncture (Oh and Kim 2022).

Plasma protein expression patterns strongly code for various health conditions, making them useful for health diagnostics. Known plasma protein levels are generally used as an indicator to provide crucial information on the function of organs and systems in the body. The effect of acupuncture on total plasma protein levels in various studies has shown an impact on the inflammatory response, including cerebral infarction and hepatic encephalopathy (Huang *et al.* 2021; Li *et al.* 2021; Oh and Kim 2022). In this study, there was no significant change in the total amount of plasma protein in rabbits given laser acupuncture because the rabbits used were healthy rabbits that were not sick. Current evidence indicates that acupuncture's effects on plasma proteins primarily manifest in diseased patients rather than healthy individuals or livestock undergoing fattening processes. While preliminary data suggest potential modulatory effects on cholesterol and plasma protein regulation, the precise mechanisms remain poorly characterized. Our results demonstrate that both laser and electro-acupuncture maintain hematological stability in healthy animal.

Acupuncture is a technological innovation that is very applicable and can be compared with rabbit farming in increasing livestock production. Acupuncture treatment system with the principle of treating specific health conditions based on the flow pattern of energy (Qi) channeled through the body's meridians. The

application of low-power laser stimulation at acupuncture points offers a rapid, straightforward, and non-invasive therapeutic approach (Araújo *et al.* 2018). Laser acupuncture employs low-intensity laser light to activate these specific points. In rabbits and other living organisms, numerous acupuncture points exist along twelve primary meridian channels, forming an interconnected network throughout the body (Xie and Wedemeyer 2012).

According to Adikara (2019), the administration of laser acupuncture will produce energy that come out of the acupuncture points, and the energy will travel through meridian pathways, resulting in physiological and biochemical changes. However, based on this research, no changes occurred in the rabbit's hematological profile. All hematological profiles, total protein, and cholesterol parameters fell within the normal range. Furthermore, Adikara (2001) stated that the changes that occur in the body when given acupuncture are in the form of changes in enzyme synthesis, hormonal synthesis and changes in organ performance. However, based on this research, no changes occurred in the rabbit's hematological profile. All hematological profile parameters fell within the normal range.

The present study showed that laser acupuncture does not significantly influence rabbits' profile hematological status. Therefore, laser acupuncture can be recommended to achieve excellent health to optimize the rabbit production. Further research is needed to determine whether there are changes in the hematological profile in livestock treated with laser acupuncture to increase livestock production or in animals given health treatment.

This study contributes to the knowledge by confirming that laser acupuncture, when applied to healthy rabbits, does not negatively impact hematological parameters. The results of this study support the potential of laser acupuncture as a safe therapeutic intervention in animal health and provide a basis for further research on the long-term effects of laser acupuncture and the application of laser acupuncture in the context of animal health and possibly human health. Future studies should include a long-term evaluation of the effects of laser acupuncture on various hematological parameters and its impact on livestock productivity. In addition, additional studies are needed to determine whether laser acupuncture therapy can affect animals with growth disorders or those with a history of gastrointestinal disorders.

CONCLUSION

This study confirms that laser acupuncture does not adversely impact the hematological parameters of healthy rabbits, with all measured values remaining within the normal range. Parameters such as hemoglobin concentration, red blood cell counts, cholesterol levels, and total plasma protein showed no abnormalities, underscoring the safety of laser acupuncture in maintaining metabolic and

hematological stability. These findings suggest that laser acupuncture is a non-invasive and safe approach to promoting livestock health. Future studies should investigate the long-term effects of laser acupuncture on animals with growth disorders or specific health conditions to understand better its broader applications in veterinary medicine and livestock productivity.

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