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Preventive and Ameliorative Role of Lactoferrin in Improving Blood, Lipid and Liver Profiles Toward Immune Homeostasis in Lipopolysaccharides (LPS) Induced Dysregulation of Bio-Vital Parameters in the Common Carp (*Cyprinus carpio*)

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ABSTRACT

This study investigated preventive and ameliorative roles of lactoferrin against LPS induced inflammation in fish common carps. A total of 160 fish were used in this study. Forty common carps were divided equally into 4 groups: control group, control treated with LF group (LF group), LPS group (LPS only) and LPS treated with LF (LPS+LF). The results showed both preventive and protective roles of LF in both LF alone and LPS+LF, respectively. Both preventive and protective role recorded an enhancing effect of LF with a dose of 6g/ kg, which was enough for restoring the liver, blood as well as lipid profiles against LPS (3µg/ ml) induced dysregulation of the bio-vital parameters. In the liver, which is one of the major organs involving in innate and adaptive immunity, a significant change was detected in the ALP, ALT and AST. On the other hand, LF regulated the lipid profile, including cholesterol, triglycerides, LDL and HDL. Additionally, LF was recorded with a significant enhancement and restoration in blood profile including RBCs, WBCs and globulin. Totally, these results suggested the enhancement role of the LF which leads to improvement of the immune- bio-vital status of the body through restoring the liver, lipid and blood profile, suggesting a beneficial and preventive role of lactoferrin as a main source for maintaining and improving industry of the aquatic life in the future.

INTRODUCTION

Fish have a wide range of nutritional benefits and are considered one of the most important parts of a healthy food. They are considered as a source of highly bioavailable nutrients including vitamins, minerals, essential fatty acids, and high-quality protein **(Douglas *et al*., 1979; Aljoburi *et al*., 2024)**. The health benefits of fish are well documented, including protection against chronic diseases, and fish aids in the growth and development of humans **(Lui, 1979; Alrudainy & Jumaa, 2016)**.

Lipopolysaccharides (LPS) is a major component of the outer membrane of Gram-negative bacteria, and this molecule can potentially induce an immune response while activating various immune-biological processes **(Bi *et al*., 2018; Alshumary *et al*., 2024; Oday *et al.,* 2024)**. On the other hand, lactoferrin (LF) is considered one of the most important glycoproteins which are involved in the innate immune system. In addition to this role, LF has an antimicrobial and an anti-cancer role raising their right to be involved in protecting the body against inflammation and other disease factors **(Aristova, 1977; Hussein & Jumma, 2024)**. In addition, LF has been showing an anti-inflammatory role **(Huriet *et al*., 1977)**. Considering the physiological capabilities of LF in host defense, in addition to current pharmaceutical and nutritional needs, LF is a nutraceutical and so far, investigators have been searching for the most convenient way to produce it **(Tall, 2008)**. Phospholipids are radical parts in the cell membrane which is involved in the metabolic activities of the lipids in the cell membrane, while HDL has been shown as an anti-atherogenic properties, decreasing inflammation, enhancing immune properties and inhibiting thrombosis. Numerous chronic diseases have been correlated with dysregulation of the lipid profile leading to an increase in low density lipoprotein (LDL) and triglycerides **(Ansell *et al*., 2005; Kubes & Jenne, 2018)**. While the liver is a significant organ in the body supporting the immune system, its failure negatively affects the immune system which profoundly leads to dysregulation of the innate and adaptive immune system. This organ is known to be a crucial barrier between the body microenvironment and the outside world **(Gomez *et al*., 2019)**.

MATERIALS AND METHODS

Fish groups

In this study, four groups of fish were divided, including control group, Lactoferrin treated group (control treated with LF), LPS group (LPS only) and LPS treated with lactoferrin (LPS +LF). Each group consisted of 40 fingerling common carp (*C. carpio*) with a total number of 160 fish and with a weight of 4.6±50g within an amount of 30L capacity tank per group during the period of April 1, 2024, until June 1, 2024.

Lipopolysaccharides and Lactoferrin exposure (LPS and LF)

In this study, four groups were separated into two groups before LPS exposure including control group and control treated with LF as a preventive and another two groups which included LPS exposure with a dose of 3µg/ ml for 7 days and LPS treated with LF after 2 hours of LPS exposure. A total number of 40 fish were considered as a control group, while another group consisted of 40 fish that were inoculated with LF as a preventive.

On the other hand, two groups which consisted of 40 common carps were exposed to LPS for 7 days, and another group was exposed to LPS for 7 days and was treated an hour later with LF.

Blood collection

2ml of blood was collected from the heart, and blood samples were then placed in a tube supplied with anti-coagulant. Complete blood picture was done by using Mindray BC- 230 advice- China.

Lipid profile

Total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using Mindray BC- 230, and all the instructions were followed according to manufacturer's instructions.

Liver enzyme

ALP, ALT and AST were measured using Mindray BC- 230 advice- China, according to manufacturer's instructions.

Statistical analysis

SPSS was used to analyze the results, where values representing the mean and standard error were obtained, then the data were analyzed using one-way ANOVA, and using Duncan's multiple-range test, the differences between groups were determined at a probability level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of our study showed a significant decrease in the liver profile including ALP, ALT and AST in the control group treated with LF compared with the control group alone. On the other hand, the results showed that the group of common carp fingerlings exposed for 7 days to LPS, which was then treated with LF (LPS+LF), recorded a significant decrease in ALP, ALT, and AST compared to the LPS group alone (Table 1).

Table 1. Lipid parameters for liver profile before and after LPS exposure

Parameter	Before LPS inoculation		After 7 Days of LPS inoculation	
	Control group	Control inoculated with LF	LPS	LPS+LF
ALP	72.31±4.3	51.3±8.32	78.21±5.1	55.12±7.1
ALT	48.32±3.21	31.42 ± 4.21	54.21±2.1	36.2±3.8
AST	306±23.1	189.32± 17.3	321±17.4	219.32

A significant decrease was detected in the lipid profile including cholesterol, triglycerides, and LDL, while it showed a significant increase in HDL, for both lactoferrin alone (LF) and lactoferrin exposed to LPS (LF+LPS) compared with the control group and LPS group, respectively (Table 2).

On the other hand, a significant decrease was registered in the blood profile including RBCs, WBCs, and globulin, for both lactoferrin alone (LF) and lactoferrin exposed to LPS (LF+LPS), compared to the control group and LPS group, respectively (Table 3).

Table 2. Lipid parameters before and after LPS exposure

Parameter	**Before LPS exposure**		**After 7days from LPS exposure**	
Lipid profile	**Control group**	**LF group**	**Control group**	**LF group**
Total cholesterol	148.21±1.9	119.34±2.41	149.1±1.1	123.2.1±2.1
Triglycerides	91.32±2.17	81.23± 1.01	92.41±3.1	83.41±2.1
High-density Lipoprotein (HDL)	53.29±1.1	78.21±2.1	51.31±41	75.21±2.1
Low-density lipoprotein (LDL)	61.1±1.7	49.21±0.97	63.1±1.82	52.11±1.01

Table 3. The blood parameters before and after LPS exposure

Parameters	**Before LPS exposure**		**After 7days from LPS exposure**	
	Control group	**LF group**	**Control group**	**LF group**
RBCs (×103/mm3)	14138± 268	14746±193	13921± 389	14387± 542
WBCs (×103/mm3)	11.23±1.2	14.21±1.02	12.92±1.43	15.41±1.2
Globulin	3.11± 0.21	3.58±0.32	3.21±0.19	3.81±0.11

Aquaculture has been considered one of the most important food productions overseas which based on that, fish culturing has become one of the highest market values in the world, therefore an intensive care is required to avoid any disease conditions **(Li *et al*., 2020; Jumma, 2024)**. Among the major causes which mediate disturbing the vital parameters of the body is LPS; this disturbance can lead to inflammation and LPS can notably mediate different disease conditions **(Ahmed *et al*., 2021)**. The liver is considered the frontline vital organ which oversees mediating immune homeostasis. Immunologically, the liver is considered a complex organ which contains a diverse population of immune cells **(Li *et al*., 2021; Sabah *et al*., 2024)**. While the liver is considered immunologically the front line, it is designated to detect, capture and clear antibody as the liver is considered one of the main sources in the body of the phagocytes. Liver enzymes are significantly affected by LPS leading to liver injury **(Guan *et al*., 2024)**. In this study, we investigated both the preventive role of LF (without LPS) as well as the ameliorative role of LF against LPS inducing inflammation. Our findings indicated that LF exhibits a preventive and ameliorative role against LPS-induced inflammation. This was demonstrated through the alleviation role of ALP, ALT and AST which were all significantly decreased in both LF group and LF+ LPS group compared with control and LPS groups, respectively. Blood parameters indicated that liver parameters play a

protective role against LPS induced inflammation while the lactoferrin significantly has an anti-inflammatory impact, leading to the improvement of the body organs' functions. These results agree with those of **Ezhilarasan (2023)**, who demonstrated the protective role of LF against liver injury. Another study, which investigated the LF's mechanisms in thioacetamide (TAA)-induced chronic liver injury in rats, concurs with our results demonstrating LF's ability to improve liver functions and to alleviate liver fibrosis induced by TAA **(Garcia, 2023)**. Previous studies have shown the role of lactoferrin in enhancing phagocytic activity and modulating the ROS, TLRs in the cells incubated with LF compared to the untreated group, which eventually leads to significant improvements in the blood parameters. These results coincide with the results of the current study (Table 2) showing the preventive role in the LF alone (without LPS) and in the ameliorative role of the LPS+LF. Specifically, we observed improvements in all blood parameters, including RBCs, WBCs, and globulin compared with LPS group . The lipid profile plays such a major role in the regulation of the immune system by regulating cell activation, differentiation and expansion **(Anderson *et al*., 2023)**. Moreover, the previous authors investigated the role of LF improving the effect of lipid profile which enhances metabolism. *In vitro* studies have revealed anti-atherogenic and lipolytic activities of LF against adipocytes. Additionally, *in vivo* studies have also demonstrated that LF can decrease plasma, hepatic triglycerides and cholesterol and can reduce visceral fat which agrees with our study findings, suggesting the beneficial role of LF in improving the lipid profile in both LF alone, without LPS and LPS group treated with LF (LPS+LF). Red blood cells is considered an immune modulator and regulator of the immune system **(Hassan *et al*., 2024; Majstorovic *et al*., 2024)**. The results of our study indicated that both RBCs significantly increased in both LF treated group and LF group treated with LPS suggesting that 10 LPS works as a preventive and ameliorative against LPS induced inflammation. These results match another study which has shown that the erythrocytes of the common carp are immune sentinels that sense the pathogenies molecular patterns (PAMPs), engulf particles, as well as secreting antibacterial agents **(AL-Bayati *et al*., 2020; Majstorovic *et al*., 2024)**. Additionally, the results of our study agree with another investigation which has shown that fish erythrocytes can detect lipopolysaccharide LPS and the TLR3 against polyinosinic, polycytidylic acid, which mimics viral dsRNA suggesting that the increasing number of RBCs might be due to the role of erythrocytes in detecting lipopolysaccharides (LPS) **(Hassan & Firas, 2020; Muna *et al*., 2024)**.

CONCLUSION

In conclusion, our results have demonstrated that LF has significantly enhanced the immune biological vitality of the body through thorough improvements in the liver, lipid and blood profile. These enhancements suggest a beneficial and preventive role of

lactoferrin as a main source for maintaining and improving industry of the aquatic life in the future.

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