

Immune and lipid metabolism gene expression relationships in the liver of castrated Polish White Improved bucks supplemented with *Curcuma longa* and *Rosmarinus officinalis* extract*

Daria M. Urbńska^{1}, Marek Pawlik², Agnieszka Korwin-Kossakowska¹, Karolina Rutkowska³, Ewelina Kawecka-Grochocka¹, Michał Czopowicz⁴, Marcin Mickiewicz⁴, Jarosław Kaba⁴, Emilia Bagnicka¹**

¹ Institute of Genetics and Animal Biotechnology PAS, Jastrzębiec, Poland

² Department of Neurotoxicology, Mossakowski Medical Research Institute PAS, Poland

³ Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, 02-776 Warsaw, Poland

⁴ Department of Medical Genetics, Medical University of Warsaw, 02-106 Warsaw, Poland

(Accepted July 14, 2026)

Clarifying the relationships between cytokines, acute phase proteins (APPs), cathelicidins (CATHs), defensins, cytolytic proteins, and the proteins involved in lipid metabolism in the liver may improve existing immunotherapeutic strategies for various diseases or establish new ones. This study aimed to determine the effect of supplementation with dry turmeric-rosemary extracts on the relationships between lipid metabolism and immune system gene expression in the liver of young castrated Polish White Improved (PWI) goat bucks. The cytokine genes correlated both with those of other cytokines and with beta defensin 1 (*GBDI*), as well as some *CATH* and *APP* genes.

Most relationships were strong and positive. More associations were observed in the treated group than in the control group. Our results may mean that the tested supplementation may reduce the risk of hypertriglyceridemia and improve immunity against specific pathogens, its effect on the

*This work was supported by the National Science Center, Poland PRELUDIUM Grant grant number: 2016/21/N/NZ9/01508 and OPUS grant number: 2013/09/B/NZ6/03514.

**Corresponding author: daria.m.urbanska@gmail.com

immune system is still unclear. Moreover, the used dose of the turmeric-rosemary mixture was too high and caused sensitization or the beginning of allergic inflammation. Further studies should include different doses of the supplement and confirm or reject our supposition.

KEYWORDS: goat liver / gene expression / turmeric / rosemary / immune response / lipid metabolism

The liver serves as a central hub for detoxification, metabolism, filtration, and nutrient storage, and innate immune [Weiler-Normann and Rehmann 2004]. It synthesizes bioactive compounds that prevent bacterial, viral, and fungal infections while inhibit oncogenic transformation [Gao et al. 2008]. Strong functional relationships exist between these hepatic proteins [Carlini et al. 2023, Presti et al. 2021, Chang et al. 2020, Bergamo et al. 2019, van Harten et al. 2018, Michels et al. 2015, Ouellette 2015, Jain et al. 2011, Seegert et al. 2011, Kaliński et al. 2000, Fantuzzi et al. 1999, Marshall et al. 1996, Vasse et al. 1996], as illustrated in Figure 1. Specifically, CATHs appear to positively modulate the expression of certain cytokines. Furthermore, interleukins 6, 10, and 16 (IL-6, IL-10, IL-16), and tumor necrosis factor-alpha (TNF- α) are modulated by other immune genes and interact reciprocally. In turn, acute-phase protein (APP) expression is driven by TNF- α , specific interleukins, and interferons.

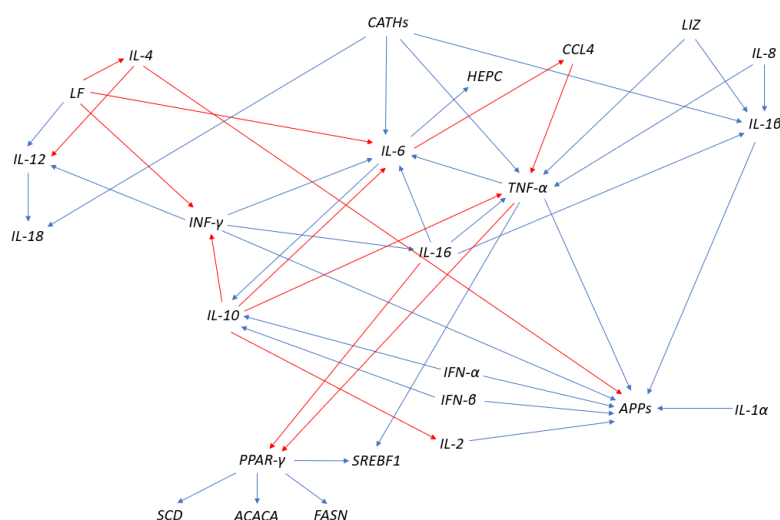


Fig. 1. Selected a positive (stimulating; in blue) and negative (inhibiting; in red) relationships between cytokines, acute phase proteins (APPs), cathelicidins (CATHs), defensins, cytolytic proteins, and lipid metabolism genes. *HP* – Haptoglobin, *FBA* – Fibrinogen α chain, *FBB* – Fibrinogen β chain, *FBG* – Fibrinogen γ chain, *BAC5* – Bactenecin 5/Cathelicidin 2, *BAC7.5* – Bactenecin 7.5/Cathelicidin 3, *MAP28* – Cathelicidin 6, *GBD1* – Beta defensin 1, *HEPC* – Hecpudin, *LF* – Lactoferrin, *LIZ* – Lysozyme, *IL-1 α* – Interleukin 1 α , *IL-1 β* – Interleukin 1 β , *IL-4* – Interleukin 4, *IL-6* – Interleukin 6, *IL-10* – Interleukin 10, *IL-12* – Interleukin 12, *IL-16* – Interleukin 16, *IL-18* – Interleukin 18, *IFN- α* – Interferon α , *IFN- β* – Interferon β , *INF- γ* – Interferon γ , *TNF- α* – Tumor necrosis factor α , *CCL4* – Chemokine 4, *ACACA* – Acetyl-coenzyme A carboxylase alpha, *SCD* – Stearoyl-CoA desaturase, *LPL* – Lipoprotein lipase, *DGAT1* – Diacylglycerol O-Acyltransferase, *SREBF1* – Sterol regulatory element binding transcription factor.

Herbs and spices have a long history of use as natural preservatives and medicines. Among them, turmeric and rosemary exhibit potent antioxidant, anti-inflammatory, and antiviral properties, largely driven by their high polyphenol content [Batta 2018, Levine *et al.* 2017]. Additionally, extracts from these plants demonstrate hepatoprotective effects [Mohamed *et al.* 2022].

Previous research on young castrated goat bucks has shown that dietary supplementation with a dried turmeric-rosemary mixture influences meat productivity and alters the hepatic expression of genes associated with the immune system, lipid metabolism, cytokines, acute phase proteins (APPs), cathelicidins (CATHs), defensins, and cytolytic proteins [Urbańska *et al.* 2023ab]. Although this herbal mixture successfully increased daily weight gain, it was also found to trigger a hypersensitivity reaction (manifested by altered expression of inflammatory markers rather than clinical symptoms) [Urbańska *et al.* 2024].

Hypersensitivity represents an excessive or abnormal organismal response to specific environmental, dietary, or biological stimuli, manifesting in clinical symptoms. While allergies represent a specific, IgE-mediated or lymphocyte-activated immune hypersensitivity, non-immunological mechanisms can also drive these reactions [Tanno *et al.* 2016]. This distinction implies that all allergies are hypersensitivity reactions, but not all hypersensitivity reactions are allergic in nature.

In these previous studies, the turmeric-rosemary mixture significantly altered the expression of isolated genes, including IL-12p35, IL-18, and IFN- γ [Urbańska *et al.* 2023ab]. However, those findings only described single gene expression profiles and did not elucidate the regulatory networks between them. Because immune and lipid metabolism genes interact dynamically in the liver, analyzing isolated expression levels fails to capture how the supplement affects the system as a whole. Currently, there is a critical lack of comprehensive data on the specific interactions and networks that emerge between the immune system and lipid metabolism under herbal supplementation. Investigating the correlations and regressions between these genes is therefore essential to verify systemic homeostasis or evaluate the risk of hypersensitivity.

This study aims to determine the effect of a dry turmeric-rosemary mixture supplement on the relationships between lipid metabolism and immune system gene expression in the liver of young castrated Polish White Improved (PWI) goat bucks

Material and methods

A group of 20 eight-month-old PWI goat bucks, castrated at three months of age, was randomly divided into two identical groups: control (CG) and treated (TG) groups, with 10 animals per group. All animals had similar live weights and were maintained under identical conditions and on the same basic diet. The goats were kept in groups in designated pens, with several animals housed in each enclosure to ensure appropriate living conditions and freedom of movement. During the grazing season, the animals were regularly released onto pasture, where they had access to

natural forage resources. The TG group was supplemented with a turmeric-rosemary dry mixture (Selko® AOmix; Amersfoort, The Netherlands, ratio of 896:19), at a dose of 1.6 g/buck/day. The study design and original animal procedures were approved by the 3rd Local Ethics Committee for Animal Experimentation in Warsaw (permission No. 31/2013, 22 May 2013).

Crucially, the present study is based entirely on a secondary analysis of existing datasets compiled during those original experiments. The comprehensive details regarding animal welfare, husbandry, husbandry management, and ethical compliance were fully described in our primary publications [Urbańska *et al.* 2023ab, 2024]. In accordance with the 3R principles (specifically Reduction), no new animal experiments were conducted, and no additional animals were sacrificed for the purpose of the current study. Consequently, the methodological procedures applied in this work are only briefly summarized below, as the full experimental design and molecular protocols are available in detail within those aforementioned publications.

To ensure clarity and flow, the fundamental aspects of the methodology are briefly recapitulated here. Liver tissue samples (approximately 30 mg per sample) were obtained during slaughter procedures conducted in a certified slaughterhouse, then immediately snap-frozen in liquid nitrogen and preserved at -80°C pending further analyses. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The quantity and quality of the extracted RNA were evaluated using a NanoDrop spectrophotometer. Subsequently, reverse transcription was carried out with the Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) using 1 µg of total RNA. The obtained cDNA was diluted to a final concentration of 50 ng/µL and used as a template for quantitative real-time PCR (RT-qPCR) analysis performed using a LightCycler®480 system (Roche, Basel, Switzerland), with the LightCycler®480 Probes Master mix and specific primers under standard cycling conditions (95°C for 10 min, followed by 45 cycles of 95°C for 10 s and 60°C for 30 s) as described by Urbańska *et al.* [2023a].

The expression of four APPs, three CATHs, one defensin, three cytolytic proteins, 13 cytokines, and five lipid metabolism genes in the livers of the bucks was studied. However, the expression levels of serum amyloid (*SAA*), C-reactive protein (*CRP*), lactalbumin (*LALBA*), α1-acid glycoprotein (*AGP*), ceruloplasmin (*CP*), caprine bacterenecin 3.4 (*ChBac3.4*), cathelicidin 7 (*CATH7*, *MAP34*), beta-defensin-2 (*GBD2*), interleukin 2 (*IL-2*), interleukin 8 (*IL-8*), adipose triglyceride lipase (*ATGL*) and peroxisome proliferator-activated receptor gamma (*PPARγ*) were below the detection level in the previous studies. The specific primer sequences and characteristics for all evaluated genes are summarized in Supplementary Table S2 [Urbańska *et al.* 2023ab, 2024].

To evaluate the relationships among the parameters, Pearson's correlation coefficients were calculated separately within the control and treated groups using the PROC CORR module of the SAS package (SAS/STAT 9.4, 2002-2012). The resulting correlation matrices are presented in a combined table within the manuscript, and

their comprehensive graphical representations are provided as color-coded correlation heatmaps in the Figure S1, S2 and S3.

Linear and fourth-degree polynomial regression analyses were performed using the PROC REG module of the SAS package. Cathelicidin 2 (*CATH2*, *BAC5*), lactoferrin (*LF*), fibrinogen α , β , and γ chain (*FGA*, *FGB*, *FGG*), interleukin 12p35 (*IL-12p35*), interleukin 18 (*IL-18*), and Interferon γ (*IFN- γ*) were selected as explanatory (independent) variables due to their differential expression between the studied groups [Urbańska *et al.* 2023ab]. The remaining evaluated genes acted as the dependent variables.

To evaluate the simultaneous effects of these factors, multiple linear and polynomial regression analyses were performed for each dependent variable using the SAS package. Values of $P \leq 0.05$ were considered statistically significant, and the optimal polynomial degree (linear, quadratic, cubic, or quartic) was determined hierarchically via sequential F-tests. Model assumptions, including the normality and homoscedasticity of the residuals, were verified using the Shapiro-Wilk test and residual diagnostics. Because the initial residuals were not normally distributed, the expression data were log-transformed to restore normality.

To visualize the complex relationships derived from these multiple regression equations, three-dimensional (3D) surface plots were generated using the Matplotlib library (v3.8) in the Python programming environment (v3.11). Additionally, to ensure visual clarity and avoid the aesthetic complexity of overlapping surfaces, the significant individual relationships from the global models were visualized using two-dimensional (2D) coordinate plots. These specific curves and lines, representing the mathematical impact of each separate significant predictor while controlling for other variables, were plotted to highlight distinct variable interactions. Both the 2D and 3D regression plots, as well as the correlation heatmaps, are provided in the Supplementary Materials.

Results and discussion

Correlations

The correlations between the studied genes in both groups are shown in Tables 1 and 2. To complement these summaries and provide a full overview of all analyzed relationships, including non-significant interactions, comprehensive correlation heatmaps containing exact correlation coefficients for all gene pairs are provided in the Supplementary Materials (Fig. S4 and S5).

While interleukin 10 (*IL-10*) did not correlate with any other studied genes in either groups, IFN- γ showed no significant correlations with other immune system genes (Tab. 1). However, IFN- γ was significantly and positively correlated with the lipid metabolism gene *SREBF1* in the treated group (Tab. 2). The other cytokine genes correlated both with those of other cytokines and with beta-defensin-1 (*GBDI*), as well as some *CATH* and *APP* genes (Tab. 1). Most relationships were strong and positive, apart from those between interleukin 16 and 18 (*IL-16* and *IL-18*) (-0.92 , $P \leq 0.01$),

Table 1. Correlations between the expressions of selected immune system genes in the control and treated groups

GROUP	CONTROL																								
	GENE	APPs					CATHs			DEFs		Cytolytic proteins			Cytokines										
		Gene symbol	HP	FGA	FGB	FGG	BAC3	BAC7.5	MAP28	GBD	HEP	LF	LIZ	IL-1a	IL-1β	IL-4	IL-6	IL-12p35	IL-16	IL-18	IFN-α	IFN-β	TNF-α	CCL4	
TREATED	APPs	HP		NS	NS	NS	0.77*	NS	0.72*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
		FGA	0.95*		NS	0.85*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.95**	NS	NS	NS	NS	NS	
		FGB	NS	0.99**		NS	0.92**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.91**	NS	NS	NS	NS	NS	
		FGG	NS	0.99**	0.99**		NS	NS	NS	NS	NS	0.77*	NS	NS	NS	NS	NS	NS	0.93**	NS	NS	NS	NS	NS	
		BAC5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	CATHs	BAC7.5	NS	0.98*	NS	0.97*	NS		0.73*	0.74*	-0.69*	NS	NS	NS	NS	NS	0.95**	0.85*	NS	NS	0.73*	0.75*	NS	NS	
		MAP28	0.97*	0.97*	0.96*	NS	0.95*	NS		NS	NS	NS	0.92**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
		GBD1	NS	0.97*	NS	NS	NS	NS	0.98*		NS	NS	NS	NS	NS	NS	0.86**	NS	0.74*	NS	0.76*	0.87**	NS	NS	
		HEPC	NS	0.98*	0.99**	1.00**	NS	0.97*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.74*	NS	NS	NS	
		LF	NS	0.98*	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
Cytokines	Cytolytic	LIZ	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
		IL-1a	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
		IL-1β	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	0.860*	
		IL-4	NS	0.96*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.72*	
		IL-6	NS	NS	NS	NS	NS	NS	NS	0.99**	NS	NS	NS	NS	NS	NS	0.78*	NS	NS	NS	NS	0.85**	NS	NS	
		IL-12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		IL-16	NS	NS	NS	NS	NS	NS	NS	0.97*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		IL-18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		IFN-α	NS	0.99**	0.97*	0.96*	NS	0.99**	0.98*	0.99**	0.95*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.95**	NS	NS	NS
		IFN-β	NS	0.96*	NS	NS	NS	NS	NS	0.99**	NS	NS	NS	NS	NS	1.00**	NS	NS	NS	0.98*	NS	NS	NS	NS	NS
TNF-α	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
CCL4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		

NS – not significant; ** – $P \leq 0.01$; * – $P \leq 0.05$
APPs – acute phase proteins: HP – Haptoglobin; FGA – Fibrinogen α chain; FGB – Fibrinogen β chain; FGG – Fibrinogen γ chain;
CATHs – cathelicidins: BAC5 – Bactenecin 5/cathelicidin 2; BAC7.5 – Bactenecin 7.5/cathelicidin 3; BACP28 – Cathelicidin 6;
DEF – defensin: GBD1 – Beta defensin 1;
Cytolytic protein genes: HEPC – Hepsidin; LF – Lactoferrin; LIZ – Lipozyme;
Cytokines: IL-1a – Interleukin 1a; IL-1β – Interleukin 1β; IL-4 – Interleukin 4; IL-6 – Interleukin 6; IL-10 – Interleukin 10; IL-12p35 – Interleukin 12p35; IL-16 – Interleukin 16; IL-18 – Interleukin 18; IFN-α – Interferon α; IFN-β – Interferon β; TNF-α – Tumor necrosis factor α; CCL4 – Chemokine

interleukin -1β (*IL-1β*) and chemokine (*CCL4*) (-0.72, $P \leq 0.05$), hepcidin (*HEPC*) and interferon α (*IFN-α*) (-0.74, $P \leq 0.05$), and cathelicidin 3 (*BAC7.5*) and *HEPC* (-0.69, $P \leq 0.05$) in CG, and between *IL-1β* and *IL-12p35* (-0.97, $P \leq 0.05$) in the TG.

The correlations between the lipid metabolism and immune system genes in the CG and TG are given in Table 2. In the CG, the expression of two lipid metabolism

Table 2. Correlations between the expressions of selected lipid metabolism and immune system genes in the control and the treated groups

Immune gene group	Gene symbol	Lipid metabolism gene			
		control group		treated group	
		<i>SCD</i>	<i>LPL</i>	<i>DGAT1</i>	<i>SREBF1</i>
Acute phase proteins	<i>HP</i>	NS	NS	0.964*	NS
	<i>FGA</i>	NS	NS	1.000**	NS
	<i>FGB</i>	NS	NS	0.994**	NS
	<i>FGG</i>	NS	NS	0.990*	NS
Cathelicidins	<i>BAC7.5</i>	0.758*	0.794*	0.974*	NS
	<i>MAP28</i>	NS	NS	0.991**	NS
Defensin	<i>GBD1</i>	0.929**	0.794*	0.958*	NS
Cytolytic proteins	<i>HEPC</i>	NS	NS	0.979*	NS
	<i>LF</i>	NS	-0.737*	NS	NS
Cytokines	<i>IL-4</i>	0.842**	0.736*	NS	NS
	<i>IL-12p35</i>	0.737*	0.752*	NS	NS
	<i>INF-α</i>	NS	NS	0.985*	NS
	<i>INF-γ</i>	NS	NS	NS	0.959*
	<i>TNF-α</i>	NS	NS	NS	0.970*
Lipid metabolism genes		<i>SCD</i>	-	0.829**	NS

NS – not significant, ** – $P \leq 0.01$; * – $P \leq 0.05$. *HP* – Haptoglobin; *FGA* – Fibrinogen α chain; *FGB* – Fibrinogen β chain; *FGG* – Fibrinogen γ chain; *BAC7.5* – Bactenecin 7.5/cathelicidin 3; *MAP28* – Cathelicidin 6; *GBD1* – Beta defensin 1; *HEPC* – Hepcidin; *LF* – Lactoferrin; *IL-4* – Interleukin 4; *IL-12p35* – Interleukin 12p35; *INF-α* – Interferon α; *INF-γ* – Interferon γ; *TNF-α* – Tumor necrosis factor α; *SCD* – Stearoyl-CoA desaturase; *LPL* – Lipoprotein lipase; *DGAT1* – Diacylglycerol O-Acyltransferase; *SREBF1* – Sterol regulatory element binding transcription factor.

genes (stearoyl-CoA desaturase, SCD, and lipoprotein lipase, LPL) were positively correlated with each other (0.83, $P \leq 0.01$) as well as with two cytokines (IL-12p35 and IL-4), GBD1, and BAC7.5. The only negative correlation was found between LPL and LF (-0.74, $P \leq 0.05$). In the TG, diacylglycerol O-Acyltransferase (DGAT1) was positively correlated with many immune system genes (*Hp*, *FGA*, *FGB*, *FGG*, *BAC7.5*, *MAP28*, *GBD1*, *HEPC*, and *IFN-α*), but only one cytokine: *IFN-α*. The expression of sterol regulatory element binding transcription factor (*SREBF1*) was correlated only with *IFN-γ* and *TNF-α* (0.96, 0.97, respectively, $P \leq 0.05$).

Regressions

The mathematical parameters of the regression models are summarized in Table 3. To fully visualize these relationships, both the multi-variable 3D surface plots and the corresponding individual 2D coordinate plots showing the impact of each separate significant predictor are provided in the Supplementary Materials (Fig. S2 and S3).

The results of the regressions of *HP*, *FGA*, *FGB*, *FGG*, cathelicidin 6 (*CATH6*, *MAP28*), *LF*, and *DGAT1* gene expression (dependent variable) on *BAC5*, *LF*, *FGG*, *IL-12p35*, *IL-18*, and *IFN-γ* (independent variable) in CG and the TG are given in Supplementary Figure S1 A-N. The results of the regressions of *IL-16*, *LF*, *GBD1*, *SCD*, *BAC5*, and *LPL* gene expression (dependent variable) on those of *FGG*, *BAC5*, *IL-18*, and *LF* (independent variable) in CG are given in Supplementary Figure S2 A-F. The regressions of the expressions of *IL-1α*, *HEPC*, *CCL4*, *BAC7.5*, and *SREBF1*

Table 3. Multiple linear and polynomial regression models for hepatic immune and lipid metabolism gene expressions in young castrated Polish White Improved goat bucks from the control and treated groups

Gene symbol / name	Regression equation	R ²	P-value
Control group			
HP / Haptoglobin	$y = 0.11 + 0.48x_{BAC5} + 0.96x_{IFN\alpha}^2$	0.63	0.022
FGA / Fibrinogen Alpha Chain	$y = 0.59 - 0.91x_{IL18} + 1.72x_{FGG}^3$	0.80	0.039
FGB / Fibrinogen Beta Chain	$y = 0.08 + 1.82x_{FGG}^3$	0.68	0.012
FGG / Fibrinogen Gamma Chain	$y = 0.05 + 7.72x_{LF}^3$	0.70	0.009
MAP28 / Cathelicidin 6	$y = 0.05 + 0.44x_{BAC5} + 2.17x_{IL-12p35}^2 - 0.26x_{IL-18}^2$	0.60	0.026
LF / Lactoferrin	$y = 0.32 - 0.35x_{IFN\gamma} + 1.25x_{FGG}^3 - 0.33x_{IL-18}^3$	0.96	0.050
DGAT1 / diacylglycerol O-Acyltransferase	$y = 0.19 - 0.16x_{IL-18}^3$	0.67	0.037
IL-16 / Interleukin 16	$y = -0.08 + 1.89x_{FGG}^3$	0.84	0.004
SCD / Stearoyl-CoA Desaturase	$y = 0.49 - 0.75x_{BAC5}^2$	0.52	0.044
LPL / Lipoprotein Lipase	$y = -0.10 + 8.72x_{LF}^3$	0.76	0.005
GBD1 / Goat β -Defensin-1	$y = -0.02 - 0.28x_{BAC5}^2$	0.56	0.033
BAC5 / Bactenecin 5	$y = -0.13 - 0.63x_{IL-18}^2$	0.51	0.049
Treated group			
HP / Haptoglobin	$y = -0.50 + 0.77x_{LF}^2 + 3.48x_{IL-12p35}^2$	0.78	0.049
FGA / Fibrinogen Alpha Chain	$y = -1.46 + 1.18x_{FGG} + 6.35x_{IL-12p35}^2$	0.99	0.041
FGB / Fibrinogen Beta Chain	$y = 0.88 + 1.07x_{FGG}^3 - 2.77x_{LF}^2$	0.99	0.036
FGG / Fibrinogen Gamma Chain	$y = 0.39 - 2.05x_{LF}^4$	0.95	0.028
MAP28 / Cathelicidin 6	$y = -0.27 + 2.13x_{IL-12p35}^2$	0.98	0.010
LF / Lactoferrin	$y = -0.96 + 4.27x_{IL-12p35}^2$	0.94	0.029
DGAT1 / diacylglycerol O-Acyltransferase	$y = -0.01 + 0.53x_{FGG}^3 + 3.70x_{IL-12p35}^2$	0.91	0.029
IFNG / Interferon Gamma	$y = 0.31 + 0.55x_{FGG}^3$	0.63	0.042
CCL4 / C-C Motif Chemokine Ligand 4	$y = 0.18 + 1.08x_{FGG}^3$	0.92	0.043
HEPC (HAMP) / Hepcidin Antimicrobial Peptide	$y = 0.80 + 0.91x_{FGG}^3 - 2.41x_{LF}^2$	0.98	0.050
BAC7.7 (CATHL3) / Cathelicidin-3	$y = 0.33 + 0.65x_{FGG}^3$	0.94	0.030

(dependent variable) on those of *FGG* and *IFN- γ* (independent variable) in TG are shown in Supplementary Figure S3 A-E.

In CG, increasing *IL-12p35* expression was associated only with a decrease in *MAP28* and then a quick increase. While both *HP* and *MAP28* expression were associated with the same genes in the same way (Fig. S1). As *IL-18* increased, *BAC5* increased and then fell off; similarly, as *BAC5* increased, the expressions of *GBD1* and *SCD* increased rapidly at first, but then dropped abruptly (Fig. S2). In TG, higher *IL-12p35* transcript levels were associated with changes in *Hp*, *FGA*, *MAP28*, *LF*, and *DGAT1* expression. In all cases, their expressions initially decreased, but then rapidly increased as *IL-12p35* expression increased. Increased *FGG* expression was also accompanied by increased *IFN- γ* , *HEPC*, *CCL4*, and *BAC7.5* expression (Fig. S3).

Immune system genes

Strong positive correlations were observed between the three FG chain genes in the CG and TG; this was not unexpected. Moreover, the regression analyses indicated that *IL-12p35* as a covariate was significant for *FGA* in the CG and for *IL-18* in the TG, while the regression of *FGG* on *LF* was significant in both groups. In a previous study, *FGG* expression was higher in the CG than in the TG, while both groups demonstrated similar expression of *FGA* and *FGB* [Urbńska et al. 2023a]. It was previously reported that *IL-6* expression modulated *FGG* expression in the liver and lung epithelium during the acute phase response [Duan and Simpson-Haidaris 2006]; however, no such relationship was noted in our study, potentially because no acute

inflammation was observed in the present group. In contrast, Carty *et al.* [2010] did not find any strong evidence of gene-gene interaction between *FG* and *IL-6* based on transcript levels in the blood. The lack of correlation between *IL-6* and *FGs* observed in our study may suggest that under baseline physiological conditions in healthy animals, transcriptional co-regulation between these parameters is not heavily pronounced, and remains unaltered by dry turmeric-rosemary extract supplementation. However, due to the limited sample size within the experimental groups, these transcriptomic patterns should be interpreted with caution, as they do not definitively preclude potential biological cross-talk or post-transcriptional regulations that may occur independently of mRNA correlation trends [Urbańska *et al.* 2023b].

In our study, *FGG* was only found to correlate with *LF* in the CG. However, in both groups, the regression on *LF* was found to be significant for *FGG*, albeit with different patterns. Unfortunately, no other studies have examined the relationship between *FGG* and *LF* in goats. As such, it is difficult to interpret the significance of the reduction in *LF* and *FGG* expression observed in the TG [Urbańska *et al.* 2023a], and the positive correlation and mutual regression observed between *FGG* and *LF* in the CG and mutual regression in the TG. Moreover, strong positive correlations were observed between *FG* and *HEPC* expression, and between *MAP28* and *IFN- α* . In addition, the regression models suggested a significant relationship between *FGG*, *LF*, and *HEPC* in the TG. As such, it cannot be definitively concluded that the reduced *FG* expression observed in our present findings indicates that the supplement has a negative impact on immunity in the bucks. Its decreased level presented by Urbańska *et al.* [2023a] may rather reflect a state of transcriptional stabilization in the organisms of bucks from the TG, suggesting that the turmeric-rosemary dry mixture influences the relationship between *FGs* and other immune system gene at the mRNA levels.

Previous studies have noted a strong correlation between *HEPC* and *FG* in random human blood samples [Pechlaner *et al.* 2015]. In contrast, Fathy and Raafat [2019] report no such correlation between *HEPC* and *FG* in the blood plasma of healthy rats or those with chronic renal failure. However, our present findings only concern the mRNA levels, and post-transcriptional or post-translational modifications of proteins can inhibit or enhance protein production; therefore, further study at the protein level is needed.

Our present findings indicate a strong positive correlation between *FGG* and *HEPC*, and regression of *HEPC* on *FGG* in TG. The latter suggests that *FGG* has a direct impact on *HEPC*, and that the supplement affects both genes. Thus, the low level of *FGG* in TG [Urbańska *et al.* 2023a] may suggest that the supplement inhibits the first factors of the cellular response, maybe because no inflammatory process was taking place in the liver tissue, or that supplementation may mitigate the inflammation in the liver. Regression of *HP* on *IL-18*, *IFN- γ* , or *BAC5* was significant in CG and on *IL-12p35* and *LF* in TG. Only the regression of *HP* on *IL-18* was negative. Thus, it seems that both groups demonstrated similar *HP* gene expression, albeit under the control of different factors. It may also mean that supplementation influences homeostasis and

that CG and TG express different sets of immune proteins, and this would influence the activity of the APPs. Moreover, whilst previous research indicates decreased *IL-12p35* and *BAC5* expression and slightly lower expression of *IL-18* and *IFN-γ* in TG [Urbańska *et al.* 2023b], no such correlation was noted in either the TG or CG in the present study, which may indicate a lack of synergy between their protein products.

Similar to our results, Barbé *et al.* [2011] did not find any correlations between HP and IL-12, IL-18, or IFN-γ in serum or broncho-alveolar lavage fluid (BALF) from pigs inoculated with swine influenza A virus (SIV) (Swine/Belgium/1/98 (H1N1) strain). Nor did they find any relationships between IL-18 and IFN-γ in serum, or between IL-18 and IFN-γ, or IL-12 in BALF. Barranco *et al.* [2012] report higher expression of IL-12 and IFN-γ in mediastinal and retropharyngeal lymph nodes and tonsils at three to seven and 14 to 17 days after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). IL-12 activates hepatic natural killer T (NKT) cells, which prevent liver regeneration. Wu *et al.* [2015] found that treatment with anti-IL-12 antibodies inhibits excessive activation of NKT cells and restores liver regeneration in mice after partial hepatectomy. While the rosemary-turmeric mixture lowered the expression of *IL-12p35* and *IFN-γ* [Urbańska *et al.* 2023b], it does not appear to affect the correlation between their transcripts. The reduced expression of *IL-12p35* observed in TG may have a positive influence on immune defense and liver restoration, and homeostasis was not disturbed in TG.

Interestingly, a very strong negative correlation was noted between *IL-18* and *IL-16* in CG but not in TG; however, in TG, these two genes correlated positively with some others: *IL-18* with *IL-1β*, and *IL-16* with *GBD1*, *IL-4*, or *IFN-β*, that, except *GBD1*, are pro-inflammatory cytokines. High levels of IL-1β and IL-18 cause severe autoinflammation disorders and various allergies [Tsutsumi *et al.* 2019]. The *IL-18* gene also demonstrated increased expression in TG [Urbańska *et al.* 2023b]. In the presence of IL-12 or IL-15, it reinforces the Th1 response and demonstrates synergy with IL-12 in the Th1 response in keratinocytes, mainly by inducing IFN-γ. However, in the absence of IL-12, IL-18 can stimulate the Th2 response, inducing allergies [Lee *et al.* 2015]. Therefore, it is possible that the positive correlation observed between *IL-18* and *IL-1β*, the negative correlations between *IL-12p35* and *IL-18*, and the absence of *IFN-γ* may hypothetically indicate a subclinical sensitization or the beginning of allergic inflammation in response to the turmeric-rosemary supplementation; the dose may be too high for young animals. It supports our previous suppositions [Urbańska *et al.* 2023b], though these observations must be treated strictly as hypotheses. On the other hand, IFN-γ stops the cell cycle of hepatocytes and inhibits their proliferation [Robinson *et al.* 2016]. Higher *IFN-γ* expression was noted in TG [Urbańska *et al.* 2023b], but this did not correlate with the other studied genes in both groups; this might suggest that excessive doses of herbs could potentially influence the ability of the liver to regenerate and silence its immune response. Hence, further studies are needed to confirm whether the dose of the supplement was too high and caused sensitivity. However, since the present study is based exclusively on mRNA expression and statistical correlations,

these interpretations remain speculative. Further research incorporating protein-level validation, biochemical blood profiles, and histopathological examinations of liver tissues is strictly necessary to confirm or reject these suppositions.

Lipid metabolism and immune system genes

Urbańska *et al.* [2024] report no differences in the expression of lipid metabolism genes between TG and CG. Similarly, no correlations were noted between lipid metabolism genes in either group, except for a strong positive correlation between *LPL* and *SCD* in CG. *SCD* and *LPL* are considered to be the most important enzymes limiting the biosynthesis of critical fatty acids in lipid metabolism; as such, their high expression would have a negative effect. Thus, the lack of any differences between groups regarding their expression, and the lack of correlation between them in TG, may indicate that supplementation does not have any negative or positive effect on the metabolism of fatty acids in the livers of the young castrated bucks. Contradictory results were obtained in a study of sheep; lower levels of *SCD* and *LPL* transcripts in the fat tissue of animals fed a diet supplemented with fresh grass or polyphenol-rich red clover compared to a standard complete pelleted mixture were found. These differences may depend on the diet or the species. The relationship between these genes observed in CG confirms that they play a key role in regulating lipid metabolism in healthy goats.

No relationship was found between *ACACA* and other lipid metabolism genes in either group. This may also confirm that the supplement does not affect lipid metabolism; *ACACA* participates in the biosynthesis of saturated fatty acids, and its increased activity causes the differentiation of adipocytes and the deposition of adipose tissue [Dong *et al.* 2023].

Our present findings indicate higher expression of *LF* and a strong negative correlation between *LPL* and *LF* gene expression in CG, which may play an important role in maintaining cholesterol and triglyceride homeostasis. A previous study found LF to reduce hepatic cholesterol deposition in mice fed a high-fat and high-cholesterol diet containing cholate, a specific steroidal lipid; LF increased serum HDL level and decreased total hepatic cholesterol content [Ling *et al.* 2019]. Urbańska *et al.* [2023a] note lower *LF* mRNA expression in bucks receiving the studied mixture compared to CG, but similar *LPL* expression in both groups; this, together with the lack of correlation between *LF* and *LPL* observed in TG in the present study, indicates that supplementation reduces *LF* expression in the liver but does not influence *LPL* expression. As such, while these specific transcriptomic patterns suggest that the supplement does not actively stimulate baseline lipogenic profiles, they are insufficient to evaluate systemic metabolic safety.

In TG, no relationship was noted between *SREBF1* and *DGAT1* expression; however, each gene was associated with the expression of other immune system genes (*DGAT1* with those of *APPs*, *GBD1*, *CATHs*, and *IFN- α* ; *SREBF1* with *TNF- α* and *IFN- γ*). *IFN- γ* is a proinflammatory cytokine, while *TNF- α* is an inducer of maturation for *SREBP-1* in human hepatocytes [Lawler *et al.* 1998]. In turn, *SREBP-1* regulates the production of

lipids and cholesterol, its activity being regulated by cell sterol levels [Balasubramaniyan 2016]. Hao *et al.* [2013] found that IFN- γ induces lipid deposition and increases the mRNA and protein levels of *SREBP-1* in a mouse mesangial cell line in a time-dependent manner; the increase of IFN- γ was noted previously in bucks receiving supplementation [Urbańska *et al.* 2023b]. Studies on the immune response in hepatocellular carcinoma found SREBP1 to be activated by IFN- γ . While IFN- γ is generally considered to have anticancer properties, and it may also have immunosuppressive effects [Xie *et al.* 2023]. Therefore, the administration of the rosemary-turmeric mixture may have health-promoting properties: treatment was found to result in higher expression of IFN- γ without any difference in *SREBF1* expression [Urbańska *et al.* 2023b]; a positive correlation was also noted between IFN- γ and *SREBF1*, and the regression of *SREBF1* on IFN- γ . However, this supposition contradicts previous data [Urbańska *et al.* 2023b, 2024], and further research is necessary.

The most correlations with immune system genes were found for *DGAT1*, and these occurred only in TG. In hepatocytes, long-chain fatty acids are esterified to produce triacylglycerol (TAG), which is stored in lipid droplets or secreted as very low-density lipoprotein (VLDL). Blocking the release of TAG causes its accumulation in the liver, steatosis, and the development of non-alcoholic steatohepatitis. DGAT1 activity blocks TAG accumulation, thus preventing the further accumulation of fatty acids and the development of non-alcoholic fatty liver disease. So far, it has been found that inhibition of DGAT1 is lipotoxic and that its overexpression increases VLDL secretion [Li and Wang 2020].

The relationship between the expression of *DGAT1* and its dependent genes (*HP*, *FGs*, *BAC7.5*, *MAP28*, *GBD1*, *HEPC*, *IFN- α*) in goat tissue remains unknown. In addition, supplementation with the studied supplement did not influence the expression of *DGAT1*, nor the immune system genes, compared to CG [Urbańska *et al.* 2023ab, 2024]. However, the influence of *IL-12p35* and *FGG* on the expression of *DGAT1* seems interesting. It is possible that the supplement did not affect *DGAT1* expression because its expression is dependent on those of *IL-12p35* and *FGG*, which were lower in TG.

SREBP1 presence is critical for the resolution of TLR-mediated inflammation [Yeudall *et al.* 2022]. Therefore, the presence of *DGAT1* and *SREBP1* in the livers of TG bucks indicates that the supplementation may improve the immune response.

Summing up, the rosemary-turmeric dry extract mixture might cause a sensitivity reaction; however, it may also reduce the risk of hypertriglyceridemia and improve immune response. It has also a positive effect on daily weight gain, cold carcass weight, and slaughter performance, with no negative effect on metabolic processes; the meat was also paler, which is desirable for consumers [Urbańska *et al.* 2024]. However, further research is needed with a wider range of mixture doses. Moreover, it must be emphasized that these findings reflect altered transcript levels, and further comprehensive studies including serum lipid biochemical profiles, protein-level verification, and histopathological analysis of liver tissue are required to validate these metabolic hypotheses.

Conclusions

FG was found to be a pro-inflammatory agent, and a lower level of its transcripts in the TG may suggest an altered inflammatory baseline rather than a definitive clinical health improvement. Although supplementation with turmeric-rosemary dry extract may reduce the risk of hypertriglyceridemia, further research at the protein level is needed to confirm this supposition. On the other hand, the positive correlation between *IL-18* and *IL-1β*, the negative correlations between *IL-12p35* and *IL-18*, and the absence of *IFN-γ* may hypothetically indicate that the used dose of the turmeric-rosemary mixture was too high and caused sensitization or the beginning of allergic inflammation. However, since these findings reflect exclusively mRNA expression profiles, further studies should treat them strictly as molecular hypotheses, include different doses of the supplement, and validate them at the protein and tissue levels.

Ethical Approval

The authors declare that this study is a secondary analysis of existing datasets [Urbańska *et al.* 2023ab, 2024]. Ethical approvals were secured during the primary data collection. No new animals were subjected to experimental procedures for this study.

REFERENCES

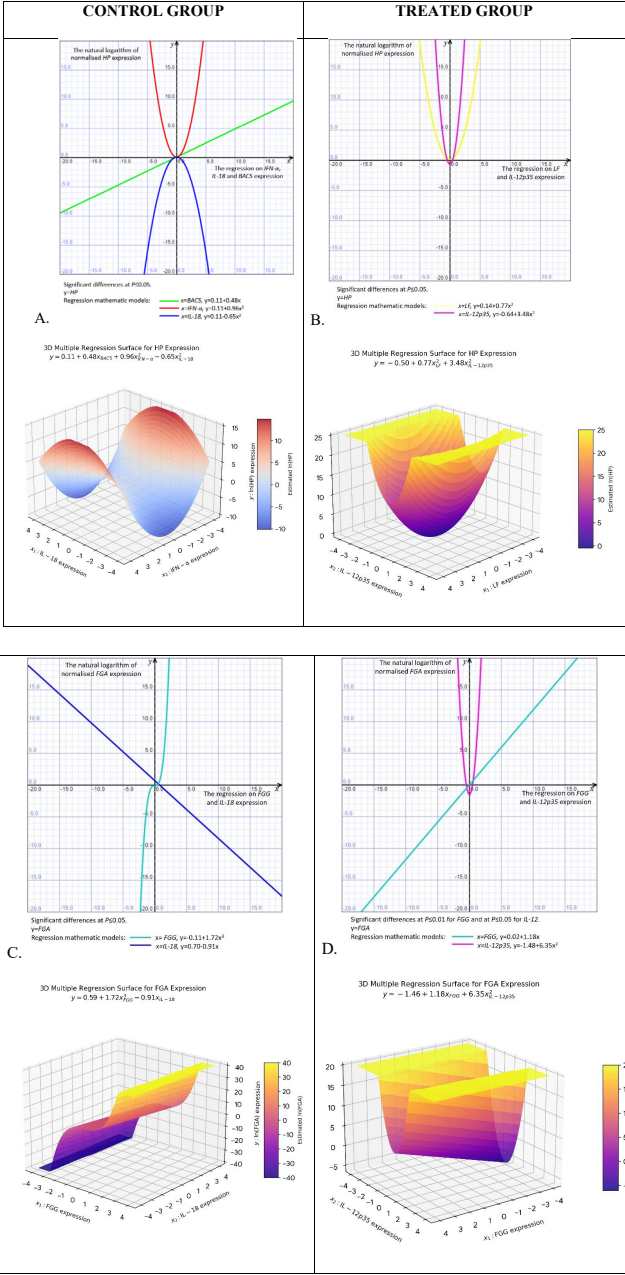
1. BALASUBRAMANIYAN V., MANJU V., NALINI N., GOWDA G., NAYAK B., RAO V.R., SARAVANAN T., SATISHCHANDRAN M., THANGAVELU K., MURTHY S.B., PRABHAKARAN M., 2016 - Cholesterol regulation by leptin in alcoholic liver disease. In: Patel, V.B. (Ed.), *Molecular Aspects of Alcohol and Nutrition*, 1st ed. Academic Press, pp. 187-200.
2. BARBÉ F., ATANASOVA K., VAN REETH K., 2011 - Cytokines and acute phase proteins associated with acute swine influenza infection in pigs. *Veterinary Journal* 187, 48-53.
3. BARRANCO I., GÓMEZ-LAGUNA J., RODRÍGUEZ-GÓMEZ I.M., QUEREDA J.J., SALGUERO F.J., PALLARÉS F.J., CARRASCO L., 2021 - Immunohistochemical expression of IL-12, IL-10, IFN- α and IFN- γ in lymphoid organs of PRRSV-infected pigs. *Veterinary Immunology and Immunopathology* 149, 262-271.
4. BATTA A., 2018 - Antioxidant rich spices and herbs. *British Journal of Medical and Health Research* 5, 1-11.
5. BERGAMO A., GERDOL M., PALLAVICINI A., GRECO S., SCHEPENS I., HAMELIN R., ARMAND F., DYSON P.J., SAVA G., 2019 - Lysozyme-induced transcriptional regulation of TNF- α pathway genes in cells of the monocyte lineage. *International Journal of Molecular Sciences* 20, 5502.
6. CARLINI V., NOONAN D.M., ABDALALEM E., GOLETTI D., SANSONE C., CALABRONE L., ALBINI A., 2023 - The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. *Frontiers in Immunology* 14, 1161067.
7. CARTY C.L., HEAGERTY P., HECKBERT S.R., JARVIK G.P., LANGE L.A., CUSHMAN M., *et al.*, 2010 - Fibrinogen and IL6 gene variants and IL-6 levels in relation to plasma fibrinogen concentration and cardiovascular disease risk in the Cardiovascular Health Study. *Annals of Human Genetics* 74, 1-9.
8. CHANG T.T., YANG H.Y., CHEN C., CHEN J.W., 2020 - CCL4 inhibition in atherosclerosis: effects on plaque stability, endothelial cell adhesiveness, and macrophage activation. *International*

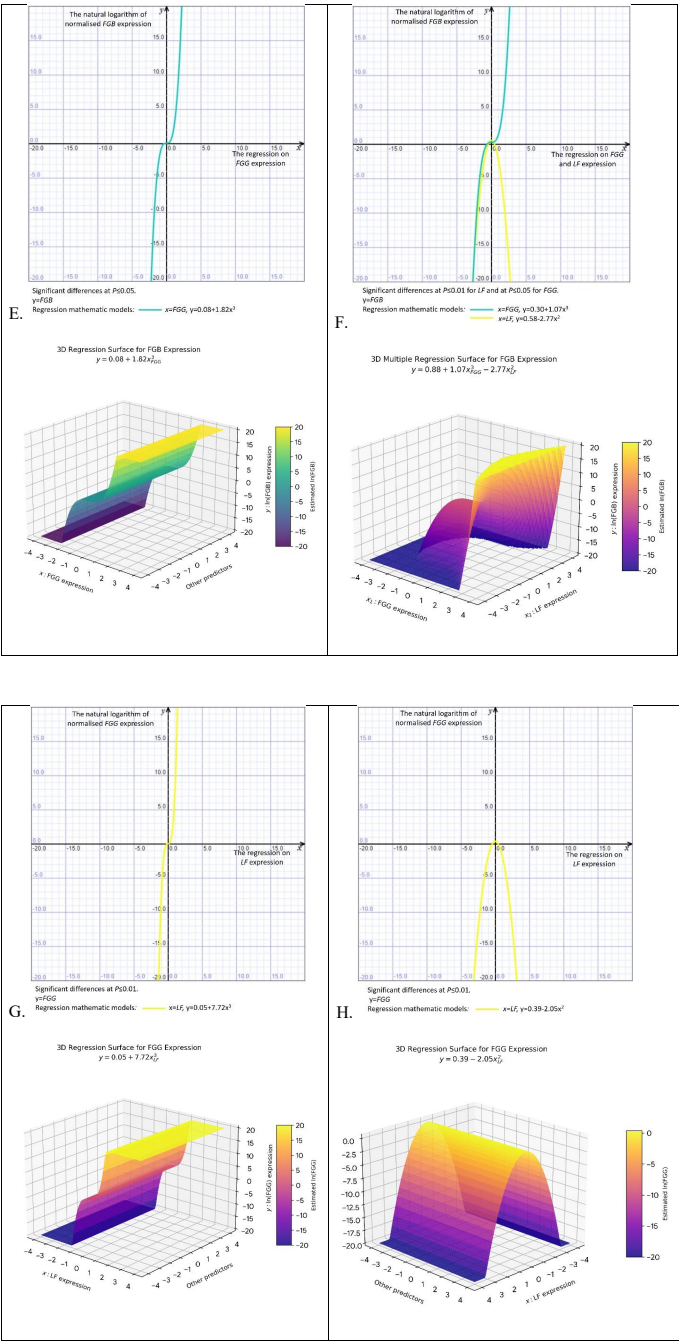
- Journal of Molecular Sciences* 21, 6567.
9. DONG J., LI M., PENG R., ZHANG Y., QIAO Z., SUN N., 2024 - ACACA reduces lipid accumulation through dual regulation of lipid metabolism and mitochondrial function via AMPK–PPAR α -CPT1A axis. *Journal of Translational Medicine* 22, 196.
 10. DUAN H.O., SIMPSON HAIDARIS P.J., 2006 - Cell type-specific differential induction of the human γ -fibrinogen promoter by interleukin-6. *Journal of Biological Chemistry* 281, 12451-12457.
 11. FANTUZZI G., REED D.A., DINARELLO C.A., 1999 - IL-12-induced IFN- γ is dependent on caspase-1 processing of the IL-18 precursor. *Journal of Clinical Investigation* 104, 761-767.
 12. FATHY M.A., RAAFAT N.A., 2019 - Possible association between serum hepcidin and hemostatic parameters in chronic renal failure rat model: influence of inhibition of ACE. *Medical Journal of Cairo University* 87, 335-349.
 13. GAO B., JEONG W., TIAN Z., 2008 - Liver: an organ with predominant innate immunity. *Hepatology* 47, 729-736.
 14. GREULICH J., UPCHURCH C.M., SEEGREN P.V., PAVELEC C.M., HARRIS T.E., DESAI B.N., HOEHN K.L., 2022 - Macrophage acetyl-CoA carboxylase regulates acute inflammation through control of glucose and lipid metabolism. *Science Advances* 8, eabq1984.
 15. HAO J., ZHANG Y., LV X., XU N., LIU Q., ZHAO S., FENG X., XING L., KANG P., LI G., LIU S., 2013 - IFN- γ induces lipogenesis in mouse mesangial cells via the JAK2/STAT1 pathway. *American Journal of Physiology - Cell Physiology* 304, C760-767.
 16. Jain S., Gautam V., Naseem S., 2011 - Acute phase proteins: as diagnostic tool. *Journal of Pharmacy and Bioallied Sciences* 3, 118-127.
 17. KALIŃSKI P., SMITS H.H., SCHUITEMAKER J.H., VIEIRA P.L., VAN EIJK M., DE JONG E.C., WIERENGA E.A., KAPSENBERG M.L., 2000 - IL-4 is a mediator of IL-12p70 induction by human Th2 cells: reversal of polarized Th2 phenotype by dendritic cells. *Journal of Immunology* 165, 1877-1883.
 18. LAWLER J.F. JR, YIN M., DIEHL A.M., ROBERTS E., CHATTERJEE S., 1998 - Tumor necrosis factor- α stimulates the maturation of sterol regulatory element binding protein-1 in human hepatocytes through the action of neutral sphingomyelinase. *Journal of Biological Chemistry* 273, 5053-5059.
 19. LEE J.H., CHO D.H., PARK H.J., 2015 - IL-18 and cutaneous inflammatory diseases. *International Journal of Molecular Sciences* 16, 26172-26195.
 20. LEVINE C.B., BAYLE J., BIOURGE V., WAKSHLAG J.J., 2017 - Cellular effects of a turmeric root and rosemary leaf extract on canine neoplastic cell lines. *BMC Veterinary Research* 13, 289.
 21. LI X., WANG H., 2000 - Multiple organs involved in the pathogenesis of nonalcoholic fatty liver disease. *Cell & Bioscience* 10, 1-15.
 22. LING C.J., XU J.Y., LI Y.H., TONG X., YANG H.H., YANG J., YUAN L.X., QIN L.Q., 2019 - Lactoferrin promotes bile acid metabolism and reduces hepatic cholesterol deposition by inhibiting the farnesoid X receptor mediated enterohepatic axis. *Food & Function* 10, 7359-7369.
 23. MARSHALL J.S., LEAL BERUMEN I., NIELSEN L., GLIBETIC M., JORDANA M., 1996 - IL-10 inhibits long term IL-6 production but not preformed mediator release from rat peritoneal mast cells. *Journal of Clinical Investigation* 97, 1122-1128.
 24. MICHELS K., NEMETH E., GANZ T., MEHRAD B., 2015 - Hepcidin and host defense against infectious diseases. *PLOS Pathogens* 11, e1004998.
 25. MOHAMED M.E., YOUNIS N.S., EL BELTAGI H.S., MOHAFAEZ O.M., 2022 - The synergistic hepatoprotective activity of rosemary essential oil and curcumin: the role of the MEK/ERK pathway. *Molecules* 27, 1123.
 26. OUELLETTE A.J., 2015 - Defensins in enteric mucosal immunity. In: Mestecky, J., Strober, W., Russell, M.W., Kelsall, B.L., Cheroutre, H., Lambrecht, B.N. (Eds.), *Mucosal Immunology*, 4th ed.

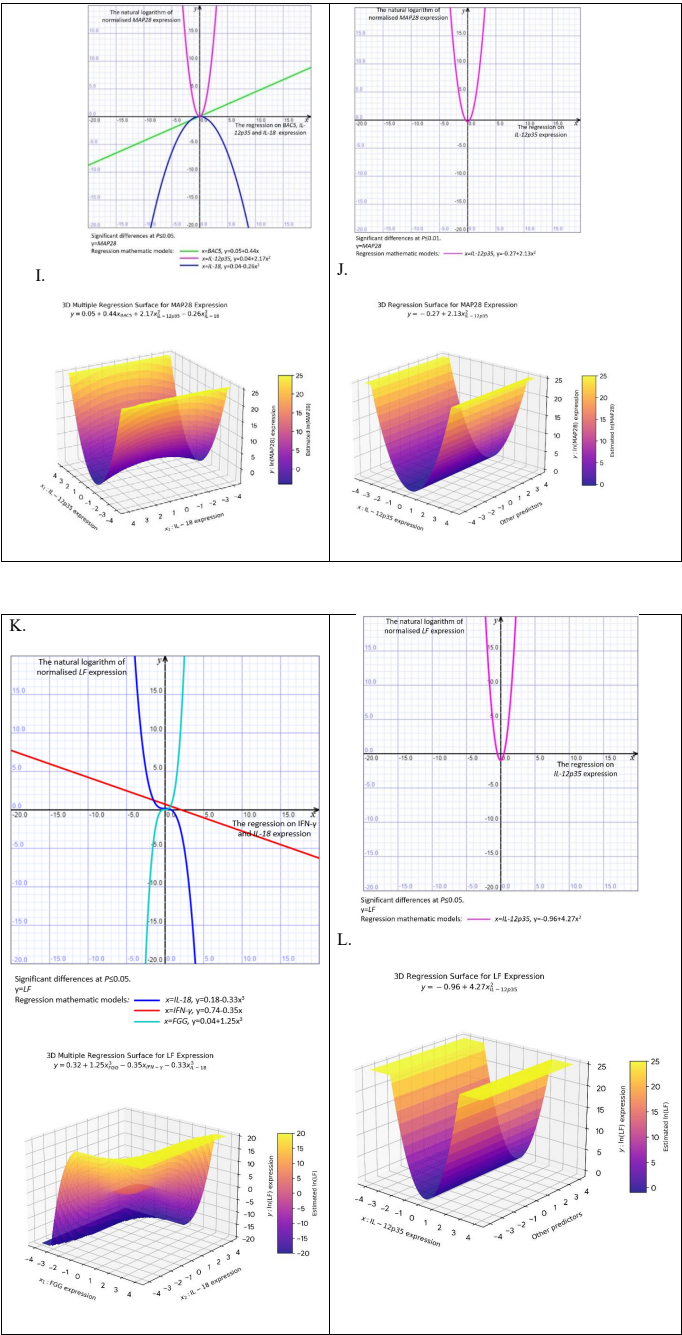
Academic Press, San Diego, pp. 331-350.

27. PECHLANER R., KIECHL S., MAYR M., SANTER P., WEGER S., HASCHKA D., BANSAL S.S., WILLEIT J., WEISS G., 2015 - Correlates of serum hepcidin levels and its association with cardiovascular disease in an elderly general population. *Clinica Chimica Acta* <https://doi.org/10.1515/cclm-2015-0068>.
28. PRESTI S., MANTI S., PARISI G.F., PAPALE M., BARBAGALLO I.A., LI VOLT G., LEONARDI S., 2021 - Lactoferrin: cytokine modulation and application in clinical practice. *Journal of Clinical Medicine* 10, 469.
29. ROBINSON M., HARMON C., O'FARRELLY C., 2016 - Liver immunology and its role in inflammation and homeostasis. *Cellular & Molecular Immunology* 13, 267-276.
30. SEEGERT D., ROSENSTIEL P., PFAHLER H., PFEFFERKORN P., NIKOLAUS S., SCHREIBER S., 2001 - Increased expression of IL-16 in inflammatory bowel disease. *Gut* 48, 326-332.
31. TANNO L.K., CALDERON M.A., SMITH H.E. SMITH H.E., SANCHEZ-BORGES M., SHEIKH A., DEMOLY P., 2016 - Dissemination of definitions and concepts of allergic and hypersensitivity conditions. *World Allergy Organ J* 9, 24.
32. TSUTSUMI N., YOKOTA A., KIMURA T., KATO Z., FUKAO T., SHIRAKAWA M., OHNISHI H., TOCHIO H., 2019 - An innate interaction between IL-18 and the propeptide that inactivates its precursor form. *Scientific Reports* 9, 16735.
33. URBAŃSKA D.M., PAWLIK M., KORWIN-KOSSAKOWSKA A., CZOPOWICZ M., RUTKOWSKA K., KAWECKA-GROCHOCKA E., MICKIEWICZ M., KABA J., BAGNICKA E., 2023a - Effect of supplementation with Curcuma longa and Rosmarinus officinalis extract mixture on acute phase protein, cathelicidin, defensin and cytolytic protein gene expression in the livers of young castrated Polish White Improved bucks. *Genes* 14, 1024.
34. URBAŃSKA D.M., PAWLIK M., KORWIN-KOSSAKOWSKA A., CZOPOWICZ M., RUTKOWSKA K., KAWECKA-GROCHOCKA E., MICKIEWICZ M., KABA J., BAGNICKA E., 2023b - Effect of supplementation with Curcuma longa and Rosmarinus officinalis extract mixture on selected cytokine gene expression in the liver of young castrated bucks of Polish White Improved breed. *Animals* 13, 1201.
35. URBAŃSKA D.M., PAWLIK M., KORWIN-KOSSAKOWSKA A., CZOPOWICZ M., RUTKOWSKA K., KAWECKA-GROCHOCKA E., MICKIEWICZ M., KABA J., BAGNICKA E., 2024 - Effect of supplementation with a mixture of Curcuma longa and Rosmarinus officinalis extracts on growth performance, meat quality and lipid metabolism gene expressions in young castrated Polish White Improved bucks. *Journal of Animal and Feed Sciences* 33, 137-148.
36. VAN HARTEN R.M., VAN WOUDEBERGH E., VAN DIJK A., HAAGSMAN H.P., 2018 - Cathelicidins: immunomodulatory antimicrobials. *Vaccines* 6, 63.
37. VASSE M., PAYSANT J., SORIA J., COLLET J.P., VANNIER J.P., SORIA C., 1996 - Regulation of fibrinogen biosynthesis by cytokines, consequences on the vascular risk. *Haemostasis* 26, 145-153.
38. WEILER NORMANN C., REHERMANN B., 2004 - The liver as an immunological organ. *Journal of Gastroenterology and Hepatology* 19 (Suppl. 1), S279-S283.
39. WU X., SUN R., CHEN Y., ZHENG X., BAI L., LIAN Z., et al., 2015 - Oral ampicillin inhibits liver regeneration by breaking hepatic innate immune tolerance normally maintained by gut commensal bacteria. *Hepatology* 62, 993-1005.
40. XIE L., LIU M., CAI M., HUANG W., GUO Y., LIANG L., CAI C., LIU J., LIANG W., TAN Y., LAI M., LIN L., ZHU K., 2023 - Regorafenib enhances anti-tumor efficacy of immune checkpoint inhibitors in colorectal cancer. *Biomedicine & Pharmacotherapy* 159, 114254.
41. YEUDALL S., UPCHURCH C.M., SEEGREN P.V., PAVELEC C.M., GREULICH J., LEMKE M.C., HARRIS T.E., DESAI B.N., HOEHN K.L., LEITINGER N., 2022 - Macrophage acetyl-CoA carboxylase regulates acute inflammation through control of glucose and lipid metabolism. *Science Advances* 8, eabq1984.

Supplementary files







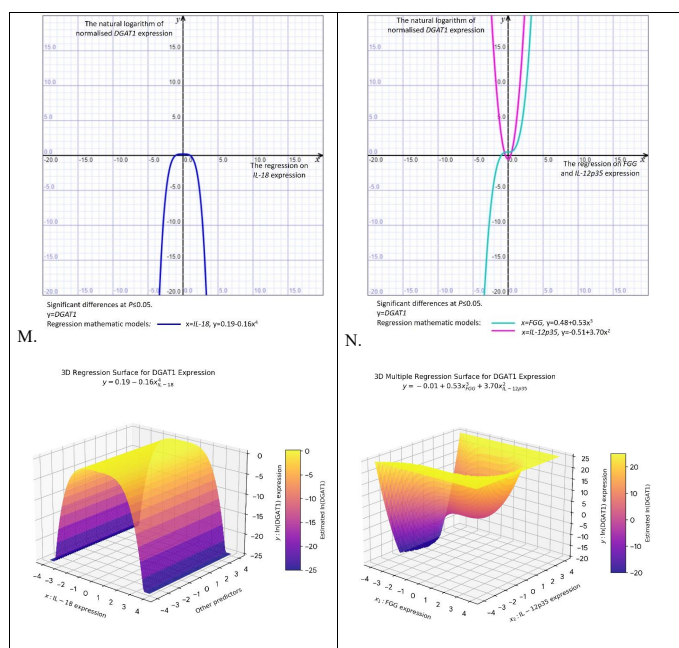
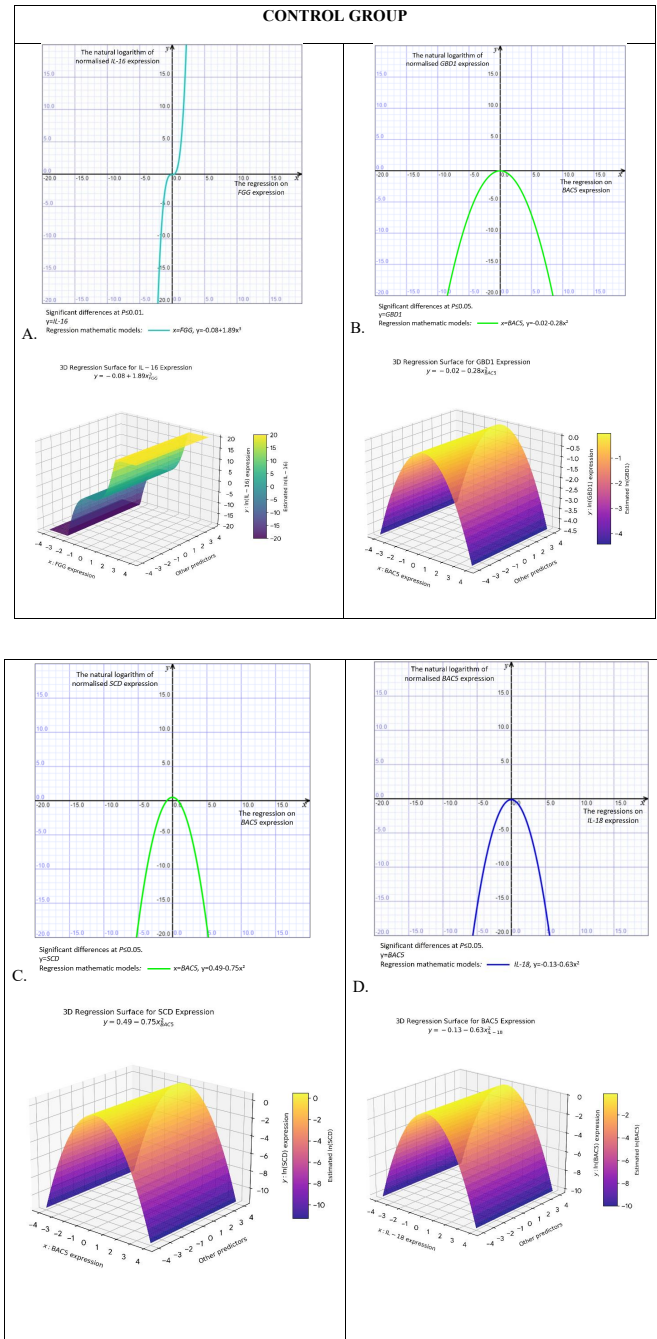


Fig. S1. The regression of the expression of the selected immune system and lipid metabolism genes on those of interferon gamma (*IFN- γ*), interleukin 12p35 (*IL-12p35*), interleukin 18 (*IL-18*), bactenecin 5 (*BAC5*), fibrinogen gamma (*FGG*) and lactoferrin (LF) in the liver of young castrated goat bucks: A, C, E, G, I, K, M – control group, and B, D, F, H, J, L, N – treated group. *IFN- γ* – Interferon γ ; *IL-12p35* – Interleukin 12p35; *IL-18* – Interleukin 18; *BAC5* – Bactenecin 5/cathelicidin 2; *HP* – Haptoglobin; *FGA* – Fibrinogen α chain; *FGB* – Fibrinogen β chain; *FGG* – Fibrinogen γ chain; *MAP28* – Cathelicidin 6; *LF* – Lactoferrin; *DGAT1* – diacylglycerol O-acyltransferase.



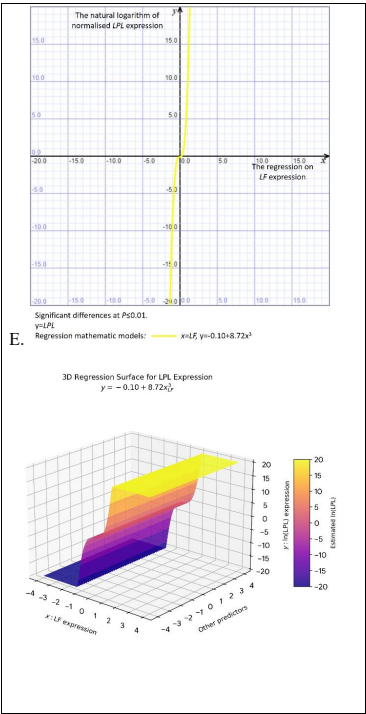
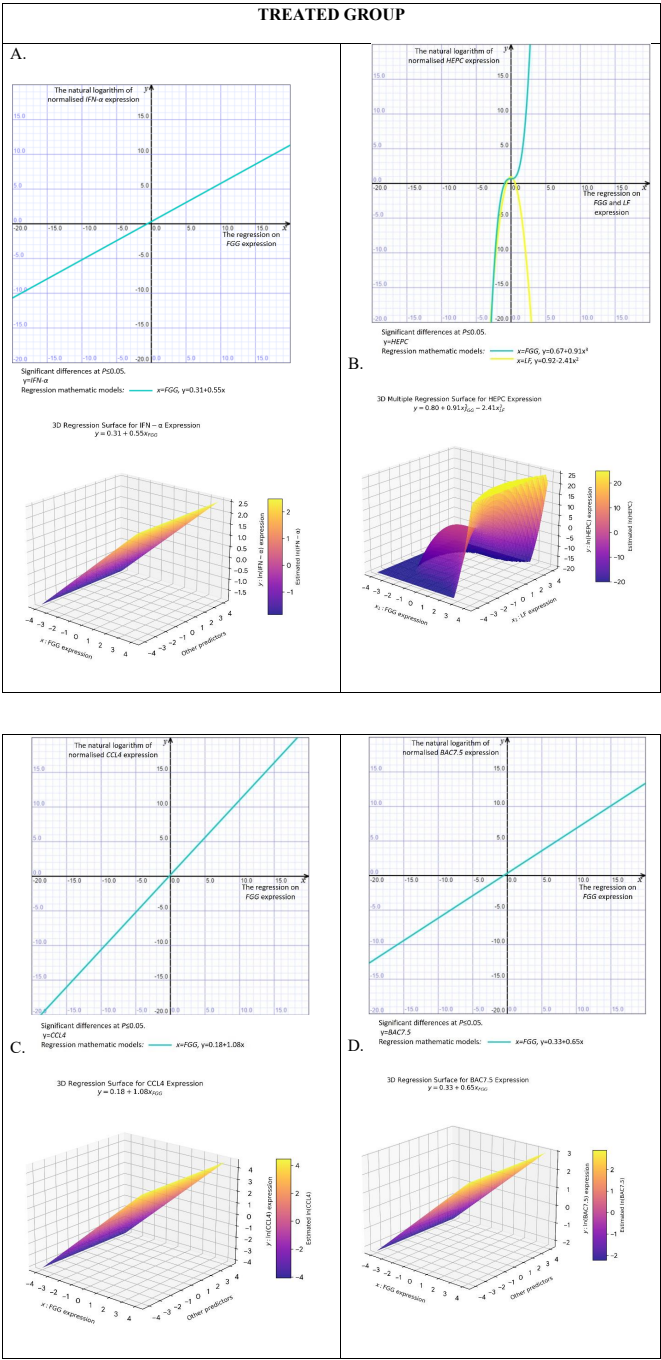


Fig. S2. The regression of the expression of selected immune system and lipid metabolism genes on those of fibrinogen gamma (*FGG*) (A, B), batenecin 5 (*BAC5*) (C, D), interleukin 18 (*IL-18*) (E), and lactoferrin (*LF*) (F) in the control group. *IL-18* – Interleukin 18; *FGG* – Fibrinogen γ chain; *BAC5* – Batenecin 5/cathelicidin 2; *LF* – Lactoferrin; *IL-16* – Interleukin 16; *GBD1* – Beta defensin 1; *SCD* – Stearoyl-CoA desaturase; *LPL* – Lipoprotein lipase.



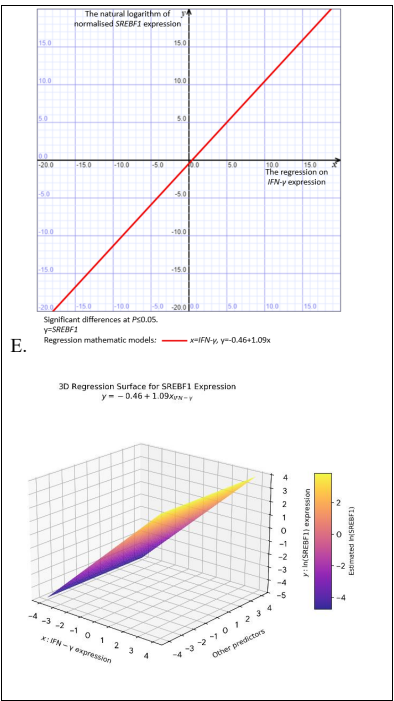


Fig. S3. The regression of the expression of selected immune system and lipid metabolism genes on those of fibrinogen gamma (*FGG*) (A-D) and interferon gamma (*IFN-γ*) (E) in the treated group. *IFN-γ* – Interferon γ ; *FGG* – Fibrinogen γ chain; *SREBF1* - Sterol regulatory element binding transcription factor; *IFN-α* – Interferon α ; *HEPC* – Hepcidin; *CCL4* – Chemokine 4; *BAC7.5* – Bactenecin 7.5/cathelicidin 3.

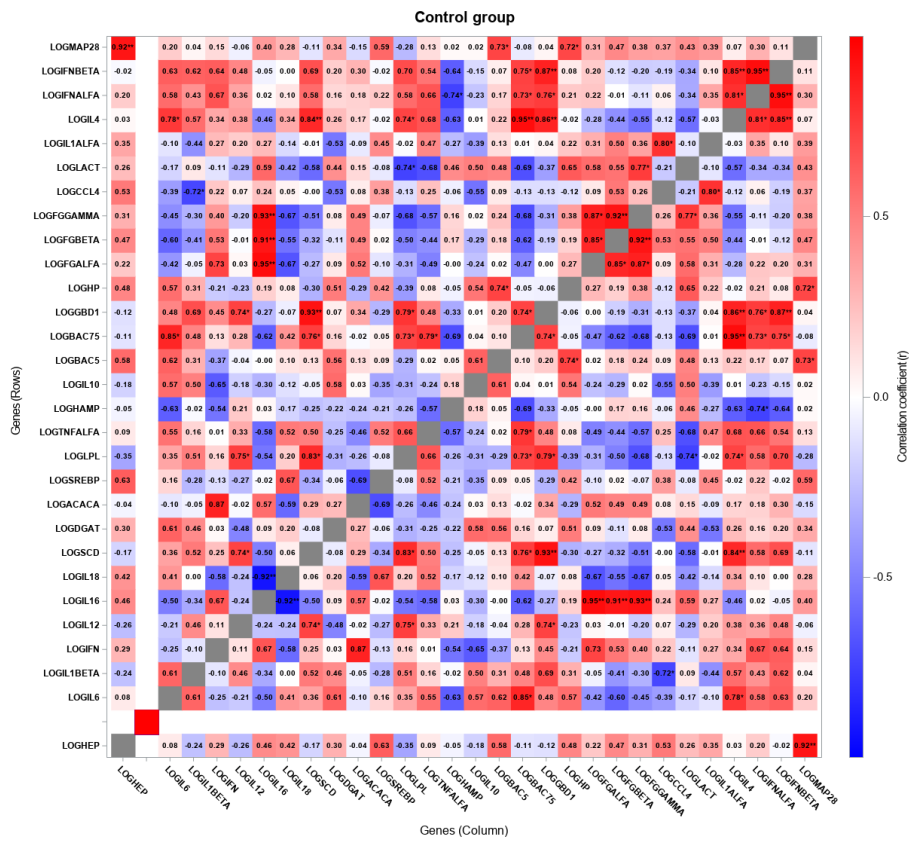


Fig. S4. Heatmap of the gene correlation matrix in the control group. Cell colors represent the value of Pearson's correlation coefficient (r), where red denotes a strong positive correlation, blue indicates a strong negative correlation, and white represents no correlation ($r = 0.00$). Numerical values inside the cells display the exact correlation coefficients. Statistical significance is indicated by asterisks: * $p \leq 0.05$; ** $p \leq 0.01$. The diagonal elements (self-correlation) have been excluded from the analysis for clarity.

