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Effect of n-6 and n-3 fatty acids on expression of pro-inflammatory cytokines in lambs vaccinated against foot and mouth disease virus

Ali Ghorbani[1], Ali Asghar Sadeghi[*1], Parvin Shawrang[2], Mohammad Chamani[1] and Farhad Foroudi[3]*

[1]*Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Agriculture Nuclear Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj, Iran*
[3]*Department of Animal Science, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran*

[*]Corresponding author,
Tel: +989195579663
E-mail address:
a.sadeghi@srbiau.ac.ir

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ORCID
Ali Ghorbani
0000-0003-2005-9184
Ali Asghar Sadeghi
0000-0002-5755-3752
Parvin Shawrang
0000-0002-7263-7858
Mohammad Chamani
0000-0002-4073-3597
Farhad Foroudi
0000-0002-8926-6766

Abstract The current study was aim to the effects of n-6 and n-3 polyunsaturated fatty acids (PUFA, or palmitic acid sources) on expression of pro-inflammatory cytokines in lambs vaccinated against foot and mouth disease (FMD) virus. A total of fifteen Sangsari male lambs with an age of 6 ± 1 months and body weight of 42 ± 1 kg were randomly assigned to one of three experimental treatments as follows: 1) PO: received calcium soap of palm oil fatty acid (FA) in the diet as source of palmitic acid (16:0), 2) SO: received calcium soap of sunflower oil FAs in the diet as source of linoleic acid (n-6 18:2) and 3) LO: received calcium soap of linseed oil FA in the diet as source of α-linolenic acid (n-3 18:3). The lambs were offered an iso-energetic and iso-nitrogenous diet for 28 days (including 21 days of adaptation and 7 days of sampling). The lambs were individually housed and had access to water *ad libitum*. The expression of interleukin-1β (IL-1β) mRNA was lower in LO when compared with PO and SO ($P<0.05$). The expression of tumor necrosis factor-α (TNFα) mRNA was higher in PO when compared with SO and LO and the lowest expression of TNFα mRNA was measured in LO ($P<0.05$). Lower concentrations of serum cholesterol and triglyceride (TG) were measured in vaccinated lambs on LO diet when compared with vaccinated lambs on palmitic FA diet ($P<0.05$). There was no significant difference between SO and LO groups in serum cholesterol or TG levels. There were no significant differences between treatments ($P>0.05$) in serum level of total proteins and albumin. The findings from the current study showed feeding α-linolenic acid diet following vaccination against FMD resulted in a decrease in serum levels of cholesterol and TG due to higher n-3 PUFA intake and suppressed the pro-inflammatory cytokine expression (IL-1 and TNFα).

Keywords: acute-phase proteins, alpha-linolenic acid, conjugated linoleic acid, foot and mouth disease, linseed

Introduction

Foot and mouth disease (FMD) is a highly contagious acute-vesicular disease of ruminants(Park et al., 2006, Orsel et al., 2007). It is caused by foot and mouth disease virus (FMDV:-family *Picornaviridae*; genus *Aphthovirus*), a single-stranded, positive-sense RNA (De Los Santos et al., 2007). Foot and mouth disease is an economically devastating disease and causes significant production losses in ruminants (Belsham, 1993). It has been shown that the L proteinase of FMDV inhibited interferons (IFN-α and IFN-β) production by infected cells and shut off host protein translation

(Chinsangaram et al., 1999).

Macrophages have a critical role in antigen processing and presentation to other immune cells (Kovacsovics-Bankowski et al., 1993; Colonna et al., 2002). Alternatively activated macrophages (M2) are poorly microbicidal and have anti-inflammatory properties while classically activated macrophages (M1) are microbicidal and pro-inflammatory (Benoit et al., 2008; Mosser and Edwards, 2008). M1 macrophages are stimulated by PAMPs, damage-associated molecular patterns and inflammatory cytokines like TNF-α and IFN-γ, whereas M2 macrophages are stimulated by anti-inflammatory cytokines like IL-10, IL-4, and IL-13 (Hirayama et al., 2018). M1 macrophages also release tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) which further aid in the elimination of pathogens (Al-Khalaifah, 2020). Pro-inflammatory cytokines reduce the synthesis of albumin and lipoproteins (Loor et al., 2013) and increase the synthesis of acute-phase proteins (APPs) such as haptoglobin (Hp), serum amyloid A (SAA), C-reactive protein and ceruloplasmin (Cp) in the liver (Powanda, 1980). Also, a significant decrease in kidney and liver function maybe happen during bacterial and viral disease.

The RNA molecules generated by FMDV are sensed by the cellular receptor for PAMPs (Ma et al., 2018), induce M1 polarization in macrophages (Sebastian et al., 2020) and activate NF-κB signaling events (De Los Santos et al., 2007). Once activated, NF-κB translocates to the nucleus driving the expression and release of pro-inflammatory proteins and cytokines including TNFα, pro-interleukin-1β, pro-IL-18, IL-6, IL-8, neutrophil chemo-attractants, monocytes and T-lymphocytes chemo-attractants and cell adhesion molecules (Nian et al., 2004, Coggins and Rosenzweig, 2012).

Major stressors associated with animal production trigger the production of pro-inflammatory cytokines (Lee et al., 2016). Also, various nutrients can affect the metabolism and function of leukocytes (Schwager et al., 2015). For example, fatty acids (FAs) alter both hormone and neuropeptide concentrations and their receptors (Bhathena, 2006). Polyunsaturated fatty acids (PUFA) are precursors for eicosanoids including prostaglandins, leukotrienes, and thromboxanes, which have hormone-like activities (Lakdawala and Grant-Kels, 2015). Kaveh et al. (2019) showed preventive effect of α-linolenic acid on inflammatory markers in sensitized animals comparable to the effect of dexamethasone. However, Hadfield (2017) reported no clear benefit of supplementary n-3 PUFAs in reducing the inflammatory response.

The n-9 FAs show a tremendous series of beneficial impacts like anti-oxidative properties, improved cholesterol levels, regulating immunity responses (Simopoulos, 2002, Alagawany et al., 2020). However, the n-6 PUFA such as linoleic acid (18:2 n-6) are the precursor of arachidonic acid (20:4 n-6) which can convert to prostaglandins (such as PGE2), leukotrienes and related compounds that have important roles in inflammation and in the regulation of immunity (Yaqoob et al., 2000). The metabolism of arachidonic acid to yield these mediators can be inhibited by the long chain n-3 PUFA (Calder et al., 2002). Feeding n-3 PUFA results in partial replacement of arachidonic acid by eicosapentaenoic acid in membrane of cells involved in inflammation which leads to decreased production of arachidonic acid-derived mediators (Darwesh et al., 2019). Thus, feeding n-3 PUFA results in a decreased capacity of immune cells to synthesis series-2 prostaglandins from arachidonic acid (Yaqoob et al., 2000) and induce formation of series-3 eicosanoids which have anti-inflammatory effects compared to series-2 prostaglandins (Gulliver et al., 2012).

The aim of the current research was to investigate the effects of feeding saturated FA (16:0) and PUFA (18:2 n-6 and 18:3 n-3) rich diets on pro-inflammatory cytokines expression and blood levels of proteins and lipids in lambs vaccinated against FMDV.

Materials and methods

Animals and management

A total of fifteen healthy Sangsari male lambs with a body weight of 42 ± 1 kg and an age of 6 ± 1 months were randomly assigned to one of three experimental treatments. The lambs were considered free of infections and cutaneous disorders based on clinical inspection. Two weeks before the start of the study, lambs were treated against possible parasites with albendazole at a dose of 5 mg/kg body weight. The study started in the spring of 2018 at Karaj city, Alborz province. The average annual temperature and rainfall in this area are 15.2 °C and 457 mm.

Treatments were as follows: 1) The palm oil (PO) group which received calcium soap of palm oil FAs in the diet as source of palmitic acid (16:0), 2) the sunflower oil (SO) group which received calcium soap of sunflower oil FAs in the diet as source of linoleic acid (n-6 18:2) and 3) the linseed oil (LO) group which received calcium soap of linseed oil FAs in the diet as source of α-linolenic acid (n-3 18:3).

The diets were formulated according to nutritional requirements of sheep by Cornell Net Carbohydrate and Protein System (CNCPS) using Sheep CNCPS 1.0.21 software. The ingredients and chemical composition of the experimental diets are presented in Table 1. The lambs were individually housed and offered the iso-caloric and iso-nitrogenous diet for 28 days including 21 days of adaptation period and 7 days of sampling period. The diets were fed three times daily as total mixed rations at 08:00 and 15:00 h. Fresh drinking water was continuously provided in each pen. After the adaptation period, the lambs were vaccinated subcutaneously using 1-mL polyvalent FMD vaccine (inactivated, containing virus types O2016, A13, A15 and Asia1 strains; Razi Vaccine and Serum Research Institute).

Data and sample collection

Blood sample was collected 7 days after vaccination (on day 28 of the experiment) before morning feeding by jugular venipuncture, after proper restraint, in labelled sterile serum tubes containing 1 mg / mL ethylene diaminetetracetate (EDTA). The serum was separated by centrifugation at 3000 rpm for 15 min and kept at −20°C for later analysis of metabolites.

Serum analysis

Plasma metabolites were quantified using commercially available kits according to the manufacturer's recommendations (PishtazTeb Co., Arak, Iran). The total serum protein and total serum albumin were measured using a photometric method at a wavelength of 546 nm. Cholesterol and triglycerides in the serum samples were estimated using enzymatic cholesterol oxidase phenol 4-aminoantipyrine peroxidase method at the wavelength of 546 nm.

RNA extraction

Total RNA was isolated from blood samples using the RNeasy®Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines. The concentration and purity (260/280 nm ratio of absorbance readings) of RNA were quantified by Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Approximately100 ng/µL purified RNA was converted into complementary DNA (cDNA) using Quantitect® reverse transcription kit (Qiagen, Hilden, Germany) following the manufacturer's procedure. The genomic DNA removal was performed before the reverse transcription of RNA to cDNA. For the internal standard (housekeeping gene), GADPH gene was used to standardize the expression.

Table 1. Feed ingredients and chemical composition of the basal diet

Ingredients (g/kg DM)	
Alfalfa hay	158
Wheat straw	158
Barley grain	330
Dry corn grain	228
Soybean meal	82
Oil	28
Limestone	8
Di-calcium phosphate	2
Salt	2
Sodium bicarbonate	4
Chemical Composition (g/kg DM)	
Metabolizable energy (Mcal/kg)	2.85
Crude protein	140
Neutral detergent fiber	257
Non-fibrous carbohydrate	529
Crude fat	27
Calcium	7.9
Phosphorus	4.3

Real-time qPCR

Real-time qPCR was performed with the Bio-Rad CFX96 Real-time PCR system (Bio-Rad Laboratories, CA, USA). The cycling conditions for all genes were as follows: 5 minutes at 94°C, 45 cycles of 20 seconds at 94°C, 20 seconds at 60°C and 20 seconds at 72°C, followed by a melt curve starting at 65°C rising to 94°C at 0.3°C per second (Smeed et al., 2007). Data were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene. The primer characteristics of cytokines and reference genes are described in Table 2.

Table 2. Primer characteristics of cytokines and reference gene for real-time PCR amplification

Target gene	Primer sequence (5′ - 3′)	Product size (bp)	Gene Accession number
IL-1β	F - CCTTGGGTATCAGGGACAA R - TGCGTATGGCTTTCTTTAGG	317	X56972
TNFα	F - GAATACCTGGACTATGCCGA R - CCTCACTTCCCTACATCCCT	238	X56756
GADPH	F - GGTGATGCTGGTGCTGAGTA R - TCATAAGTCCCTCCACGATG	265	AF030943

F=Forward, R=Reverse, GADPH=Glyceraldehyde3-phosphate dehydrogenase, IL-1β=Interleukin-1β, TNFα =Tumor necrosis factor-α

The analysis of melting curve was performed at the end of the amplification cycle to confirm the specificity of amplification. The relative expression levels of IL-1 and TNFα transcripts were measured by quantitative Real time-PCR. Comparative ΔΔCt method was used for quantification of Real-Time PCR outputs. The efficiency of amplification of target and housekeeping genes was determined by a 5-fold serial dilution of cDNA as a standard curve. Using the GenEx enterprise software, the fold changes were statistically analyzed. All the standard curves showed good PCR amplification efficiencies between 90 to 120%.

Statistical analysis

Data were subjected to ANOVA using the MIXED procedure of SAS (Statistical Analysis System) version 9.2 (SAS Institute, USA). The model included fixed effe-

cts of the treatments, and the lamb as a random effect. Differences between treatment means were considered significant at *P*<0.05 using Tukey's HSD test.

Results

The data for real-time PCR (qRT-PCR) revealed that there were significant differences in pro-inflammatory cytokines IL-1β and TNF-α (Table 3, P<0.05). The expression of IL-1β mRNA was lower in LO groups when compared with PO and SO (P<0.05). However, there was no significant difference between PO and SO in IL-1β mRNA expression. The minimum expression of TNFα mRNA was measured in LO (P<0.05). Also, the TNFα mRNA expression was higher in PO when compared with SO and LO.

Table 3. The relative gene expression of TNFα and IL-1β in lambs fed palmitic acid (PO), linoleic acid (SO) or α-linolenic acid (LO) diets (n=3)

	PO	SO	LO	SEM	P value
IL-1β	1.52[a]	1.48[a]	0.67[b]	0.19	0.0001
TNFα	1.41[a]	1.07[b]	0.78[c]	0.11	0.0001

PO: palm oil treatment (palmitic acid); SO: sunflower oil treatment (n-6 PUFA, linoleic acid); LO: linseed oil treatment (n-3 PUFA, α-linolenic acid); SEM: standard error of means.
a, b: within rows, means with common superscript do not differ (P>0.05).

Lower concentration of serum cholesterol (Table 4) was measured in vaccinated lambs on LO diet compared with the vaccinated lambs on palmitic FAs diet (P<0.05). Additionally, a significant decrease (P<0.05) in the level of triglyceride (TG) was also observed in LO compared with PO. However, the concentration of serum cholesterol and TG were not significantly different between palmitic acid and 18:2 n-6 FA (*P*>0.05). Also, there was no significant difference between SO and LO in serum cholesterol or TG levels. Serum levels of total proteins and albumin were not affected by treatments.

Table 4. The serum metabolite levels (mg/dL) in vaccinated lambs fed palmitic acid (PO), linoleic acid (SO) or α-linolenic acid (LO) diets (n=5)

	PO	SO	LO	SEM	P value
Cholesterol	79.4[a]	75.4[ab]	65.5[b]	8.16	0.0419
Triglycerides	28.4[a]	24.1[ab]	22.4[b]	3.61	0.0460
Total proteins	4.63	4.85	4.79	0.32	0.5422
Albumin	2.20	2.21	2.37	0.22	0.4299

PO: palm oil treatment (palmitic acid); SO: sunflower oil treatment (n-6 PUFA, linoleic acid); LO: linseed oil treatment (n-3 PUFA, α-linolenic acid); SEM: standard error of means.
a, b: within rows, means with common superscript do not differ (P>0.05).

Discussion

In this study the lambs were considered free of infections and cutaneous disorders based on clinical inspection. Also, the environmental condition was same for all groups and all animals had 3 weeks of adaptation to diets and housing. Then, differences among treatments on gene expression of cytokines, was related to the effects of sources of fatty acid in diets on responses of immune system to FMD vaccination. Our finding on IL-1, are in agreement with Zeng et al. (2016) who found that a high ratio of α-linolenic acid to linoleic acids in the diet significantly increased the complement C3 contents, promoted interleukin-10 mRNA abundance, whereas suppressed pro-inflammatory cytokines (like TNFα and IFNγ2, IL-1β, IL-8) and reduced signal molecules mRNA levels in the intestine of juvenile fish. In another study, the levels of IL-1 and TNF-α and expression of IL-1β increased in heat stressed lambs (Shi et al., 2020).

Winnik et al. (2011) in a human study confirmed that the mRNA levels of TNFα, IFNγ and IL-4 decreased in response to α-linolenic acid. Matsuyama et al. (2005) showed that TNFα and IL-8 levels decreased significantly in the n-3 group, while there was no significant change in the n-6 group. Darwesh et al. (2019) implied that the anti-inflammatory and anti-fibrotic effects of n-3 PUFAs and their metabolites are ascribed to their ability to: (1) incorporate into the cell membrane and displace arachidonic acid as an alternative substrate for phosholipase A2, (2) alter the lipid raft restricting the dimerization and pro-inflammatory signaling of TLR, (3) activate G-protein-coupled receptor mediated signaling that stimulates PPARs and inhibit NF-kB activity, (4) undergo cytochrome P450 epoxygenase mediated metabolism into the corresponding anti-inflammatory oxylipins, (5) inhibit the NLRP3 inflammasome cascade, (6) prevent the activation of the profibrotic TGF-β signaling pathway, and, (7) undergo metabolism into anti-inflammatory and pro-resolving lipid mediators resolvins, protectins, and maresins. Our findings in this research suggested that the PUFA, especialyy n-3 PUFA, could reduce the pro-inflammatory cytokine production in lambs vaccinated against FMD.

Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 induce catabolic metabolism, muscle proteolysis, adipose tissue lipolysis, insulin resistance, increase the synthesis of acute-phase proteins and decrease in protein synthesis in the liver (Powanda, 1980, Bertoni et al., 2015). Also, it was shown that diets with increased concentration of saturated FAs could elevate cholesterol, TG and low-density lipoprotein (LDL) concentrations in blood plasma (Rowe et al., 1999). In ruminants, the transcription factors such as SREBP1, PPARα and PPARγ regulate the expression of the stearoyl-CoA desaturase (SCD) gene (Dervishi et al., 2010). Moreover, dietary PUFAs undergo the greatest changes during their passage from feed to dairy products due to the extensive biohydrogenation taking place in the rumen. Conjugated linoleic acid originates from ruminal biohydrogenation of dietary *cis*-C18:2 and linolenic acid (Griinari et al., 2000). The most represented isomer in milk lipids of ruminants is cis-9, trans-11 CLA (rumenic acid). CLA and rumen protected form of n-6 and n-3 PUFA can decrease cholesterol and TG in plasma (Majewska et al., 2016). Ebrahimi et al. (2014) showed that goats fed diets supplemented with high α-linolenic acid had an up-regulation of the PPARα, PPARγ and down regulation of the SCD gene compared to those fed a diet supplemented with high linoleic acid. In support of this concept, cellular TG accumulation decreased when hepatocytes were exposed to PUFA in rats (Ikeda et al., 1998, Kumamoto and Ide, 1998). Moreover, production of TNF-α inhibits the lipoprotein lipase which results in elevated plasma TG (Feingold et al., 1989). Although our findings showed that feeding PUFA especially n-3 FA resulted in lower TNF-α and IL-1 expression in FMD vaccinated lambs which may improve cholesterol and TG synthesis in the liver, however the decrease in serum cholesterol and TG in PUFA-lambs shows that lower expression of main enzymes which are involved in TG and cholesterol synthesis had occurred in lambs on n-6 and n-3 diets when compared with lambs on saturated FA diet.

A significant decrease in kidney and liver function maybe happen during bacterial and viral infections. Pro-inflammatory cytokines reduce the synthesis of albumins, lipoproteins, retinol-binding protein and paraoxonase (Loor et al., 2013) and increase the synthesis of AAPs such as Hp, SAA, C-reactive protein and Cp in the liver (Powanda, 1980). It was reported that serum albumin and urea was lower, but serum globulin, cholesterol, TG, aspartate transaminase, and alanine transaminase activities were significantly higher in the Brucellosis affected cattle (Nath et al., 2014). Seo et al. (2019) showed that blood urea nitrogen was significantly increased, but albumin was decreased after FMD vaccination of Holstein steers. Also Ghanem and Abdel-Hamid (2010) showed that the total protein and albumin were significantly reduced in cattle suffered from FMD.

It was found that TNF-α and APPs were increased in FMD virus-challenged cattle compared with healthy animals (Bertoni et al., 2008). Albumin is classified as a negative APP, which is dramatically decreased in inflammatory conditions. Albumin might also be decreased in FMD infection because it usually decreases during inflammation (Ghanem and Abdel-Hamid, 2010). Anil and Gurudutt (2011) reported that the serum levels of total protein, albumin and globulin decreased in FMD affected sheep compared with the normal control. Barkakati et al. (2015) showed that FMD caused significant decrease in total protein, albumin and blood urea nitrogen in cattle. Also, Nahed (2010) showed a significant decrease in serum concentration of total protein and albumin in FMD infected cattle. Seo et al. (2019) showed that the vaccination of FMD decreased the hepatic albumin production. However, in this study there were no significant differences between treatments in serum levels of total proteins and albumin. Our findings showed that FA source of diet did not have any significant effect on serum levels of total protein and albumin.

Conclusion

Feeding α-linolenic acid diet to lambs that were vaccinated against FMD resulted in a decrease in serum levels of cholesterol and TG due to higher n-3 PUFA intake, and also suppressed the pro-inflammatory cytokine expression (IL-1 and TNFα).

Declaration of competing interest

None.

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References

Al-Khalaifah, H., 2020. Modulatory effect of dietary polyunsaturated fatty acids on immunity, represented by phagocytic activity. *Frontiers in Veterinary Science* 7, 672-681.

Alagawany, M., Elnesr, S.S., Farag, M.R., El-Sabrout, K., Alqaisi, O., Dawood, M.A., Soomro, H., Abdelnour, S.A., 2020. Nutritional significance and health benefits of omega-3,-6 and-9 fatty acids in animals. *Animal Biotechnology* 1-13.

Anil, G., Gurudutt, J., 2011. Metabolic profile of foot and mouth disease stressed sheep in semi arid region. *Journal of Stress Physiology and Biochemistry* 7, 25-34.

Barkakati, J., Sarma, S., Kalita, D., 2015. Effect of foot and mouth disease on haematological and biochemical profile of cattle. *Indian Journal of Animal Research* 49, 713-716.

Belsham, G.J., 1993. Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; aspects of virus protein synthesis, protein processing and structure. *Progress in Biophysics and Molecular Biology* 60, 241-260.

Benoit, M., Desnues, B., Mege, J.L., 2008. Macrophage polarization in bacterial infections. *The Journal of Immunology* 181, 3733-3739.

Bertoni, G., Minuti, A., Trevisi, E., 2015. Immune system, inflammation and nutrition in dairy cattle. *Animal Production Science* 55, 943-948.

Bertoni, G., Trevisi, E., Han, X., Bionaz, M., 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *Journal of Dairy Science* 91, 3300-3310.

Bhathena, S.J., 2006. Relationship between fatty acids and the endocrine and neuroendocrine system. *Nutritional Neuroscience* 9, 1-10.

Calder, P.C., Yaqoob, P., Thies, F., Wallace, F.A., Miles, E.A., 2002. Fatty acids and lymphocyte functions. *British Journal of Nutrition* 87, 31-48.

Chinsangaram, J., Piccone, M.E., Grubman, M.J., 1999. Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *Journal of Virology* 73, 9891-9898.

Coggins, M., Rosenzweig, A., 2012. The fire within: Cardiac inflammatory signaling in health and disease. *Circulation Research* 110, 116-125.

Colonna, M., Krug, A., Cella, M., 2002. Interferon-producing cells: On the front line in immune responses against pathogens. *Current Opinion in Immunology* 14, 373-379.

Darwesh, A.M., Sosnowski, D.K., Lee, T.Y., Keshavarz-Bahaghighat, H., Seubert, J.M., 2019. Insights into the cardioprotective properties of n-3 pufas against ischemic heart disease via modulation of the innate immune system. *Chemico-Biological Interactions* 308, 20-44.

De Los Santos, T., Diaz-San Segundo, F., Grubman, M.J., 2007. Degradation of nuclear factor kappa b during foot-and-mouth disease virus infection. *Journal of Virology* 81, 12803-12815.

Dervishi E, Serrano C, Joy M, Serrano M, Rodellar C, Calvo J.H. 2010. Effect of the feeding system on the fatty acid composition, expression of the Δ9-desaturase, Peroxisome Proliferator-Activated Receptor Alpha, Gamma, and Sterol Regulatory Element Binding Protein 1 genes in the semitendinous muscle of light lambs of the Rasa Aragonesa breed. *BMC Veterinary Research* 6, 1-11.

Ebrahimi, M., Rajion, M.A., Goh, Y.M., 2014. Effects of oils rich in linoleic and α-linolenic acids on fatty acid profile and gene expression in goat meat. *Nutrients* 6, 3913-3928.

Feingold, K., Soued, M., Staprans, I., Gavin, L., Donahue, M., Huang, B., Moser, A., Gulli, R., Grunfeld, C., 1989. The effect of tnf on lipid metabolism in the diabetic rat: Evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for tnf induced hyperlipidemia. *The Journal of Clinical Investigation* 83, 1116-1121.

Ghanem, M.M., Abdel-Hamid, O.M., 2010. Clinical, haematological and biochemical alterations in heat intolerance (panting) syndrome in egyptian cattle following natural foot-and-mouth disease (fmd). *Tropical Animal Health and Production* 42, 1167-1173.

Griinari, J., Corl, B., Lacy, S., Chouinard, P., Nurmela, K., Bauman, D., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by δ9-desaturase. *The Journal of Nutrition* 130, 2285-2291.

Gulliver, C.E., Friend, M.A., King, B.J., Clayton, E.H., 2012. The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. *Animal Reproduction Science* 131, 9-22.

Hadfield, J.M., 2017. Characterization of the immune response to lipopolysaccharide in early pregnant ewes as a model to study bacterial infection induced embryonic loss. *Graduate Theses, Dissertations, and Problem Reports. 5731.*

Hirayama, D., Iida, T., Nakase, H., 2018. The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. *International Journal of Molecular Sciences* 19, 92-104.

Ikeda, I., Cha, J.-Y., Yanagita, T., Nakatani, N., Oogami, K., Imaizumi, K., Yazawa, K., 1998. Effects of dietary α-linolenic, eicosapentaenoic and docosahexaenoic acids on hepatic lipogenesis and β-oxidation in rats. *Bioscience, Biotechnology, and Biochemistry* 62, 675-680.

Kaveh, M., Eftekhar, N., Boskabady, M.H., 2019. The effect of alpha linolenic acid on tracheal responsiveness, lung inflammation, and immune markers in sensitized rats. *Iranian Journal of Basic Medical Sciences* 22, 255-261.

Kovacsovics-Bankowski, M., Clark, K., Benacerraf, B., Rock, K.L., 1993. Efficient major histocompatibility complex class i presentation of exogenous antigen upon phagocytosis by macrophages. *Proceedings of the National Academy of Sciences* 90, 4942-4946.

Kumamoto, T., Ide, T., 1998. Comparative effects of α- and γ-linolenic acids on rat liver fatty acid oxidation. *Lipids* 33, 647-654.

Lakdawala, N., Grant-Kels, J.M., 2015. Acrodermatitis enteropathica and other nutritional diseases of the folds (intertriginous areas). *Clinics in Dermatology* 33, 414-419.

Lee, I.K., Kye, Y.C., Kim, G., Kim, H.W., Gu, M.J., Umboh, J., Maaruf, K., Kim, S.W., Yun, C.-H., 2016. Stress, nutrition, and intestinal immune responses in pigs—a review. *Asian-Australasian Journal of Animal Sciences* 29, 1075.

Loor, J.J., Bertoni, G., Hosseini, A., Roche, J.R., Trevisi, E., 2013. Functional welfare–using biochemical and molecular technologies to understand better the welfare state of peripartal dairy cattle. *Animal Production Science* 53, 931-953.

Ma, X.-x., Ma, L.-n., Chang, Q.-y., Ma, P., Li, L.-J., Wang, Y.-y., Ma, Z.-r., Cao, X., 2018. Type i interferon induced and antagonized by foot-and-mouth disease virus. *Frontiers in Microbiology* 9, 1-8.

Majewska, M.P., Pająk, J.J., Skomiał, J., Kowalik, B., 2016. The effect of different forms of sunflower products in diets for lambs and storage time on meat quality. *Animal Feed Science and Technology* 222, 227-235.

Matsuyama, W., Mitsuyama, H., Watanabe, M., Oonakahara, K.-i., Higashimoto, I., Osame, M., Arimura, K., 2005. Effects of omega-3 polyunsaturated fatty acids on inflammatory markers in copd. *Chest* 128, 3817-3827.

Mosser, D.M., Edwards, J.P., 2008. Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology* 8, 958-969.

Nahed, S., 2010. Investigation of serum insulin and cortisol concentrations in foot and mouth disease-infected cattle in relation to changes in serum biochemical variables and protein electrophoretic fractionation profile. *Global Veterinaria* 4, 450-455.

Nath, R., Das, S., Sarma, S., Devi, M., 2014. Comparison of blood profiles between healthy and brucella affected cattle. *Veterinary World* 7, 668-670.

Nian, M., Lee, P., Khaper, N., Liu, P., 2004. Inflammatory cytokines and postmyocardial infarction remodeling. *Circulation Research* 94, 1543-1553.

Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A., De Jong, M.C.M., 2007. Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination. *Vaccine* 25, 2673-2679.

Park, J.H., Kim, S.J., Oem, J.K., Lee, K.N., Kim, Y.J., Kye, S.J., Park, J.Y., Joo, Y.S., 2006. Enhanced immune response with foot and mouth disease virus vp1 and interleukin-1 fusion genes. *Journal of Veterinary Science* 7, 257-262.

Powanda, M., 1980. Host metabolic alterations during inflammatory stress as related to nutritional status. *American Journal of Veterinary Research* 41, 1905-1911.

Rowe, A., Macedo, F., Visentainer, J., Souza, N., Matsushita, M., 1999. Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. *Meat Science* 51, 283-288.

Schwager, J., Richard, N., Riegger, C., Salem, N., 2015. Ω-3 pufas and resveratrol differently modulate acute and chronic inflammatory processes. *BioMed Research International* 2015, 1-11.

Sebastian, R., Sravanthi, M., Umapathi, V., Krishnaswamy, N., Priyanka, M., Dechamma, H., Ganesh, K., Basagoudanavar, S.H., Sanyal, A., Reddy, G., 2020. Foot and mouth disease virus undergoes non-progressive replication in mice peritoneal macrophages and induces m1 polarization. *Virus Research* 281, 197906.

Seo, J., Song, M., Jo, N., Kim, W., Jeong, S., Kim, J., Lee, S., Seo, S., 2019. The co-injection of antioxidants with foot-and-mouth disease vaccination altered growth performance and blood parameters of finishing holstein steers. *Asian-Australasian Journal of Animal Sciences* 32, 792-799.

Shi, L., Xu, Y., Mao, C., Wang, Z., Guo, S., Jin, X., Yan, S., Shi, B., 2020. Effects of heat stress on antioxidant status and immune function and expression of related genes in lambs. *International Journal of Biometeorology* 64, 2093-2104.

Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition* 21, 495-505.

Smeed, J.A., Watkins, C.A., Rhind, S.M., Hopkins, J., 2007. Differential cytokine gene expression profiles in the three pathological forms of sheep paratuberculosis. *BMC Veterinary Research* 3, 1-11.

Winnik, S., Lohmann, C., Richter, E.K., Schäfer, N., Song, W.-L., Leiber, F., Mocharla, P., Hofmann, J., Klingenberg, R., Borén, J., 2011. Dietary α-linolenic acid diminishes experimental atherogenesis and restricts t cell-driven inflammation. *European Heart Journal* 32, 2573-2584.

Yaqoob, P., Pala, H., Cortina-Borja, M., Newsholme, E.A., Calder, P.C., 2000. Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *European Journal of Clinical Investigation* 30, 260-274.

Zeng, Y.-Y., Jiang, W.-D., Liu, Y., Wu, P., Zhao, J., Jiang, J., Kuang, S.-Y., Tang, L., Tang, W.-N., Zhang, Y.-A., 2016. Dietary alpha-linolenic acid/linoleic acid ratios modulate intestinal immunity, tight junctions, anti-oxidant status and mrna levels of nf-κb p65, mlck and nrf2 in juvenile grass carp (ctenopharyngodon idella). *Fish and Shellfish Immunology* 51, 351-364.