Journal of Oil Palm Research Vol. 37 (4) December 2025 p. 741-750
DOI: https://doi.org/10.21894/jopr.2025.0014

DIETARY INCLUSION OF CRUDE PALM OIL, RED PALM OIL, REFINED PALM OIL, PALM KERNEL OIL AND SOYBEAN OIL ON MODULATION OF INTESTINAL BARRIER INTEGRITY AND IMMUNITY IN LAYING HENS

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ABSTRACT

Oils derived from plants have unique and distinct properties based on the compounds present. Prominent chemical compositions in different oils include fatty acids, tocopherols, tocotrienols and carotenoids. Different compounds can affect the regulation of gut immune response and barrier function. Feeds containing either crude palm oil (CPO), red palm oil (RPO), refined palm oil (RBD), palm kernel oil (PKO) and soybean oil (SBO) were fed to 150 laying hens. The feeding trial lasted for 16 weeks. Jejunum mucosa was collected at the end of week 16 to measure the proteins associated with barrier function using ELISA and genes related to immunity and barrier integrity by real-time polymerase chain reaction (PCR). In terms of barrier function proteins, various oils did not affect the concentration of intestinal trefoil factor (ITF) and diamine oxidase (DAO). However, the concentration of IgA was higher in CPO and RPO in contrast to SBO and the alkaline phosphatase (ALP) concentration was higher in RPO and PKO. In terms of gene expression, the downregulation of genes related to immunity and barrier function genes was seen in CPO, RBD, RPO and PKO. Similar trends of lower regulation were observed in CPO and RPO than in other oils. Suppression of genes associated with immunity and barrier function would suggest reducing pathogens' load and exposure to antigens in the gut. In conclusion, different sources of oil affect the regulation of intestinal mucosal immunity and barrier function.

Keywords: crude palm oil, gene expression, layer chickens, red palm oil, refined palm oil.

Received: 12 February 2024; **Accepted:** 26 November 2024; **Published online:** 7 March 2025.

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INTRODUCTION

The inclusion of lipids in poultry feed is important because it provides energy at a lower cost due to its high energy density and simultaneously supplies essential fatty acids. Lipids in poultry feed can originate from either animal or plant sources. Oils of plant origin are abundant in unsaturated fatty acids (USFA) and phytonutrients such as squalene, polyphenols, vitamins and carotenoids (Zhou et al., 2020). Palm, soybean, linseed and rapeseed (canola) oils are commonly used plant-based oils in poultry feed. *Figure 1* shows the distinctive properties of fatty acids, vitamin E and carotenoids in crude palm oil (CPO), red palm oil (RPO), refined palm oil (RBD), palm kernel oil (PKO) and soybean oil (SBO). Palm oil extracted from the mesocarp of palm fruit has a balance of saturated to USFA; saturated fatty acids are mainly palmitic acid (C16:0) at around 50%, and USFA are mainly oleic acid (C18:1) at around 40%. PKO is abundant in saturated fatty acids, with about 80% contributed by mainly lauric acid (C12:0). SBO has around 80% USFA, mainly contributed by linoleic acid (C18:2).

The gut immunity and barrier function-related pro and anti-inflammatory proteins are kept in balance in a healthy condition, exposure to foreign antigens such as pathogens will induce the immune response and elevate the levels of the related proteins. Fatty acids and phenolic compounds in the oils may affect the regulation of immune response and gut barrier function at the gut mucosa. The carotenoids and vitamin E are potent antioxidants and have been shown to reduce oxidative stress and enhance intestinal immunity and integrity (Eroglu et al., 2022; Lewis et al., 2019). Carotenoids and vitamin E have been proven to display antioxidant properties that reduce oxidative stress, prevent inflammation by lowering tumour necrosis factor alpha (TNFα) and Interleukin 6 (IL6), enhance intestinal integrity by upregulating tight junctions and mucous secretion and modulate the immune system by upregulating Immunoglobulin A (IgA) and toll-like receptors (TLR) (Eroglu et al., 2022; Lewis et al., 2019). Measurement of proteins related to gut immunity and barrier functions can be accessed by measuring the proteins and genes (messenger RNA) through ELISA and real-time PCR, respectively.

We recently reported a study on the dietary influence of SBO, RPO, CPO, RBD and PKO on egg production, egg quality and β-carotene, tocopherol and retinol deposition (Izuddin et al., 2022), antioxidant enzyme protein and gene expression (Izuddin et al., 2023a), blood lipid profiles and fatty acid profiles and metabolism (Izuddin et al., 2023b) in laying hens. Izuddin et al. (2022) reported the different sources of the aforementioned oils could not alter egg production performance and egg quality. Still, dietary CPO and RPO increased the feed's β-carotene levels, leading to greater concentrations of β-carotene in the egg yolk and liver (Izuddin et al., 2022). Thus, including CPO and RPO provides better protection against oxidation and the reduction of antioxidant enzyme production from antioxidants provided by vitamin E and carotenoids (Izuddin et al., 2023b). The inclusion of oils in the feeds influences fatty acid composition. Dietary supplementation of the oils also affects fatty acid concentration in the egg yolk, serum and liver (Izuddin et al., 2023a).

There have been no reports on the effects of palm oil and palm kernel oil on the gut barrier function and immune response in poultry. Palmitic acid has been linked to the induction of inflammation and the destruction of epithelial integrity (Ghezzal et al., 2020). Even though palm oil contains high levels of palmitic acid, it also has lower levels of oxidation-prone USFA, along with high levels of antioxidants such as carotenoids and vitamin E. PKO contains a great amount of medium-chain fatty acids (MCFA) (Izuddin et al., 2023b), which are linked with reducing inflammation and maintaining the intestinal mucosal barrier function in monogastric animals (Jia et al., 2020). The addition of MCFA into the feed reduced the number of pathogenic microbes and contributed to the performance and gut health of broiler chickens (Gomez-Osorio et al., 2021). Dietary supplements containing polyunsaturated fatty acids (PUFA) have been shown to improve immune response and gut health in chickens (Attia et al., 2020). No research has investigated the potential of palm oil and PKO on gut health related to the immune response in laying hens. Hence, this

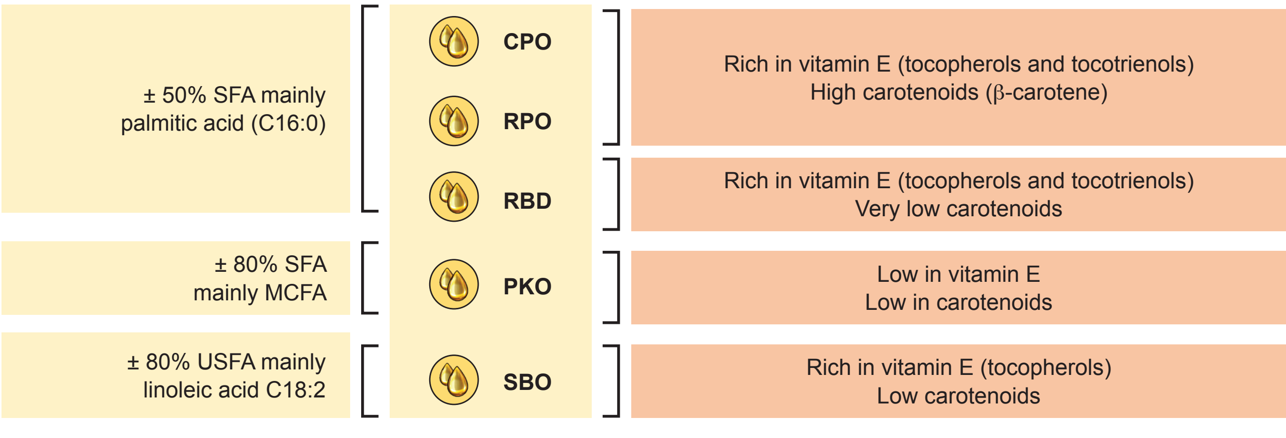


Figure 1. Key properties of the oils.

study aimed to explore the impacts of the inclusion of SBO, RPO, CPO, RBD and PKO on intestinal immunity and intestinal barrier function in laying hens.

MATERIAL AND METHODS

Ethics, Animal Management and Dietary Treatments

The Institutional Animal Care and Use Committee (IACUC) of UPM approved the care and use of commercial Hisex Brown laying hens for this experiment under AUP No.: UPM/IACUC/AUP-R013/2020. The ISO laying hens at the 22nd week of age were divided into five dietary groups. Each treatment comprised six biological replicates, with five hens per biological replicate, totalling 30 hens per treatment. Each hen was placed in an A-type battery cage with dimensions of 40 cm in height, 30 cm in width and 50 cm in depth. The lighting programme consisted of 16 hr of total light (11 hr of natural light with an additional 5 hr of LED lighting) and 8 hr of total darkness in an open house system. Five treatment groups were given isocaloric and isonitrogenous diets [2,790 kcal kg^{-1} metabolisable energy (ME), 17% crude protein] in mash form containing either 3% SBO, RPO, CPO, RBD, or PKO. The feed formulation is as detailed by Izuddin et al. (2022). Each hen was offered 120 g of feed daily in the morning and had unrestricted access to water through nipple drinkers. The feeding trial for laying hens lasted 16 weeks.

Slaughtering and Sample Collection

Six hens per treatment (one hen from each replicate) were randomly picked for slaughter and sample collection. During evisceration, approximately 3 cm of the middle section of the jejunum was excised, drenched with PBS buffer (pH 7.4) to eliminate digesta, and then cut to expose the mucosa. The exposed mucosa was then scrapped with a glass slide, collected in a microcentrifuge tube (Eppendorf) and snap-frozen.

Intestinal Mucosa IgA, ALP, DAO and ITF

The mucosal IgA was detected using a Chicken IgA ELISA kit (Qayee-Bio, Shanghai, China). Mucosal concentrations of alkaline phosphate (ALP), diamine oxidase (DAO) and intestinal trefoil factor (ITF) were determined using the Chicken ALP, DAO and ITF ELISA Kit, respectively, from Sunlong Biotech, Hangzhou, China. The ELISA procedure was conducted according to the provided protocols. The target protein concentration was determined using an equation derived from a standard curve of known concentrations of ALP, DAO and ITF (ng mL^{-1}) plotted against absorbance measured at 450 nm.

Liver and Intestinal Mucosa Immunity and Barrier Function Gene Expression

The ribonucleic acid (RNA) extraction, reverse transcription, qPCR protocol and quantification of gene expression were detailed by Azizi et al. (2023). The NucleoSpin® RNA plus kit (Machery Nagel, Dueren, Germany) was used to extract total RNA. The RNA was transcribed using a cDNA Synthesis Kit (Biotechrabbit, Berlin, Germany). The quantification of genes was performed on a qPCR machine (LightCycler® 480, Roche, Basel, Switzerland) using qPCR Green Master Mix (Biotechrabbit, Berlin, Germany). The information about the genes is summarised in *Table 1*.

Experimental Design and Statistical Analysis

The study was performed based on a completely randomised design. Statistical analysis software was used to analyse the data (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). The normality of the data distribution was confirmed using PROC UNIVARIATE and the Shapiro-Wilk. The mean difference among different oils was determined using a one-way analysis of variance (ANOVA). The significant difference was confirmed at $P<0.05$.

RESULTS AND DISCUSSION

Intestinal Mucosa Barrier Integrity Proteins

There was a significant difference in IgA and ALP levels, but no significant difference in ITF and DAO between the oils (*Figure 2*). Greater concentrations of mucosal IgA antibodies were found in CPO compared to SBO and PKO, but there was no significant difference between RBD and RPO. The SBO had lower IgA concentrations than the CPO and RPO but did not differ from the RBD and PKO. There was no significant difference in IgA levels between RPO, RBD and PKO. The PKO and RPO displayed significantly greater levels of ALP compared to other oils. The CPO, RBD and SBO did not show significant differences from each other.

The impact of the different oils on the regulation of intestinal mucosa immunity and integrity proteins was investigated by measuring protein biomarkers such as ITF, secretory IgA, ALP and DAO proteins using the ELISA approach. The intestinal mucosa is constantly exposed to external environments, including commensal and non-commensal

TABLE 1. THE INFORMATION OF GENES

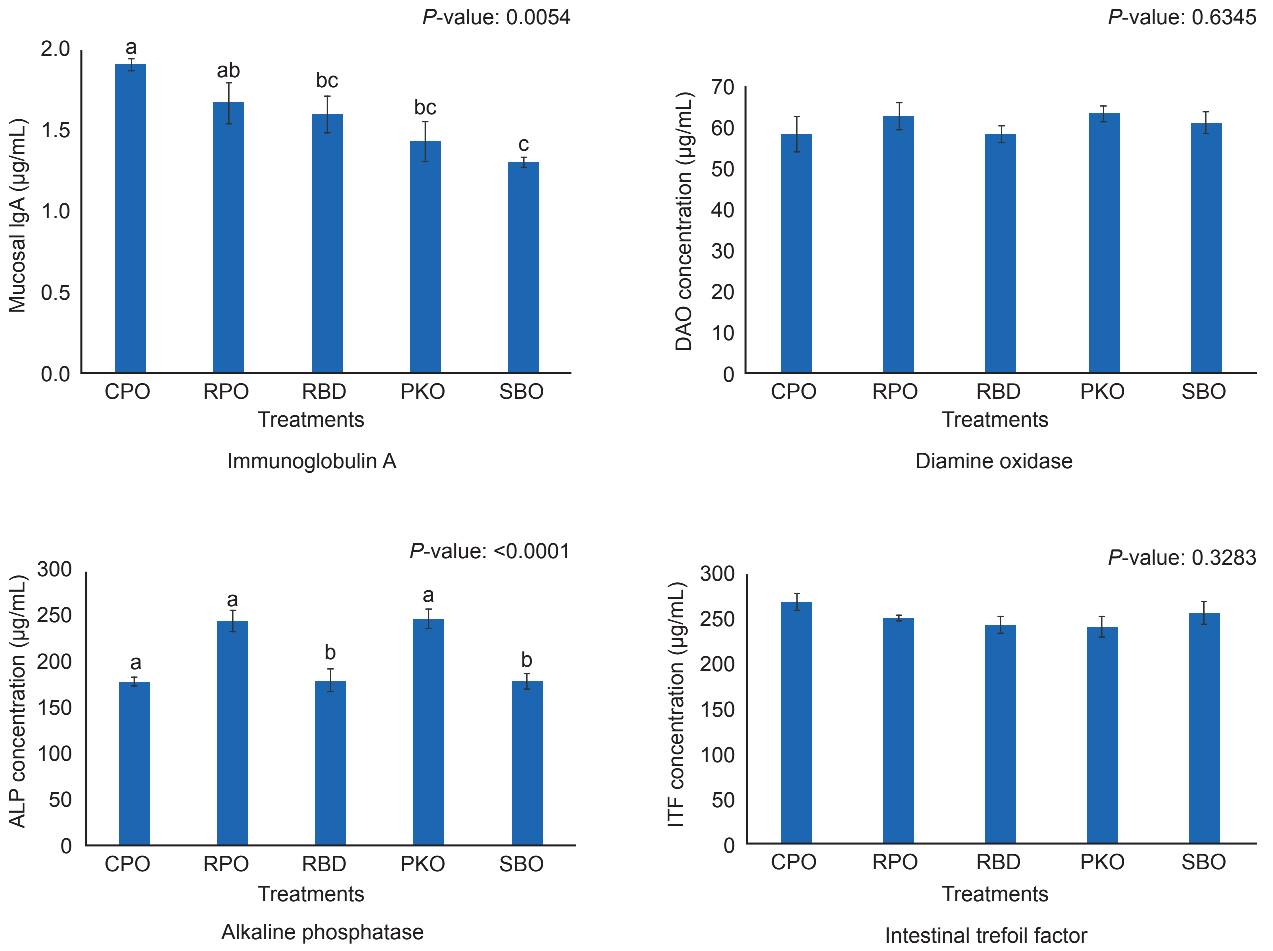
Gene	Forward and reverse primers	Accession No.	Product size (bp)	Functions
GAPDH	CTGGCAAAGTCCAAGTGGTG AGCACCACCCTTCAGATGAG	NM_204305.1	275	Housekeeping gene to normalise gene expression
IL17	TATCAGCAAACGCTCACTGG AGTTCACGCACCTGGAATG	NM_204460.1	110	Pro-inflammatory cytokine to induce immune response
IL10	TAACATCCAACTGCTCAGCTC TGATGACTGGTGCTGGTCTG	NM_001004414.2	135	Anti-inflammatory cytokine
IL6	GCTCGCCGGCTTCGA GGTAGGTCTGAAAGGCGAACAG	NM_204628.1	71	Pro-inflammatory cytokine to induce immune response
IL1β	TGCTTCGTGCTGGAGTCACCC GGCCGGTACAGCGCAATGTT	NM_204524.1	98	Pro-inflammatory cytokine to induce immune response
TLR4	TCTTTCAAGGTGCCACATCCA AGCGACGTTAAGCCATGGAA	NM_001030693.1	132	Recognition of foreign antigens and maintenance of epithelial barrier function
TGFβ1	GCCGACACGCAGTACACCAAG GCAGGCACGGACCACCATATTG	NM_001318456.1	168	Immune homeostasis to suppress harmful proinflammatory responses
SIGA	ACCACGGCTCTGACTGTACC CGATGGTCTCCTTCACATCA	XM_003642969.5	1,446	IgA class of antibody with secretory function that recognises specific antigens
TNFα	GCTGTTCTATGACCGCCCAGTT AACAACCAGCTATGCACCCCA	NM_204267.1	140	Pro-inflammatory cytokine and principle mediator of immune response
MUC2	TTCATGATGCCTGCTCTTGTG CCTGAGCCTTGGTACATTCTTGT	XM_040673077.1	93	Forming mucous barrier to prevent direct contact between epithelial cells and luminal content
CLDN1	CATACTCCTGGGTCTGGTTGGT GACAGCCATCCGCATCTTCT	NM_001013611.2	100	Transmembrane protein of tight junction barrier between cells
OCLD	ACGGCAGCACCTACCTCAA GGGCGAAGAAGCAGATGAG	NM_205128.1	123	Transmembrane protein of tight junction barrier between cells
ZO1	CTTCAGGTGTTTCTCTTCCTCCTC CTGTGGTTTCATGGCTGGATC	XM_040680632.1	131	Transmembrane protein of tight junction barrier between cells

Note: *GAPDH* - glyceraldehyde 3-phosphate dehydrogenase; *CLDN1* - claudin 1; *IL17* - interleukin 17; *IL10* - interleukin 10; *IL6* - interleukin 6; *IL1β* - interleukin 1-beta; *MUC2* - mucin 2; *OCLD* - occluding; *SIGA* - secretory immunoglobulin 1; *TLR4* - toll-like receptor 4; *TNFα* - tumour necrosis factor-alpha; *TGFβ1* - tumour growth factor-beta 1; *ZO1* - zona occludens 1.

microorganisms in intestinal content, mainly from dietary intake and it needs to balance the inflammatory response (Spencer & Belkaid, 2012). Therefore, it is essential to maintain the integrity of mucosal tissue and immune response to defend the tissues from invading non-commensal pathogenic microorganisms and other antigens.

In maintaining good gut condition, trefoil factors are essential components to mucin at the mucosal surfaces, contributing to mucosal integrity, healing and barrier function. Similarly, the apical layer of intestinal mucosa contains DAO, which is secreted into the lamina propria from the intestinal epithelia and binds to the basolateral membrane for the maintenance of mucosal tissue barriers (Wollin et al., 1998). The current study discovered no differences in ITF and DAO concentration of jejunal mucosa between different oils. The differences in the oil properties, such as fatty acid profiles, vitamin E and carotenoid content, did not contribute to ITF and DAO production. It indicates no adverse impacts on feeding oil containing high palmitic acid (C16:0) in palm oil or MCFA in PKO to laying hens.

Another important peptide, secretory IgA is the major class of antibodies in the mucous membrane of the intestinal mucosa. It acts as a passive immunity and first line of defence that protects the intestinal barrier from the adhesion and penetration of pathogenic microorganisms (Pietrzak et al., 2020). In this study, a greater concentration of secretory IgA was seen in CPO and RPO. Higher secretory IgA indicates stronger protection of mucosal surfaces against various antigens. The induction of higher production of secretory IgA in mucosal



Note: SBO - soybean oil; PKO - palm kernel oil; RBD - refined palm oil; RPO - red palm oil; CPO - crude palm oil.
a,b,c with different letters between means on a row denote a significant difference ($P<0.05$).

Figure 2. Immunoglobulin A, diamine oxidase jejunum mucosa, alkaline phosphatase and intestinal trefoil factor concentrations.

can be attributed to the presence of a palmitic acid (C16:0)-rich diet contributed by palm oils (RPO and CPO) compared to MCFA-rich PKO and PUFA-rich SBO. In a mice model, intestinal IgA was greater in mice receiving palm oil than in soybean and coconut oils, but rapeseed oil did not show an increment in intestinal IgA production despite having a comparable amount of oleic acid (C18:1) as palm oil (Kunisawa et al., 2014). The same study suggested that coconut oil contains higher SFA in lauric (C12:0) and myristic (C14:0) acids but did not give effects similar to palm oil containing higher palmitic acid (C16:0). Kunisawa et al. (2014) then enriched the SBO with palmitic acid (C16:0) and they discovered an increase in the faecal IgA concentration in mice fed palmitic acid (C16:0) enriched SBO, suggesting that palmitic acid (C16:0) is a crucial fatty acid in enhancing intestinal IgA production.

Intestinal ALP is essential in promoting intestinal homeostasis and health by regulating the intestinal microbiota through a relationship between mucosa, microbiota and diet. The ALP protects the host by detoxifying harmful microbial ligands (Estaki et al., 2014). Our study suggests that RPO and PKO contribute to the higher production of ALP in the jejunal mucosa. The presence of palmitic acid (C16:0) and carotenoids in RPO and MCFA-rich PKO seems to induce the regulations of intestinal ALP and leads to better intestinal health.

Intestinal Mucosa Immunity Gene Expression

All target genes showed differences in expression between the oils (*Table 2*). The expression of *TNFα* was lower in palm oils (RBD, CPO and RPO) and PKO, with no significant differences observed among them. In palm oils (RBD, CPO and RPO) and PKO, the expression of *TLR4*, *IL1β*, *IL6*, *IL10* and *IL17* was downregulated. The CPO and RPO showed lower fold changes than PKO but showed no difference compared to RBD. In palm oils (RBD, CPO and RPO) and PKO, the expression of *TGFβ1* was downregulated, with the lowest expression in CPO and no difference in expression between RPO, RBD and PKO.

TABLE 2. JEJUNAL MUCOSA IMMUNITY GENE EXPRESSION

Parameters	CPO	RPO	RBD	PKO	SBO	SEM	*P*-value
TNFα	0.280[b]	0.307[b]	0.383[b]	0.490[b]	1.000[a]	0.075	<0.001
TLR4	0.005[c]	0.040[c]	0.182[bc]	0.304[b]	1.000[a]	0.101	<0.001
IL1β	0.010[c]	0.070[c]	0.246[bc]	0.386[b]	1.000[a]	0.100	<0.001
IL6	0.002[c]	0.024[c]	0.170[bc]	0.253[b]	1.000[a]	0.101	<0.001
IL10	0.004[c]	0.027[c]	0.169[bc]	0.253[b]	1.000[a]	0.101	<0.001
IL17	0.003[c]	0.023[c]	0.124[bc]	0.261[b]	1.000[a]	0.101	<0.001
TGFβ1	0.032[c]	0.136[bc]	0.189[b]	0.216[b]	1.000[a]	0.094	<0.001

Note: SBO - soybean oil; PKO - palm kernel oil; RBD - refined palm oil; RPO - red palm oil; CPO - crude palm oil; SEM - standard error of means; *TLR4* - toll-like receptor 4; *IL1β* - interleukin 1-beta; *IL10* - interleukin 10; *TNFα* - tumour necrosis factor-alpha; *IL6* - interleukin 6; *IL17* - interleukin 17; *TGFβ1* - tumour growth factor-beta 1. [a,b,c] with different letters between means on a row denote a significant difference ($P<0.05$).

Cytokines are small-size signalling proteins or peptides secreted by immune cells as a signal to activate and regulate the immune system and inflammatory responses. The current study explored the anti-inflammatory *IL10* and several pro-inflammatory cytokines such as *TNFα*, *IL1β*, *IL6*, *IL17* and pattern recognition receptor TLR4. The further elucidation of the regulation of mucosal immunity and barrier functions was determined by quantifying the expression of those cytokine genes.

Oxidative stress contributes to fibrogenesis by inducing cytokines belongs to pro-inflammatory groups such as *TGFβ1*, *IL6* and *TNFα* (Casas-Grajales & Muriel, 2015). *TNFα*, *IL1β* and *IL6* are important mediators and potent pro-inflammatory cytokines for host defence in response to infection and injury. Stimulating molecules from bacterial cell wall products such as peptidoglycans, lipopolysaccharides and other bacterial toxic products in the intestinal lumen activate macrophages and T lymphocytes to secrete strong proinflammatory cytokines such as *TNFα*, *IL1* and *IL6* (MacDermoit, 1996). *TGFβ1* also contributes to the inflammation response in which the presence of IL6 stimulates the production of T helper 17 (Th17) cells to induce additional inflammation (Sanjabi et al., 2009). The *IL17* involves host defence against pathogens in the mucosa. It is known to contribute to inflammatory response, but *IL17* also contributes to the fine balance between immunity tolerance and inflammatory responses in the mucosa (Guglani & Khader, 2010).

Proinflammatory cytokines upregulation responds to antigens or lipid peroxidation products that induce cell oxidative stress. The lipid sources in animal feed may influence the level of lipid peroxidation products, which are affected by the fatty acid profile, storage conditions and period and processing of the lipid source (Totani et al., 2007). In this study, the upregulation of proinflammatory cytokines in the jejunal mucosa of SBO could be associated with higher content of USFA, particularly PUFA. Higher TBARS and PUFA present in the SBO may enhance lipid peroxidation and induce oxidative stress to the mucosal tissues, thus affecting the regulation of proinflammatory cytokines. Tan et al. (2018) conducted a study on dietary supplementations of oxidised SBO in broiler chickens that upregulated the pro-inflammatory *IL22* of ileal mucosa, indicating the products of lipid peroxidation from oxidised oil-induced inflammation.

TLRs are part of the pattern recognition receptor family crucial to the initial protection because of the presence of receptors that recognise pathogen-associated molecular patterns (PAMPs) and gram-negative bacteria's lipopolysaccharide (Molteni et al., 2016). This study disclosed that the *TLR4* of jejunal mucosa was downregulated in PKO and palm oils (RBD, CPO and RPO). *TLR4* expression can be related to the recognition of lipopolysaccharides of Gram-negative bacteria that activate NFkB, leading to proinflammatory cytokine and chemokine production (Fukui et al., 2001). *TLR4* signalling upon detecting bacterial antigens generates several cytokines such as IL1β, *IL6* and *IL8* (Laurent et al., 2001). The upregulation of *TLR4* in the SBO aligned with activating other proinflammatory cytokines such as *IL17*, *IL6*, *IL1β* and *TNFα*.

Anti-inflammatory cytokines are immunoregulatory molecules essential to manage the response of proinflammatory cytokines and work in concert with specific receptors and cytokine inhibitors to regulate immune response (Gan et al., 2019). *IL10* plays a vital role as the primary regulator in suppressing the immune system's proinflammatory response. The barrier of the intestine plays a critical role in preventing mucosal damage and promoting barrier integrity by the action of *IL10* on Treg cells or macrophages (Wei et al., 2020). This study revealed the *IL10* downregulation in palm oils (RBD, CPO and RPO) and PKO. The downregulation of this anti-inflammatory was in concert with the control of proinflammatory cytokines.

Intestinal Mucosa Barrier Function Gene Expression

The *SIGA*, *CLDN1*, *OCLD* and *ZO1* genes differed between oils, except for the MUC2 gene (*Table 3*). In *SIGA*, the CPO and RBD were downregulated. The CPO had lower regulation than the RPO and PKO. The expression of *CLDN1*, *OCLD* and *ZO1* genes were downregulated in palm oils (RBD, CPO and RPO) and PKO compared to SBO. The CPO and RPO were lower in the regulation of *CLDN1* compared to PKO, with no difference from RBD. Palm oils (RBD, CPO and RPO) and PKO showed no significant differences in *OCLD*. In *ZO1*, with no difference in CPO compared to RBD and RPO and lesser *ZO1* regulation in CPO than in PKO.

The intestinal barrier comprises a mucous layer lining the epithelial cells that grip together by tight junction proteins on a lamina propria. The mucous membrane lining the epithelial cells is composed of mucin produced and secreted by goblet cells, the high composition of secretory IgA antibodies to defend against foreign antigens and the presence of commensal microbiota recognised as mutual by the host immune system. The secretory IgA presence at the mucous membrane aids in eradicating and blocking pathogenic microorganisms and antigens from the intestinal lumen by entrapping in mucous and eliminating them by mucociliary and peristaltic actions (Mantis et al., 2011).

In the current study, no influence of feeding different oils on the expression of the *MUC2* in the jejunal mucosa. The RPO, PKO and SBO had no difference in *SIGA* regulation, but CPO and RBD were downregulated relative to the SBO. However, it was inconsistent with the higher mucosal secretory IgA protein of CPO and RPO, as palmitic acid (C16:0) was suggested to raise the production of IgA in the intestinal mucosa. Kunisawa et al. (2014) fed SBO enriched with palmitic acid (C16:0). They discovered an increase in the faecal IgA concentration in mice, suggesting that palmitic acid (C16:0) is crucial in enhancing intestinal IgA production.

The intercellular barrier of epithelial cells is held together by a continuous network of tight junction proteins that secure the paracellular space and manage the permeability of substances across the epithelial tissues. Tight junction protein comprises claudins, occludins, zona-occludens-1 and actin-myosin cytoskeletal proteins (Gil-Cardoso et al., 2016). The current result discovered upregulation of *CLDN1*, *OCLD* and *ZO1* in SBO in contrast to palm oils (RBD, CPO and RPO) and PKO groups. Yan et al. (2016) found that the emulsion of SBO-based increased the intestinal permeability of lipopolysaccharides in Caco-2 cells by downregulating the P-glycoprotein gene that facilitates the permeability of lipopolysaccharides in the *in vitro* cell model. However, our study found the upregulation of barrier function genes in SBO than in other oils. Therefore, the interpretation could be that the higher regulation of tight junction proteins was in response to the upregulation of the proinflammatory cytokines such as *TNFα*, *IL1β*, *IL6*, *IL17* and *TLR4* to reinforce the intestinal mucosa barrier. The cytokines such as TNF, interferon-gamma, IL-13 and IL-17 affect the epithelial barrier function through several mechanisms, including cytoskeletal modulation, epithelial apoptosis, kinase activation, membrane trafficking and protein synthesis (Turner, 2009).

It was suggested that higher dietary supplementations of omega-6 (linolenic acid; C18:2n-6) fatty acids induce the production of intestinal proinflammatory mediators such as leukotrienes in mice (Ohtsuka et al., 1997) and increase intestinal epithelium barrier permeability and susceptibility in mice (Deol et al., 2021). Ohtsuka et al. (1997) reported that the jejunal mucosa of mice with a diet less in omega-6 or omega-3 (linolenic acid; C18:3n-3) fatty acids reduced the production of leukotrienes; leukotrienes are produced in response to mucosal damage induced by food-

TABLE 3. JEJUNAL MUCOSA BARRIER FUNCTION GENES EXPRESSION

Parameters	CPO	RPO	RBD	PKO	SBO	SEM	*P*-value
SIGA	0.168^{c}	0.763^{ab}	0.318^{bc}	0.789^{ab}	1.000^{a}	0.104	0.024
MUC2	0.474	0.219	0.455	0.421	1.000	0.095	0.078
CLDN1	0.004^{c}	0.029^{c}	0.156^{bc}	0.495^{b}	1.000^{a}	0.111	0.001
OCLD	0.331^{b}	0.528^{b}	0.516^{b}	0.421^{b}	1.000^{a}	0.073	0.008
ZO1	0.018^{c}	0.082^{bc}	0.195^{bc}	0.276^{b}	1.000^{a}	0.098	<0.001

Note: SBO - soybean oil; PKO - palm kernel oil; RBD - refined palm oil; RPO - red palm oil; CPO - crude palm oil; SEM - standard error of means; *SIGA* - secretory immunoglobulin A; *MUC2* - mucin 2; *OCLD* – occluding; *CLDN1* - claudin 1; *ZO1* - zona occludens 1. [a,b,c] with different letters between means on a row denote a significant difference ($P<0.05$).

sensitive enteropathy. The current study disclosed that SBO increased the proinflammatory cytokines in the jejunal mucosa as the SBO contains a higher fraction of omega-6 fatty acid composition than palm oils (RBD, CPO and RPO) and PKO.

Dietary supplementations of MCFA can reduce pH in the gastrointestinal, creating an unsuitable medium for some pathogens in the intestinal tract and potentially substituting the antibiotic growth promoter in poultry (Gomez-Osorio et al., 2021). The benefits of MCFA can be seen in dietary coconut oil, which is an oil with a high content of MCFA. Coconut oil contains up to 91% SFA made up of MCFA, mainly lauric acid (C12:0). Braundmeier-Fleming et al. (2020) reported that substituting SBO with coconut oil increases the growth performance and immune functions in pre-weaning and grower pigs without dietary supplementations of antibiotics. Similarly, PKO contributes to higher MCFA in the diet and might positively influence the regulation of pathogens in the intestinal cavity and provide a lesser pro-inflammatory response to the intestinal mucosa.

CONCLUSION

In conclusion, the dietary inclusion of different oils in laying hens did not affect mucosal ITF and DAO protein, but dietary palm oils increased mucosal IgA and ALP protein compared to SBO. In contrast to proteins, all mucosal immunity (*TNFα*, *TLR4*, *TGFβ1*, *IL1β*, *IL6*, *IL10* and *IL17*) and barrier function (*SIGA*, *MUC2*, *CLDN1*, *OCLD* and *ZO1*) genes were downregulated in palm oil (CPO, RPO and RBD) and PKO, with the lowest regulation observed in CPO and RPO compared to other oils. Lower gene expression associated with proinflammatory cytokines (*TLR4*, *TNFα*, *IL1*, *IL6*, *IL17*) would suggest reduced mucosal exposure to antigens in the gut environment.

ACKNOWLEDGEMENT

The oils used in this study were kindly provided by Palm-Tech Product Sdn. Bhd., for which the authors are grateful. No financial or non-financial competing interests or conflicts of interest are declared by the authors.

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