

Use of immune stimulators, such as essential oils is one solution to improve immunity.



# Managing immune response in broilers



**TEERA TIYASATKULKOVIT\*** reports that feed supplementation with thymol and carvacrol enhanced digestive enzyme activities, and improved immune response of broilers.

Poultry are constantly under threat from disease-causing pathogens. Protection from such diseases is achieved through the immune system, the primary defense mechanism that protects the bird from antigens entering its body.

## Immune system

The bird's immune system is composed of two types: innate and adaptive. These two systems work together to support the bird when a pathogen enters its body.

### *Innate immune system*

The innate immune system is the first line of defense against a variety of pathogens. This system is non-specific nor targeted, but it offers an immediate and fast response once

the body detects a pathogen threat in its body. The innate immune response has several components, including physical and chemical barriers, blood protein and cellular components.

### *Adaptive immune system*

On the other hand, the adaptive immune system or acquired immune system works in a more complicated way. It takes more time to respond to pathogens but has more efficacy than the innate immune system. The adaptive immune system has the ability to recognize the pathogen through immunological memory thus improving the overall reaction time whenever the same pathogen is detected in the bird's system. ▷

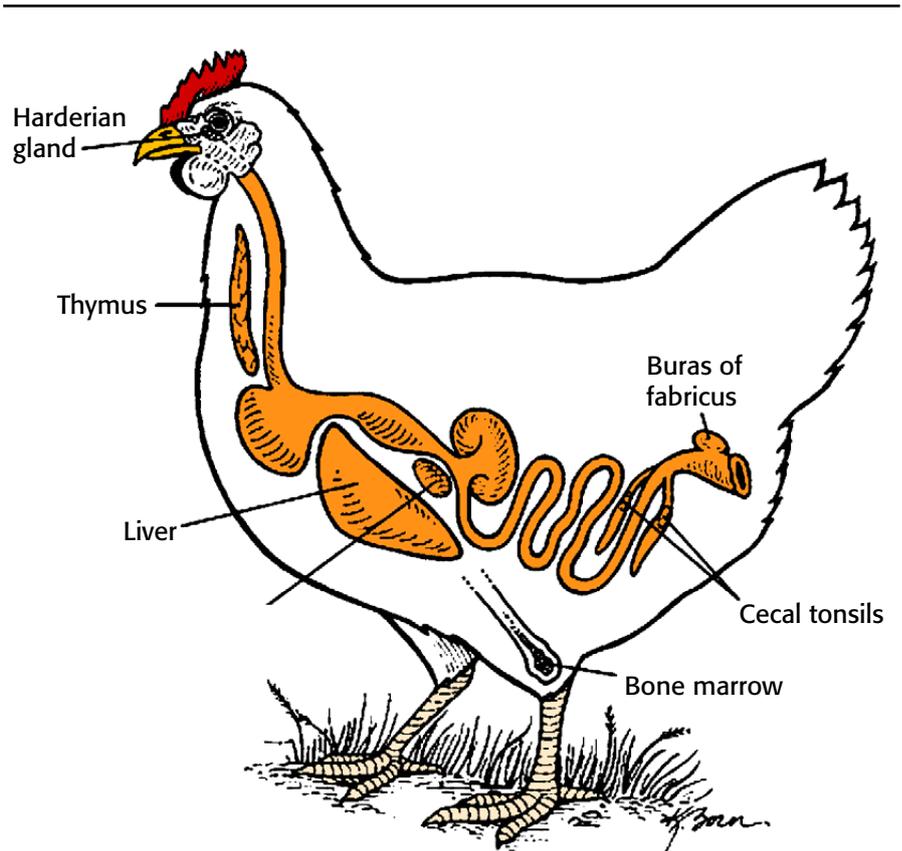
## Immune system organs

In birds, the organs related to immune system can be classified into primary lymphoid organs such as the thymus, bursa of Fabricius and the bone marrow. Bursa of Fabricius is a unique lymphoid organ located in rump of the bird and is responsible for the amplification, differentiation, and maturation for B cell, a type of white blood cell. Even though this organ is very active in young birds, it will disappear when the bird starts to mature. The secondary lymphoid organs are the spleen, bone marrow, Harderian gland, pineal gland, and other lymphoid tissues. Bronchial associated lymphoid tissue (BALT) and gut associated lymphoid tissue (GALT) are respectively found along the bronchus and intestines. Birds also have head-associated lymphoid tissues (HALT) with their major component located behind the bird's eyeballs. These lymphoid tissues are strategically located where foreign matter entering the body from either the skin or a mucosal surface can be trapped. In addition, birds have lymphatic circulatory system and capillaries that works together with the primary and secondary lymphoid organs by providing communication to the blood supply and transportation of the lymph fluid throughout the bird's body.

### Broiler starter immunity

Although chicks begin to develop their own defense mechanisms during embryonic life, they still hatch with an incomplete immune system. They have to use the maternal immunity immunoglobulin that is present in the amniotic fluid and yolk of the egg until their immune system fully develops a few weeks

Figure 1: Hen immune organs.



Julie Gauthier, Rob Ludlow

later. The secondary immune organs such as the spleen, caecal tonsils and Meckel's diverticulum are incomplete at hatch causing the chick to have very low resistance to pathogens at this stage. Maternal nutritional status and early nutrition feeding play a very significant role in the modulation of the nutritional immune system in chicks. Nutrients in the feed supports the growth of both the primary and the secondary lymphoid organs.

In birds, the gastrointestinal track is an important immune organ that represents approximately 70% of the immune system. The intestinal epithelial lining (IEL) has a variety of gut-associated lymphoid tissue

GALT population that produces and stores immune cells to fight against pathogens. The innate population of the GALT supports immunity with the release of cytokines upon activation. Cytokines are small cell-signaling protein secreted by cells that play an important role in the activation and regulation of other cells and tissues during inflammation and immune response.

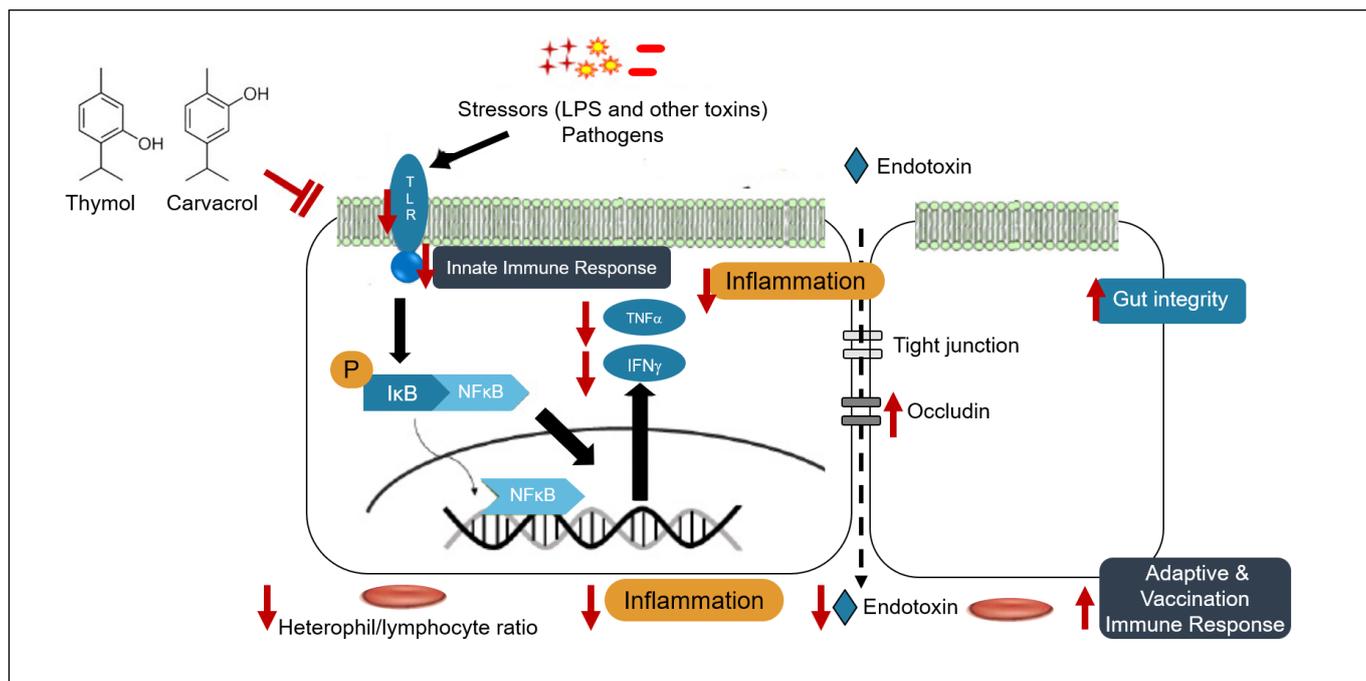
Upon hatching, the immune system and mucosal immune system both require sufficient feed intake for their rapid development. Research supports that a chick's delayed access to feed has an effect on its intestinal development and GALT

Table 1: Summary of essential oil benefit trials in poultry.

Essential Oil Blend	10 Day G:F	24 Day Intestine Enzyme		24 Day Pancreatic Enzyme		10 Day Hypersensitivity	
		Trypsin	Lipase	Trypsin	Lipase	Trypsin	Lipase
Control	840	44.2	24.7	60.4	53.5	0.87	0.81
60	900	46.7	30.2	67.4	54.4	0.95	0.86
100	917	46.8	32.9	73.3	54.5	1.13	0.99
200	958	52.2	34.5	73.5	55.2	1.2	1.13

Modified from H. Hashemipour, 2013 Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens.

**Figure 2: Reaction of body defense system versus stressors and the role of essential oils in inflammatory response.**



H. Wu, K. Jiang 2017, Huang and Lee 2018, M. Chamanara, A. Abdollahi 2019

□ like the bursa of Fabricius, caecal tonsils and Meckel's diverticulum. Therefore, early feeding is not only associated with the immune organ development but also with the functioning of the immune system in chick hatchlings. In addition to protein and energy, it is also critical for early feeding to include nutrients such as minerals and vitamin as well as other feed additives like prebiotic, probiotic, organic acids and essential oils.

### Essential oil support in broiler starter immunity

Research by Hashemipour and team showed that the addition of a thymol and carvacrol essential oil blend (Next Enhance 150 from Novus International, Inc.) in broiler starter feed, improved performance of birds during their first 10 days of life over birds fed a control diet (Table 1).

#### Digestive enzyme activity

Increased inclusion rate of the essential oil demonstrated continuous improvement on bird performance. This research also found that birds fed the essential oil blend had a higher number of enzymes produced from the intestine and pancreases compared to control and had better immunity response on day 10 than those

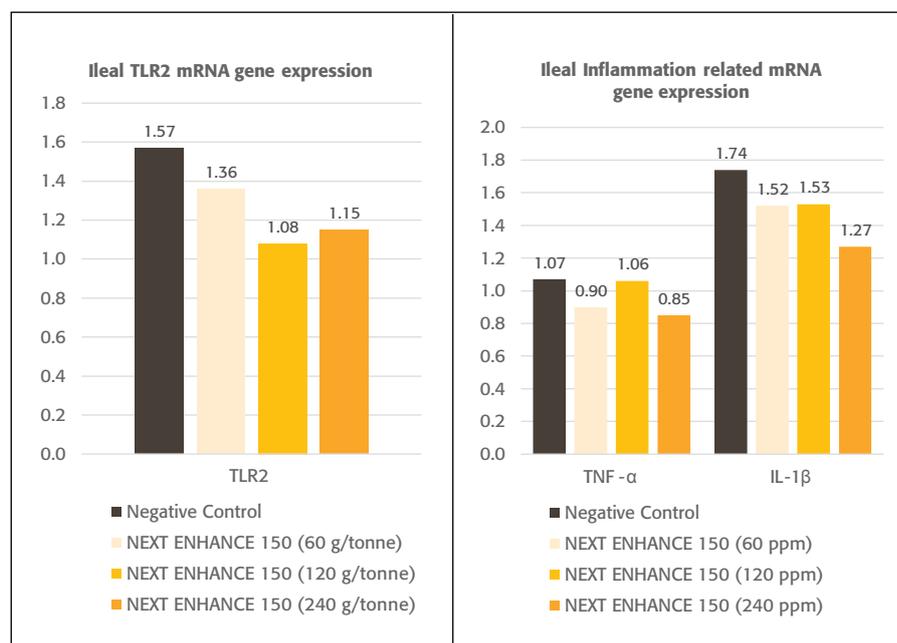
in the control group. This can be referred to as better GALT and immune system respectively.

#### Immune response

When pathogens or other stressors are released into the bird's gut, TLR (Toll-like receptors) proteins located on the cell membrane detect the invasion and stimulate an innate

immune response, which includes an inflammatory response (Figure 2). As the TLRs are stimulated, many signaling pathways are also activated through these adaptor proteins. The NFκβ (nuclear factor kappa β) pathway involves many physical reactions and are extremely important in the modulation of immunity, inflammation, and apoptosis. Under

**Figure 3. Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*.**



E. Du, W. Wang, 2016. Challenged *C. perfringens* in crop from d 14 to d 20 ( $1.0 \times 10^8$  cfu/mL).

normal physical conditions, NFkB bind with the inhibitor of kappa B (IκB), but when the cell is activated by the invasion stressor, NFkB is released from IκB-inhibited form. As a result, NFkB activation induces DNA and generation of TNF alpha (Tumor necrosis factor) and IFN gamma (Interferon gamma), the molecules that enhance inflammation. Because of the inflammation, the tight junction is compromised, and the endotoxin can pass to the bloodstream causing damage to the internal organs which can lead to health issues and mortality.

The thymol and carvacrol essential oil blend is shown to reduce the TLR expression by obstructing them and helping in the NFkB pathway inhibition. These reactions result in a decrease of TNF alpha and IFN gamma concentration that is released from the cell. Thymol and carvacrol exert an anti-inflammatory effect via innate immune system modulation. They also act positively on the tight junction, enhance barrier function, and increase occludin concentration. Occludin is a protein

located at the tight junction and is considered a staple of tight junctions. Due to these actions, the path of endotoxin is prevented, and a lesser concentration is detected in serum. The heterophil/lymphocyte ratio also decreases thereby improving the overall gut integrity. As a result, the blood levels of non-specific immune cells such as heterophils (neutrophils) are also lowered, and lymphocyte and antibody production are promoted to defend against invading pathogens.

The combination of thymol and carvacrol (Next Enhance 150) is also shown to be effective in reducing the effect of pro-inflammatory cytokines and the inflammation. When birds challenged with *Clostridium perfringens* were fed thymol and carvacrol, they showed lower gene expression related to TLRs and inflammation compared with the control group (Figure 3). The result shows that the essential oil blend containing thymol and carvacrol has the potential to protect birds challenged with *Clostridium perfringens* by assisting the bird's defend mechanism and developing

a positive effect on its intestinal integrity.

## Conclusion

With the increased focus by producers to reduce or completely remove antibiotics, the role of the immune system is critical especially during the young animal or starter stage. Essential oil blends, specifically thymol and carvacrol (Next Enhance 150), have been shown to effectively improve the performance and health of birds under certain pathogen and disease challenges, through the modulation of specific immune responses in the bird's body. **Ap**

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