AviPod

An Update for Poultry Professionals

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Stress and Bird Immunity

With intensive production management, there is increasing stress in poultry production. Many diseases, including infectious bursal disease (IBD), salmonellosis, avian influenza, Newcastle disease, coccidiosis, mycoplasmosis, aspergillosis and mixed infections of various diseases cause continual trouble. In addition, poultry farming faces a considerable number of environmental stresses, including heat stress, cold stress, high stocking density, toxic substances, drug effects, inadequate nutritional factors etc., which lead to low immune levels or abnormal development of immune organs, resulting in abnormal immune function as well as several deleterious changes. Owing to imperfections in immune system, immune barrier of body is easily broken and certain pathogenic factors can easily invade body causing variety of diseases, production loss and even mortality.

A stress-free environment is main target in poultry production as stress reduces feed intake and growth, as well as impairs immune response and function, resulting in high disease susceptibility. These effects of stress are correlated with higher corticosteroid levels that modulate several immune pathways such as cytokine-cytokine receptor interaction and Toll-like receptor signaling along with induction of excessive production of reactive oxygen species (ROS) and thus oxidative stress.

Understanding about the impact of stress on immune system of birds and the transcriptome of oxidative stress and immune-related genes are beneficial for implementing effective strategy for enhancing bird immunity.

Stress and its Impact on Immune System

Stress has many different definitions; however, it can be defined as an adaptive response to hazards that threaten bird's homeostasis. Stressors can be external or internal stimuli, and bird's response differs according to severity and duration of stressor and bird's physiological status. Stressors include temperature (e.g., heat and cold), air quality (e.g., ammonia, ozone, hydrogen sulphide, carbon dioxide), light (e.g., ultraviolet light) and photoperiod, parasitic infestation, environmental contaminants (e.g., mycotoxins such as Aflatoxin B1; pesticides), general sanitation including infectious agents, heavy metals, feed composition changes, changing rearing systems, farm locations, transportation, handling and stocking density.

Stress and Bird Immunity

Dear Readers,

Birds, like other animals have a complex immune system with both innate and adaptive components, relying on physical and chemical barriers, as well as specialized cells and molecules to defend against pathogens.

In birds, stress whether from environmental factors, social interactions or disease exposure often leads to reduction in immune function. When birds experience stress, it activates hypothalamic-pituitary-adrenal (HPA) axis, leading to increased production of corticosteroids, which suppress immune system by reducing activity of immune cells like lymphocytes, macrophages, NK cells and impairing production of antibodies. As a result, stressed bird become more susceptible to infections, diseases, and may experience slower growth rates, lower production and even unable to recover from illness. Additionally, stress can alter balance of beneficial gut microbiota, which is crucial for overall immune health.

Exposure to heat stress is one of the potential environmental stressors that can modulate immune response and influence severity of infectious diseases. Heat stress is reported to suppress humoral as well as cell-mediated immunity of chickens. Hence, managing stress is crucial for maintaining a healthy immune system. Providing optimal environmental conditions, proper nutrition, minimizing transportation stress and reducing handling are key strategies. Specific interventions, such as use of immune-enhancing feed additives help mitigate negative effects of stress on immunity.

This issue of AviPod explores impact of stress on immune system of birds and the transcriptome of oxidative stress and immune-related genes, in addition, strategies to enhance immunity through nutrition and environmental management as well as how bioactive derivative of yeast such as beta-glucans and nucleotide supplementations modulate or mitigate stress-associated effects.

We hope that the issue will be worthwhile for you. Kindly scan the below QR code to share your valuable feedback and suggestions or else reach out to us *via* email at aviglo@intaspharma.com.

We value and await your feedbacks.

Regards

Temperature Stressors

Birds can maintain a relatively constant internal temperature only within the thermoneutral zone (approximately at 21-28°C). Exceeding these limits results in heat or cold stress.

- Birds are more sensitive to heat stress than other domestic animals due to the absence of sweat glands and their feather covering. Resistance to heat stress varies among chicken strains, with high growth rate birds being less resistant. Rapid growth and higher metabolic rates make commercial broilers more sensitive to heat stress.
- Heat stress negatively impacts poultry production (feed intake, FCR, body weight), product quality (meat or eggs), reproduction and disease resistance. High temperatures inhibit lymphocyte-mediated responses, particularly lymphocyte proliferation, causing variable changes in humoral immune responses.
- Heat stress lowers the relative weights of immune organs and decreases levels of circulating antibodies (IgM and IgY) and lymphocytes.
- High temperatures (34.5°C for 14 days) damage the thymus cortex and bursal follicles, disturbing maturation of T and B lymphocytes.
- Heat stress causes inflammation of jejunal epithelium and triggers more pathological lesions of *Salmonella enteritidis,* possibly due to altered macrophage function.
- Additionally, heat stress modulates the immune response by inducing heat shock protein (HSP) expression in immune cells such as heterophils, macrophages and lymphocytes.

Exposure to heat stress is a potential environmental stressor that can modulate immune response and influence the severity of infectious diseases.

Stress-associated modulation of HPA axis and immune response

- Heat stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 1), increasing corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), which elevate corticosteroid levels. This leads to increased fatty acid synthesis, fat accumulation, protein catabolism and impaired gastrointestinal functions, such as decreased jejunum villi height and increased permeability to microorganisms. Consequently, birds become more susceptible to diseases like necrotic enteritis (NE) and colibacillosis.

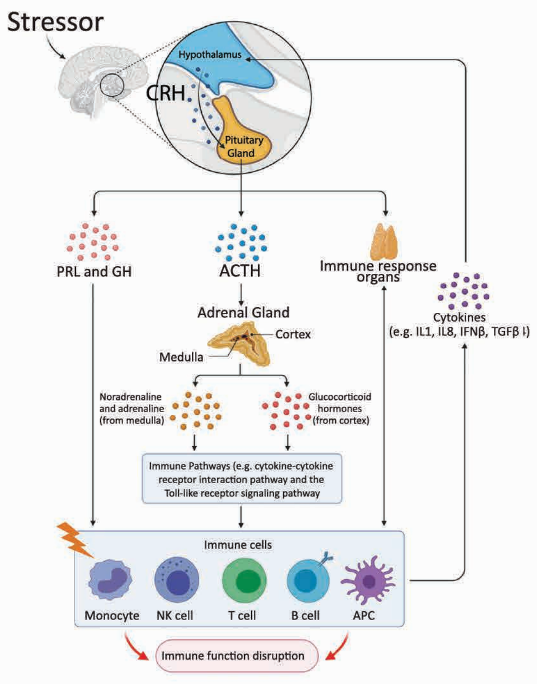

Fig. 1: Stress-associated modulation of HPA axis and immune response by central nervous system
(GH-growth hormone, PRL-prolactin, IL-interleukin, IFN-interferon, TGFβ4-transforming growth factor beta 4)

- Stress triggers a series of hormonal releases. For instance, elevated corticosteroid levels due to stress induce several immune pathways (e.g., cytokine-cytokine receptor interaction and Toll-like receptor signalling pathways). These reactions impair immune function and elevate levels of immune mediators such as interleukins.

Effect of stress hormones on immune system

The impact depends on the duration of exposure to stressors.

- Short exposure to stressors increases corticosterone, raising the H/L (heterophils/lymphocytes) ratio and inducing pro-inflammatory cytokines (IL1β, IL6, IL18, TGFβ 4) and chemokines (CCLi2, CCL5, CCL16, CXCLi1). Dexamethasone induces thymic and bursal lymphocyte apoptosis.
- Long-term exposure (e.g., 7 days) to corticosterone, dexamethasone and ACTH decreases immune organ size and function, shifting the immune system to an immunosuppressed state. This reduces antibody response, phytohemagglutinin (PHA) response, lymphocyte proliferation, IL2 and gamma interferon (INFγ) production and monocyte phagocytic activity.

- In ovo exposure to stress hormones affects future immune responses. Elevated glucocorticoids in hens lead to higher levels of glucocorticoids in egg yolk, resulting in chicks with decreased cutaneous basophil hypersensitivity responses, increased adrenocortical responses to stress and slower growth rates.

Transcriptomic profile changes of immune-related genes during stress

- Stress reduces the growth and function of bursa, thymus and spleen, leading to lower counts of blood leukocytes, lymphocytes, and serum immunoglobulins (IgM, IgY, IgA), impairing immune responses and disease resistance.
- In broilers, acute heat stress modulates thymic transcriptome responses to lipopolysaccharide, affecting thymocyte survival and trafficking.
- Stress increases corticosterone, disrupting physiological functions and suppressing immune responses. In a corticosterone-induced stress model, stress upregulated 199 genes and downregulated 1235 genes in the bursa, affecting key genes like MYD88, VEGFA, TLR4, and IL15, which are crucial for TLR signaling and cytokine-cytokine receptor interaction pathways. This leads to elevated TNFα, IL1β, IL6, and decreased CD3+ and CD4+, causing an inflammatory response. This inflammatory response is associated with upregulation of CATHB1. CATHB1 provides anti-inflammatory action through IL10 induction.
- During Newcastle disease virus (NDV) infection, heat-stressed chickens show downregulation in immune pathways such as IL8 signaling, NF-κB signaling and B cell receptor signaling in lung tissue.
- Stress is linked to high levels of corticosteroids and reactive oxygen species (ROS), suppressing immune system function.

Transcriptomic profile changes during oxidative stress

Exposure to stressors like heat, toxins, heavy metals, transportation, handling and infections induces oxidative damage through excess ROS production, exceeding antioxidant defense capacity and leading to cell death.

- Oxidative stress enhances transcriptional regulatory cascades, including **Nrf2, NF-κB,** and **HSFs** (Fig. 2). Nrf2 modulates stress response genes upon activation by high ROS levels, inducing protective molecules and detoxifying genes (SOD, GPx, CAT, GST, HO-1, NQO1, G6PD). Excessive stress downregulates Nrf2, inhibiting its protective pathway.
- NF-κB modulates pro-inflammatory responses and high levels impair cellular function, causing tissue damage.
- HSPs monitor immune response during stress by modifying cytokine expression, preventing inflammatory damage and inhibiting NF-κB pathways. HSPs have protective actions with anti-apoptosis properties, immune and cellular functions. Environmental factors like temperature and stocking density disrupt levels of various HSPs; e.g., they increase HSP70 expression and circulating corticosterone in broilers.

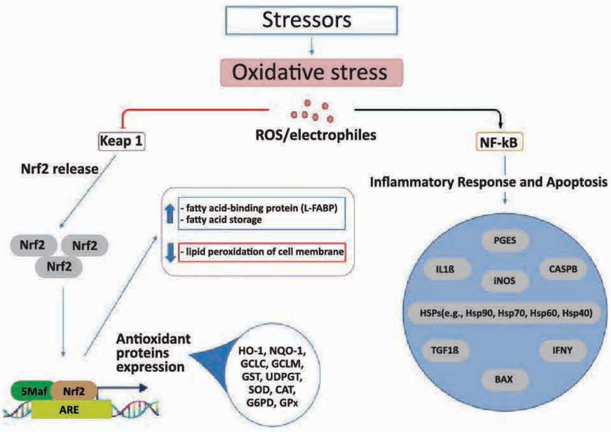

Fig. 2: Induction of oxidative stress by, e.g., cold or heat stressors. Oxidative stress triggers Nrf2/ARE signaling and NF-κB and induces antioxidant protein expression, and inflammatory response and apoptosis.

(Keap 1: Kelch-like ECH-associated protein 1)

- Antioxidant defense dysfunction leads to non-native protein aggregation and cell death. Cells increase chaperone proteins (HSPs and HSFs) to alleviate protein aggregation. HSP and HSF expressions are regulated by stressor duration, severity and type.
- Mycotoxins like Aflatoxin B1 cause oxidative damage, impaired mitochondrial function, increased ROS, and downregulated Nrf2, NQO1 and SOD. Aflatoxin B1 also induces inflammation and upregulates apoptosis genes (caspase-9, caspase-3, Bax) while downregulating Nrf2, HO-1 and protein in broiler liver.
- Heavy metals induce oxidative stress by interfering with Nrf2 expression. Mercury reduces Nrf2 protein levels and suppresses the Nrf2/Keap1 pathway in ovary, liver and kidney tissues of laying hens, increasing Keap1 protein expression.
- Stressors mainly induce oxidative stress, characterized by ROS overproduction, closely interacting with immune response.

Harmful Gases

Air in poultry houses may be polluted with harmful gases like ammonia (NH_3), hydrogen sulphide (H_2S), and carbon dioxide (CO_2). Permissible concentrations are $NH_3 \leq 25$ ppm and $CO_2 \leq 3,000$ ppm.

- NH_3 at 70 ppm reduces spleen weight, lysozyme and globulin concentrations, and limits lymphocyte proliferation. High NH_3 levels decrease mRNA expression of genes related to oxidative stress (e.g., GPx) and inflammation in the thymus (e.g., IL-1B, IL-6). NH_3 toxicity impairs immune response, affecting the bursa. NH_3 at 15 ppm alters tracheal microbiota, increasing upper respiratory infections. NH_3 increases *E. coli* in lung tissue, activating inflammation.
- H_2S is a toxic gas causing oxidative stress, inhibiting energy metabolism and stimulating inflammatory reactions in the spleen, impairing immune function. Similar changes occur in the bursa and thymus at 20 ppm H_2S.

Other Environmental Stressors

Changing rearing systems, stocking density, farm location and other physical environments can induce stress response and thus modulate immune response. In general, unfavourable environments suppress normal antibody and cell-mediated responses. In addition, exposure to contaminations or a pathogenic challenge during development may significantly increase impact of other stressors.

Enhancing Immunity

Several approaches have been considered to boost bird immunity to overcome stress-associated effects. Of these, dietary supplementation of certain nutrients and management modifications are commonly considered. Dietary supplementations improve bird immunity by improving development of lymphoid tissues and triggering beneficial immune modulators and responses.

Immunomodulators

Immunomodulators, natural or synthetic, regulate the immune system, crucial for poultry growth, disease resistance, FCR, body weight gain, and overall health. They enhance disease resistance, vaccine and antibiotic efficacy, and improve digestive tract microflora.

With rising antibiotic resistance and bans on growth stimulants, immunomodulation offers a promising alternative for improving poultry health and production. These solutions should be natural and environmentally safe.

Popular bioactive additives include **prebiotics, probiotics, synbiotics, minerals, vitamins, organic acids** and **herbs.** Immunomodulators or biological response modifiers (BRMs), act on the immune system rather than directly on microbes, providing broad-spectrum benefits. They stabilize the immune system, with studies showing positive effects on production, phenotypic features, and bird health.

Prebiotics

- These are substances that support functioning of probiotics and have positive effect on immune system, and have ability to modulate gut microbiota. Fructooligosaccharides (FOS), galactooligosaccharides (GOS) and mannooligosaccharides (MOS) are mainly used.
- Beneficial effect of prebiotics on immunity has been demonstrated by enhancing humoral and cellular immune responses (e.g. increased lymphocytes and activity of phagocytes), and weight of thymus and bursa.

Probiotics

- These are most often belong to genus Lactobacillus, Bacillus, Lactococcus, Enterococcus, Bifidobacterium and Saccharomyces. Many studies have confirmed health-promoting effect of probiotics on birds in aspect of digestive tract, immune system, intestinal microbiota or by preventing bacterial, viral or protozoan infections.
- Immunomodulatory properties of probiotics include changes in levels of interleukins (IL-2, IL-10) and immunoglobulins (IgY, IgA, IgM), as well as increase in weight of immune organs (spleen, thymus, bursa etc.) and proliferation of T and B lymphocytes.

Synbiotics

It is the combination of a probiotic and a prebiotic.

- They effect health of birds, biochemical blood indicators and modulate functioning of immune system. These substances can modify intestinal microbiota by increasing number of beneficial bacteria and concentration of lactic acid in intestines.
- A significant effect of synbiotics on immune system has been confirmed by increase in serum antibody titers in IB, IBD and ND.

Minerals

- **Selenium:** Se is critical for immune enzyme function as it is a fundamental part of selenoproteins (e.g., GPxs). Such enzymes play essential roles in fighting oxidative damage through free radical scavengers; protection of biomolecules such as lipid, protein and DNA; and maintenance of membrane cellular integrity. Supplementation of Se elicits immune response, increases disease resistance and improves growth and reproductive performance. However, Se deficiency increases risk of exudative diathesis (a disorder arising from Vitamin E deficiency) in chicks, immunodeficiency, pancreatic dystrophy, nutritional muscular dystrophy, myopathy and reduced performance.
- **Copper:** Cu is a part of structure of several metalloenzymes such as cytochrome oxidase and SOD, as well as Cu neutralizes free radicals. Cu is also involved in enhancing immune response to combat infection and repair damaged tissue.

Vitamins

- **Vitamin A:** Plays a critical role in development of immune system function post hatch. Vit A activates innate immune cells (macrophages and dendritic cells). In mucosal sites such as lungs and gut, it increases mucin and secretory IgA antibody production. Also maintains mucosal integrity.
- **Vitamin E and C:** Act as antioxidants that reduce oxidative stress and support immune health.

Organic Acids

Another type of feed additive with health-promoting and immunostimulating effect.

- Formic, acetic, propionic, malic, fumaric and citric acids are used primarily. These substances are an additive that has positive effect on feed hygiene through their preservative impact and inhibition of development of pathogenic microorganisms as a result of lowering pH of the environment.
- Beneficial effects of formic acids on broilers have been demonstrated in production indicators (body weight gain, feed intake, or FCR), gastrointestinal tract (elongation of intestinal villi) and immunological functions (increase in number of lymphocytes in spleen, antibody titers against IBD). Similarly, in laying hens, administration of formic acid at 1 or 1.5 ml/L of water increased quality of eggs and immunity of birds against ND.

Herbs

Herbs have long been used in human medicine and for feeding livestock and poultry. Main advantages of herbs are high content of biologically active substances that effect digestion and absorption of nutrients and enhance immune function. Most of the herbs are having antibacterial, antifungal and antioxidant properties. Important herbs used in poultry are

- **Amla** (*Phyllanthus emblica*): Amla is well known for its nutritional qualities. It is rich in polyphenols, minerals and is regarded as one of the richest source of Vitamin C. Amla acts as strong immunity booster, antibacterial, antifungal, antiviral. Amla fruit powder as feed additive has been reported to possess antistress, adaptogenic, immunogenic and growth stimulating properties resulting in better performance of broiler.
- **Tulsi** *(Ocimum sanctum)*: An aromatic plant with multiple therapeutic functions. Powder or extract of tulsi is rich in essential oils and organic acids, which have positive effects on physiological

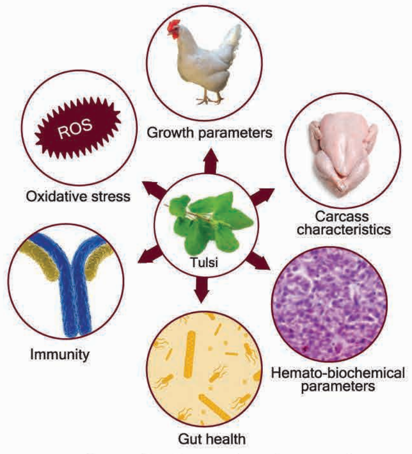

Fig. 3: Effect of tulsi on broiler production and health

functions. It has been extensively used in medicine to treat many health issues. Tulsi has displayed antimicrobial, antiprotozoal, antioxidant, antiparasitic, immunomodulatory, anti-stress and anti-inflammatory properties. It is rich in biologically active compounds, including eugenol, ursolic acid, apigenin and luteolin which have a chief role in enhancing immune response. Eugenol is a major essential oil of tulsi that shows a potent antibacterial effect. Moreover, in poultry, dietary tulsi has been recommended for enhancement of general health conditions with promising results. Supplementation of broilers with tulsi induced positive influences on bird health in terms of improved performance parameters, carcass characteristics, gut health and immune status, as well as alleviation of stress, and modifications of important blood parameters (Fig. 3).

- **Turmeric** *(Curcuma longa)*: Contains curcumin, which has anti-inflammatory benefits.
- **Garlic** *(Allium sativum)*: Has antimicrobial and immune-boosting properties

Advanced Immunostimulants

Bioactive compounds of yeast, such as beta-1,3/1,6-glucans and nucleotides, have been shown to improve intestinal health, feed efficiency, growth, immunity and gut morphology in chickens.

Beta-Glucans

Beta-glucans are naturally occurring polysaccharides known as "**biological response regulators**." They are found in the cell walls of microorganisms and cereals (oats and barley). Yeast derived β-1,3/1,6-glucans have beneficial effects as supplements, originating from the cell wall of *Saccharomyces cerevisiae* (Fig. 4). They are used as growth promoters, antimicrobial agents and immunomodulators in poultry.

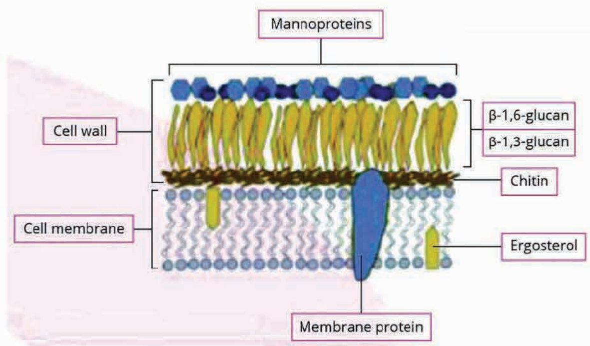

Fig. 4: Cell wall and cell membrane composition in *Saccharomyces cerevisiae*

Mechanism of β-glucans involves receptors like Toll-like receptors (TLR-2,4,6), Dectin-1, CR3, and lactosylceramide, expressed in immunocompetent cells (neutrophils, macrophages, granulocytes, dendritic cells, NK cells, T and B lymphocytes) (Fig. 5). β-glucans activate innate and acquired immunity. Studies show that adding 200–400 mg pure β-glucan/kg diet enhances innate immunity, increases lysozyme and phagocytic activity, and improves antibody response against AIV and NDV vaccines. Moreover, yeast β-glucan supplementation results in larger lymphoid organs.

Yeast β-glucans are noted for their immunomodulatory properties in both innate and adaptive immune systems.

- Supplementation of 0.1-0.2 percent β-1,3/1,6-glucan in broiler diets significantly alters gut microbial composition, increasing beneficial bacteria (Alistipes, Bacteroides, Faecalibacterium).
- β-1,3/1,6-glucan improves immune levels, increases expression of immune factors in intestinal and damaged parts, and alleviates damage post-infection.
- Yeast β-glucans protect against pathogens (*Salmonella* spp., *E. coli, Eimeria* spp.), improve gut health, and increase disease resistance.

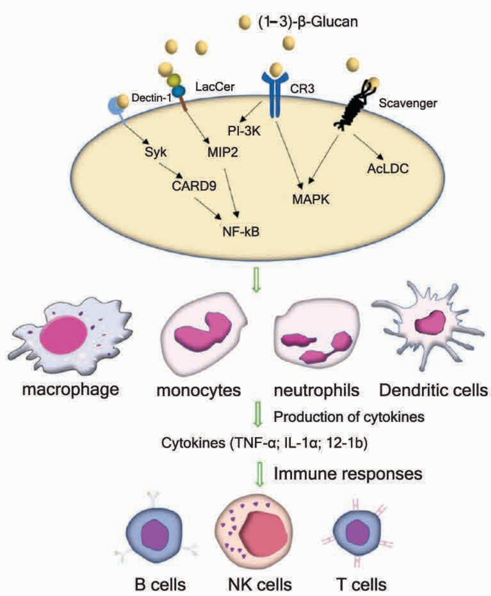

Fig. 5: Immune activation induced by beta-1,3/1,6-glucans

- Supplementation stimulates intestinal antimicrobial peptide gene expression and enhances humoral immunity.
- Yeast β-glucans enhance lysozyme and complement levels, improve phagocytic activity and alter tight junction protein expressions.
- Broilers fed yeast β-1,3/1,6-glucans show enhanced humoral and cell-mediated immune responses.

- Yeast β-glucans scavenge free radicals, increase antioxidant enzyme activity, reduce ROS and lower lipid peroxidation.
- Supplementation with yeast cell wall extract prevents production loss, regulates intestinal immune responses, decreases *C. perfringens* colonization, and reduces gut lesions after infection with coccidial oocysts and *C. perfringens*.

Nucleotides

Nucleotides are low-molecular-weight bioactive agents, either endogenously synthesized or dietary-supplemented, essential for physiological processes and immunological modulation in birds. These are provided as yeast extract or pure material.

- Nucleotides were not initially considered essential nutrients, as organisms can synthesize them *via* de novo synthesis and salvage pathways. However, during stress (rapid growth, disease, reproduction, environmental changes, recovery from injury, limited nutrient intake), their availability may limit the maturation of fast-dividing tissues like intestines and immune cells, necessitating additional nucleotides for cell proliferation.
- Nucleotides are supplemented in poultry diets due to insufficient synthesis during fast development, stress and illness. Yeast nucleotides increase chicken growth (average daily gain, FCR) and benefit broiler immunity and nutrient utilization.
- Nucleotides improve immune response in hot environments, enhance antibody titres against Newcastle disease virus, and increase bursa weight.
- Dietary nucleotides are absorbed in the intestinal tract and move to immunologic organs like the bursa, enhancing bird immunity. They elevate blood total proteins, leukocyte count, antioxidant capacity, and phagocytic index.
- Studies show upregulation of MUC2 and TFF2 gene expressions in birds supplemented with yeast nucleotides, enhancing intestinal mucosal immunity. Feeding yeast nucleotides promotes beneficial intestinal bacteria and enhances intestinal barriers.
- Exogenous dietary nucleotides aid enterocyte proliferation during growth and high protein/ DNA synthesis demand, accelerate intestinal mucosa recovery, and enhance brush-border enzyme activity.
- Dietary nucleotides reduce DNA damage induced by T-2 toxin in immune cells. Nucleotides (adenosine, guanosine, cytidine, uridine) are potential feed additives with significant roles in broiler productivity in tropical and subtropical areas.

Environmental and Management Strategies for Optimum Immune Response

- **Improved biosecurity:** Preventing pathogen entry through sanitation and controlled access.
- **Optimal housing conditions:** Providing adequate ventilation, temperature control and space.
- **Minimizing stress:** Reducing handling stress and ensuring consistent routines.

Conclusion

In intensive farming, birds face stressors like overcrowding, hot weather, toxic gases, vaccination and infections, reducing their immunity and productivity. Heat stress suppresses humoral and cell-mediated immunity. Prolonged high temperatures and elevated corticosteroids cause metabolic imbalances, diseases and lymphoid tissue dysfunction, increasing disease susceptibility. Antibiotic medications are used extensively without understanding their potential side effects on immunity, and their use faces criticism due to antibiotic residues and antimicrobial resistance.

Enhancing bird immunity requires a multifaceted approach, including proper nutrition, probiotics, prebiotics, organic acids, environmental management and natural immunostimulants. Non-drug feed additives like yeast-derived beta-glucans and nucleotides have gained popularity as alternatives to antibiotics. Evidence suggests these additives improve birds' immunological status, intestinal integrity, and growth parameters. Beta-1,3/1,6-glucans from yeast **(IMMUTAS)** are recommended during stress for their high immunomodulation capacity. Nucleotides boost the immune system during stress or illness. Implementing these strategies can significantly improve bird health and productivity.

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