NUCLEOTIDES: THE VERSATILE BIOMOLECULES IN POULTRY NUTRITION

Abstract

Varied physiological processes in animals depend on a family of bioactive nucleotides, which have low molecular weights and intracellular characteristics. They have drawn considerable attention in poultry rearing due to their diverse effects on gut health, production performance, immune system, gut health, meat quality, gene expression, immune response to vaccines etc. The future use of nucleotides in poultry depends on the evaluation of growth efficiency as well as broad and specific effects of these molecules on immunity and health at the cellular, tissue, and systemic levels. This chapter highlights the applications of nucleotides in poultry production and its implications in the sector.

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I. INTRODUCTION

The building blocks of nucleic acids, the nucleotides (NT) are crucial in the encoding, deciphering, storage and transmission of genetic information, division of cells, synthesis of proteins ,energy metabolism and cell signalling besides functioning as constituents of coenzymes, allosteric effectors as well as cellular agonists in animals. They are integral in varied physiological functions, such as lipid metabolism, growth, development and repair of tissues etc. In addition they affect the growth and development of the digestive and immune systems. Nucleotides are intracellular compounds of low-molecular-weight and are made up of three basic components: (i)a heterocyclic nitrogenous base derivative of either a pyrimidine or purine; (ii) a pentose (ribose or deoxyribose), and (iii) phosphate groups. In RNA, the thymine base is substituted by a uracil base. Active *de novo* synthesis of NT occur mainly in the liver and most animals appear to be almost independent of the supply of exogenous NT. Nevertheless, the need for exogenous NT may increase under certain contexts, e.g. tissue injury, dysfunction of liver, under disease or stress, or in fast-growth life stage. There are two major pathways for the genesis of nucleotide or nucleic acids: *de novo* synthesis and the salvage pathway. Majority of NT can be formed from precursors of amino acids(as Glutamine, Formate, Aspartic acid , Glycine etc) within cells by a *de novo* pathway . In addition, they can be synthesised through a salvage pathway. A salvage route that uses intermediates created during the breakdown of nucleotides to create new nucleotides can also be used to recycle nucleotides. In salvage pathway, pyrimidines as well as purines are formed from intermediates by the degradation of nucleotides. During the process of degradation of nucleic acids (*e.g.* RNAs and DNAs) nucleosides and/or bases are recycled for biogenesis of nucleotides. This salvage pathway is simpler and highly energy efficient than the *de novo* synthesis of NT and is modulated by the supply of free bases.It involves the re-synthesis of nucleotides from nucleosides that arise from nucleotide catabolism or dietary sources. It is especially significant in organs as the intestinal tract and bone marrow, where nucleotides synthesis capacity is limited.

It was perceived that the physiological demands of animals could be met by the nucleotides produced via the salvage pathway or *de novo* synthesis (Hoffmann, 2007).Although these pathways act as sources of NT for the maintenance of host cells , they require energy sources and amino acids and are thus metabolically costly processes. Over the past few years, numerous researchers have come to the conclusion that under certain circumstances, such as malnutrition, reproduction, recovery from injury, challenges (environmental or diseases) disturbed endogenous synthesis of nucleotides, stress , oxidative challenge ,limited nutrient intake, diseases, growth induced fast enterocyte turnover , immunosuppression, enhancement of immune responses etc , the two aforementioned mechanisms may not supply sufficient nucleotides to sustain standard biological needs.The intestinal epithelial cells and the immune system cells have little ability to synthesize nucleotides through biosynthetic pathways and are dependent on exogenous supply. In animals, NT are considered semi or conditionally essential nutrients as they are often added to animal diets as pure substances or yeast extracts. NT supplementation in diets may save the energy cost of de novo synthesis.

NT act as a elementary constituent in nucleic acid, protein, carbohydrate and fat metabolism and hence play a critical role in protein synthesis, cell mitosis, lipid metabolism, hematopoiesis, immunity and gut health. NT are essential components of ribonucleic acid and deoxyribonucleic acid and are imperative in many biochemical processes that are vital for metabolism of cells .NT and their derivatives are crucial as activated intermediates in numerous biosynthetic pathways and are integral in transferring chemical energy. When nucleoside triphosphates lose their terminal phosphate groups, they become abundant in energy and act as precursors of mononucleotides.NT and their derivatives as components of three integral coenzymes Nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) and coenzyme A (CoA) are associated with the formation and oxidation of fatty acid and pyruvate in the Citric acid cycle. NT are integral in cellular metabolism as sources of energy , besides being involved in protein synthesis and contributing to cell signalling . Dietary nucleotides play imperative role in the immune system,by influencing activation of lymphocytes.

During stress conditions , the de-novo synthesis of NT is unable to meet the increased demand of rapidly proliferating tissues, making NT essential for the growth and proliferation of tissues like the intestine that has rapid cell turnover.NT are involved in production of both antibody and cytokines cell-mediated immunity and host resistance to bacterial/fungal infections.They are known to modulate protein synthesis by enhancing the availability of precursors of RNA synthesis .NT may initiate alterations in the microflora of intestine that may alter long-chain polyunsaturated fatty acids levels, as bacteria are endowed with essential enzymes for fatty acid elongation and desaturation .In the enterocyte or in the hepatocyte, they may regulate chain elongation and desaturation, causing an improvement of phospholipid synthesis by the liver.

Dietary NT modulate gene expression, especially of the genes associated with NT metabolism. This high degree of regulation suggests that the uptake and metabolism of NT are of appreciable importance to diverse cell types. Exogenous nucleosides are known to mediate protein biosynthesis and signal membrane transduction.Their receptors may modulate the genes which have a direct impact on the levels of cytokines in the intestine. Dietary nucleotides may impact the protein biosynthesis by modulating the intracellular pool of NT. Exogenous supplementation of these compounds may be essential to support growth and maintenance functions.

Dietary nucleotides influence the lymphocyte subset populations in the blood and small intestine besides lymphocyte activation, proliferation and maturation. Additionally, they support the development of the immune system's early immunoglobulin response, which helps fight infection. Several immune system components require dietary NT to function at their best. Additionally, NT seems to promote phagocytosis and boost natural killer cell (NK) activity. Dietary NT contribute to the production of lipoproteins in the liver or enterocytes and may delay the onset of atherosclerosis. In the enterocyte or the liver, NT may regulate the lengthening and desaturation of fatty acid chains. When the body's endogenous supply is deficient, dietary NT may become a necessity for optimum function.

II. ABSORPTION OF NUCLEOTIDES

The nucleoside form of a nucleotide which is devoid of phosphate group in its structure, is the most absorbable form in the lumen of intestine. This form also reduces the metabolic burden on the cells of intestine by reducing the secretion of alkaline phosphatase, which is necessary to convert NT into nucleosides for greater absorption. Enterocytes absorb nucleosides by facilitated diffusion through Sodium dependent channels.Enzymatic hydrolysis before absorption is a pre requisite for dietary nucleic acids as these are absorbed mainly as nucleosides , bases and small molecular NT. NT which possess highly negatively charged phosphate groups inhibit absorption. The entry of NT into enterocytes mostly as nucleosides is carried out primarily by facilitated diffusion and by specific sodium ion-dependent carrier mediated processes. The circulatory system carries nucleosides, endogenous NT, and partly dietary metabolic products into muscle tissues. The reminder of the products are synthesised again for nucleotides and take part in metabolism, while a part of the products are broken down into uric acid or β-alanine and expelled. Pyrimidine nucleosides as Uridine and Cytidine are a class of non-protein nitrogenous compounds that play integral roles in biochemical processes and as constituents of RNA, DNA, coenzymes etc.

III. SOURCES OF NUCLEOTIDES

The prime constituents of poultry diets, as maize, soyabean meal , organ meats, fish, fresh seafood sources as anchovies and sardines, animal protein soluble, fish meal and fish by-products, legumes, yeast extracts, and single cell proteins (SCP), dry whole yeast etc. are sources of nucleotides. Yeast extracts have nucleic acids as key components and are typically present as mono-, di-, or triphosphoric NT. The feed containing high levels of protein have more NT content .Soyabean meal, corn, oil, and oil- seeds have comparatively lower levels of NT. Yeast cultures possess diverse immunostimulants (β-glucans, mannoproteins, chitin and nucleotides), which could generate a more general immune response to regulate the nuclear factor kappa-B signaling pathway and augment immunity in animals.

IV. EFFECT ON GUT HEALTH

The beneficial effects on the gastrointestinal tract observed in animals fed diets supplemented with dietary nucleotides may be attributable to increased nucleotide pools that augment DNA and RNA production. After injury or starvation, the enterocytes' development and differentiation are enhanced by the increased production of RNA and DNA. Therefore, while the endogenous supply of nucleotides may be constrained by mucosal injury, an exogenous supplementation of nucleotides may aid to optimise tissue function in the GI tract and boost up the activity of the brush border enzymes.

V. EFFECT OF NUCLEOTIDES ON PRODUCTION PERFORMANCE

When birds are in stress, reduction in performance and growth ensues in. The increased demand along with relatively slow supply of nucleotides by the bird itself necessitates the exogenous supply of extra nucleotides in poultry diet. Thus, providing readily accessible nucleotides through diet promotes the formation of these quickly growing cells without using energy, increasing productivity in birds. Although chickens can synthesise nucleotides *de novo*, like mammals, it is now believed that this ability, particularly in young chicks, may not be sufficient to meet demand. Due to this, NT have been referred to as conditionally necessary, and they will be most helpful at times of stress as challenges, higher stocking densities, rapid growth phases, and the substitution or removal of antibiotics. NT can also act as alternatives to artificial growth promoters to prevent performance losses in poultry production.

Profitability in broiler production could be increased by the inclusion of dietary nucleotide rich yeast extract at 0.5% level in broiler diet as it improved growth performance in terms of live body weight, gain in weight, feed intake and feed conversion ratio (FCR).During heat stress, the best performance was observed in birds fed nucleotides at the rate of 1g/kg. When birds were exposed to stress factors such as high stocking density, nucleotides had a beneficial effect on body weight gain, FCR etc. Dietary nucleotide supplementation improves the body weight gain and FCR of broilers from 7 to 20 days of age. The supplementation of 1% Glutamine enhanced body weight gain, feed conversion ratios, and intestinal villi height of turkey poults during the age of week one. The incorporation of nucleotides in feed during stress has reduced mortality in birds by approximately 30 %. Improved weight gain were also noticed when NT were fed at the age of 3 weeks. Additionally, NT supplementation improves egg production, fertility and hatchability .

An in ovo investigation on the effect of the supplementation of a combination of nucleosides (25, 50, and 100 mg/egg) in broilers revealed upregulation of homeobox (Cdx) in the chicken jejunum at 3, 7, and 14 days of age. The birds fed with nucleosides performed better and had higher energy metabolizability. Growth performance, plasma protein levels , intestinal surface as well as villous development were enhanced by the supplementation of nucleosides at 50 mg/egg in broiler chicken. Broiler chicks (Vencobb-430) fed with dietary NT rich yeast extract (NuPro®) at 0.5%, 1% and 1.5% had higher live body weight as well as cumulative body weight gain wherein inclusion of dietary nucleotide at 0.5 percent level decreased the average feed intake and FCR and improved the body weight gain and performance index.

VI. EFFECT OF NUCLEOTIDES ON THE IMMUNE SYSYEM

Addition of 1% Glutamine in the diet for 21 days in chicks resulted in higher bile, and serum IgA as well as IgG concentrations. In poultry, dietary supplementation with NT has improved the immune system of broilers by enhancing the production of leukocytes and macrophages.Exogenous NT can augment the production and gene expression of interleukins in the small intestine. Administration of 0.5% adenosine or guanosine or 0.5% combination of uridine + cytidine to broiler diet improved immune functions. The level of 1g/kg appeared to be the best level of the nucleotide for a better immune response. Combined supplementation of Adenosine, Uridine and Cytidine significantly enhanced the level of IgA and immune indices such as the comparative weight of the Bursa of Fabricius. NT are imperative for the optimum function of several constituents of the immune system. NT also facilitate phagocytosis and augment natural killer cell activity. Dietary NT might promote cellular immunity through T-helper cell-mediated effects, which increase proliferation of lymphocytes and antigen processing. A linear increase in immunological parameters as antibody against Newcastle disease virus, IL-2 and INF-γ gene expression, and lysozyme production has been observed with increasing inclusion rate of dietary nucleotides. Yeast cell wall and NT are capable of stimulating the innate immunity of broilers and help birds to furnish faster and stronger responses under pathogenic challenge conditions.

VII. EFFECT OF NUCLEOTIDES ON INTESTINAL HISTOMORPHOLOGY

The advantageous effect of NT on intestinal cell integrity, development, turnover and proliferation of crypt cells has been substantiated.They have effects on intestinal cells during development, maturation, and repair after damage induced by pathogenic challenge or stress.NT lead to increased intestinal villi corresponding to increased surface area of the intestine and higher activities of digestive enzymes, therefore, enhanced nutrient absorption and improved digestibility. NT markedly mitigated the negative effects of *C. perfringens* challenge by augmenting the intestinal barrier function and intestinal histomorphology which positively reflected on the growth performance of challenged birds. Chicks supplemented with 1% glutamine had heavier intestinal relative weights and longer intestinal villi. They enhance intestinal growth and barrier-related gene expression, besides the diverse nature and abundance of the intestinal microbiota. Supplementing NT to mycotoxin-challenged chickens was found to repairDNA damage in immune cells that are greatly sensitive to action of mycotoxin. Adenine, Uracil and Cytidine significantly increases activity of brush border enzymes (Aminopeptidase and Alkaline phosphatase).

VIII. EFFECT OF NUCLEOTIDES ON MEAT QUALITY

Elevated Hue value, lipid content, ash percentage , iron content and redness of meat were observed in NT supplemented poultry whereas the shear force values were lowered. Increase in monounsaturated acids and Linolenic acids were observed with NT supplementation along with decrease in Docosahexanoic acid and Eicosapentanoic acid.The degree of unsaturation was more in the NT group and Atherogenic index was positively impacted by the NT supplementation.The physical as well as nutritional attributes of the *Pectoralis major* muscle of broiler chickens were enhanced by nucleotide supplementation. Superior carcass yields as breast, wing, thigh and drumstick weights have also been reported. The meat of chicken fed nucleotides was redder in color and tenderer in texture making it more enticing to the consumers. The enhanced iron content and lipids with higher unsaturation levels contributes to the improved nutritional characteristics that could result in beneficial health effects in humans .

IX. EFFECT OF NUCLEOTIDES DURING DISEASE CHALLENGE

Alterations in the microbial population and metabolite levels that may have an impact on gut growth and development can result from changes in the nutrients available for fermentation in the cecum.This is particularly crucial when taking into account how Eimeria affects the microbiota, as it can suppress the growth of other bacteria like Lactobacillus and Bifidobacterium by altering the intestinal environment (for example, by increasing mucus flow in the ceca) and predispose the bird to necrotic enteritis by encouraging the growth of mucolytic *Clostridium perfringens*.It may be possible that yeast nucleotides have anti-inflammatory effects and can prevent any further inflammation brought on by *C. perfringens* based on the elevation of IL-4 in the ileum of birds receiving nucleotides. Nucleotide supplementation improves intestinal health in broilers and reduces the pathology brought on by the C. perfringens challenge, characterized by improved intestinal morphology, lesion scores, intestinal barrier function, and increased intestinal IgA production.By enhancing intestinal barrier function and histomorphology, nucleotides significantly mitigated the unfavourable effects of the *C. perfringens* challenge, which in turn improved growth performance in the challenged birds. At 0.1%, nucleotides supplementation significantly reduced lesion scores in intestine and *C. perfringens* levels while also significantly enhanced intestinal barriers and histomorphology, which in turn enhanced growth performance metrics. When Eimeria damaged performance and gut function and altered gut microbiota, yeast nucleotides were discovered to increase performance on their own, attenuate harmful effects of Eimeria on indices of gut function, and modified cecal microbiome. NT supplementation has been suggested as a way to lessen the side effects of coccidial vaccinations since they are conditionally required and their need rises during intestinal regeneration. Nucleotides are critical to expedite intestinal growth or healing during phases of rapid growth in broilers or when Eimeria damages the gut.Nucleotides improve nutrient absorption, which in turn has a positive cascade effect on the cecal microbiota, which may help to lessen the negative consequences of Eimeria.

X. EFFECT OF NUCLEOTIDES ON IMMUNE RESPONSE TO VACCINES

Intestinal development, intestinal barrier-related gene expression, intestinal microbiota, and antibody titre to infectious bronchitis virus (IBV) of specific pathogen-free hens were examined in relation to the effects of dietary yeast NT supplementation and was concluded that NT aided in furnishing a quicker and more potent immune response to the IBV vaccine. Additionally, supplementing with dietary yeast NT can strengthen intestinal development, the expression of genes in relation to barriers and the diversity and richness of the intestinal microbiota.

XI. EFFECT OF NUCLEOTIDES ON GENE EXPRESSION

Birds supplemented with nucleotides exhibited increased expression of toll-like receptors and interleukins IL-4 and IL-18 in the ileum compared to the control. When birds were fed with YCW,expression of macrophage mannose receptor and IL-18 was upregulated. The increased expression of cytokines and innate immunity-related receptors in broilers fed with nucleotides and YCW suggests that these products have immunomodulatory properties during pathogen challenge. Following microbial infections, cell-mediated immunity is induced by IL-18 in conjunction with IL-12, which is regarded as a key mediator of the Th1 response.The generation of the cytokines required for the activation of the innate immune system is mediated by the activation of PRRs by PAMPs.Therefore, higher expression of TLRs and MMR seen in the ileum of these birds may be related to upregulation of IL-18 in the ileum of birds fed nucleotides and YCW. The balance of T cell differentiation to Th-2 cells, which are essential for producing anti-inflammatory cytokines and suppressing pro-inflammatory cytokines, may be favoured by dietary nucleotides.IFN and IL-12 are two pro-inflammatory cytokines that are inhibited by interleukin-4, an anti-inflammatory mediator. As a result, the increased levels of IL-4 in the ileum of birds that received nucleotides may suggest that yeast nucleotides have anti-inflammatory effects and may even prevent future inflammation brought on by *C. perfringens*. IL-2, INF- gene expression, lysozyme production, and antibody against NDV have been shown to be positively correlated with the inclusion rate of dietary nucleotides, partially mitigating immune compromise in broiler chickens. Improved expression of the nutrient transporter cationic amino acid transporter 1 was observed on supplementation of nucleotides.

The ileum's upregulated TLRs are linked to a stronger gastroepithelial barrier against pathogenic microorganisms. The effect of feed on the growth and proliferation of tissues with quick cell turnover, such lymphocytes and enterocytes, may be the cause of the upregulation of toll-like receptors brought on by the diet rich in NT.

XII. CONCLUSION

The integration of nucleotides into contemporary poultry production will be framed by the challenge of figuring out how to use nucleotides as novel bioactive compounds. To fully understand their potential, research that focuses on identification and characterization of novel bioactive compounds will be necessary. This fundamental knowledge will then need to be validated using a variety of in vivo experiment models that evaluate growth efficiency as well as broad and specific effects on immunity and health at the cellular, tissue, and systemic levels. Once this level of comprehensive research is accomplished, the scientific community can precisely appreciate the beneficial potential of nucleotides in sustainable poultry production.