**NUCLEOTIDES: THE VERSATILE BIOMOLECULES IN POULTRY NUTRITION**

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The building blocks of nucleic acids, or nucleotides (NT), are crucial in the encoding, deciphering, storage and transmission of genetic information, cell division, protein synthesis ,energy metabolism and cell signalling besides serving as components of coenzymes, allosteric effectors and cellular agonists in terrestrial animals. They are involved in many physiological processes, such as lipid metabolism, tissue growth, development and repair. In addition they affect the growth and development of the digestive and immune systems. Nucleotides are low-molecular-weight intracellular compounds made up of three components: (i) a nitrogenous heterocyclic base derivative of either a pyrimidine or purine; (ii) a pentose (deoxyribose or ribose), and (iii) one or more phosphate groups. In RNA, the thymine base is replaced by a uracil base. Because of active *de novo* synthesis of NT mainly in liver, most animals appear to be almost independent on exogenous NT. However, the requirements for exogenous NT may increase under certain conditions, e.g. tissue injury, dysfunction of liver, under disease or stress, or in fast-growth life stage. There are two major pathways for a formation of nucleotide or nucleic acids: *de novo* synthesis and the salvage pathway. Majority of NT can be synthesized from precursors of amino acids( as Glutamine, Formate, Glycine and Aspartic acid ) within cells by a *de novo* pathway . Also, they can be formed 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 and purines are formed from intermediates from the degradation of nucleotides. During degradation of nucleic acids (*e.g.* RNAs and DNAs) nucleosides and/or bases are re-utilised for biosynthesis of nucleotides. This salvage pathway is simpler and more energy efficient than the *de novo* synthesis of NT and is modulated by the availability of free bases.It involves the re-synthesis of nucleotides from nucleosides that originate from nucleotide catabolism or dietary sources. It is especially significant in organs such 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 *de novo* synthesis or a salvage pathway (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 reproduction, malnutrition, injury recovery, environmental or disease challenge , disturbed endogenous synthesis of nucleotides, stress , oxidative challenge ,limited nutrient intake, diseases, fast enterocyte turnover induced by rapid growth, reproduction, immunosuppression and enhancement of the immune responses the two aforementioned mechanisms may not provide enough nucleotides to maintain normal biological needs. Some cells such as the epithelial cells of the intestine and the immune system cells have little ability to synthesize nucleotides through biosynthetic pathways and depend on exogenous sources. NT are considered “semi” or “conditionally” essential nutrients in animals as they are often added to animal diets in the form of pure substances or yeast extracts. Dietary nucleotide supplementation may save the energetic cost of de novo synthesis.

NT act as a fundamental component in carbohydrate, protein, fat and nucleic acid 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 participate in many biochemical processes that are vital for cellular metabolism .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 act as energy-rich precursors of mononucleotide units.NT and their derivatives as components of three integral coenzymes Nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) and coenzyme A (CoA) and are involved in the formation and oxidation of fatty acid and oxidation of pyruvate in the Citric acid cycle. NT are integral in cellular metabolism as sources of energy (ATP, GTP) besides being involved in synthesis of protein and contributing to cell signalling .All of these nucleotide derivatives are also important components in carbohydrate, protein, fat and nucleic acid metabolism. Dietary nucleotides have been reported to play a crucial role in the immune system, such as lymphocyte activation.

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

Dietary NT influence biosynthetic processes and modulate gene expression, especially of the genes associated with nucleotide metabolism. This high degree of regulation suggests that the uptake and metabolism of nucleotides are of great importance to diverse cell types. NT improve intestinal morphology and function, immune response, composition of intestinal microbiota, liver function and morphology, as well as growth performance. 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 regulating the intracellular nucleotide pool. Exogenous supplementation of these compounds may be essential to support growth and maintenance functions.

Dietary nucleotides affect 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 nucleotides may become a necessity for optimal function.

ABSORPTION OF NUCLEOTIDES

The nucleoside form of a nucleotide, which has no phosphate group in its structure, is the most absorbable form in the intestinal lumen. This form also reduces the metabolic burden on the intestinal cells by reducing the secretion of the enzyme alkaline phosphatase, which is necessary to convert nucleotides into nucleosides for greater absorption. Nucleosides are absorbed into the enterocyte by facilitated diﬀusion through Na-dependent channels .Enzymatic hydrolysis before absorption is a pre requisite for dietary nucleic acids as these are absorbed mainly in the form of nucleosides , bases and small molecular nucleotides. Nucleotides with highly negatively charged phosphate groups hinder absorption. The entry of nucleotides into enterocytes mostly in the form of nucleosides is carried out primarily by facilitated diffusion and by specific sodium ion-dependent carrier mediated processes. The circulatory system carries nucleosides, endogenous nucleotides, and partly dietary metabolic products into muscle tissue. The reminder of the products are synthesised again for nucleotides and take part in metabolism, while a portion 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 which play key roles in the biochemical processes and as constituents of DNA,RNA,coenzymes etc.

SOURCES OF NUCLEOTIDES

The major ingredients for poultry diets, as corn, soybean 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. Nucleic acids are key components of yeast extracts and typically are present as mono-, di-, or triphosphoric nucleotides. The feed containing high levels of protein have high nucleotide content .They exist at low levels in soybean meal, corn, oil, and oil- seeds. Yeast cultures contain different immunostimulants (β-glucans, mannoproteins, chitin and nucleotides), which could produce a more general immune response to regulate the nuclear factor kappa-B signaling pathway and improve immune status in animals.

EFFECT ON GUT HEALTH

The positive effect on the gastrointestinal tract in animals fed the diet supplemented with dietary nucleotides may be due to enhanced synthesis of DNA and RNA by increasing the nucleotide pools. This elevated synthesis of RNA and DNA synthesis enriches growth and differentiation of the enterocytes after injuries or malnutrition. Therefore, an exogenous supplementation of nucleotides may help optimize tissue function in the GI tract and stimulate activity of the brush border enzymes when the endogenous supply of nucleotides might be limited by mucosal injury.

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 animals, may not be sufficient to meet demand. Due to this, nucleotides have been referred to as conditionally necessary, and they will be most helpful at times of stress as health challenges, high stocking densities, rapid growth phases, and the replacement 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 addition of 1% Glutamine increased body weight gain, feed conversion ratios, and intestinal villous height of turkey poults during the first week of age. The incorporation of nucleotides in feed during stress has reduced mortality in birds by approximately 30 %. Improvements in weight gain were especially noticed when nucleotides were fed the first 3 weeks of life, indicating optimal early development of the birds will support performance later on. Additionally, nucleotide supplementation improves egg production, fertility and hatchability .

An *in ovo* study on the effect of the administration of a combination of nucleosides (25, 50, and 100 mg/egg) in broilers revealed upregulation of homeobox (Cdx) in the jejunum at 3, 7, and 14 days of age. The groups fed with nucleosides performed better and had higher energy metabolizability. Growth performance, plasma protein, intestinal surface and villi development were enhanced by the administration of nucleosides at 50 mg/egg in broiler chicken. Broiler chicks (Vencobb-430) fed with dietary nucleotide rich yeast extract (NuPro®) at 0.5%, 1% and 1.5% had higher live body weight and cumulative 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.

EFFECT OF NUCLEOTIDES ON THE IMMUNE SYSYEM

Addition of 1% Glutamine in the diet for 21 days in chicks resulted in higher bile, intestinal, and serum IgA and serum IgG concentrations. In poultry, dietary supplementation with NT has improved the immune system of broilers by enhancing the production of leukocytes and macrophages. ..Exogenous nucleotides can augment the production and gene expression of interleukins in the small intestine. Supplementation 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. Combination of Adenosine, Uridine and Cytidine signiﬁcantly enhanced the level of IgA and immune indices such as the relative weight of the Bursa of Fabricius. NT are important for the optimal function of several constituents of the immune system. NT also appear to facilitate phagocytosis and increase natural killer cell (NK) activity. Dietary nucleotides might promote cellular immune responses through T-helper cell-mediated effects, which increase antigen processing or lymphocyte proliferation.A linear increase in immunological parameters (including antibody against NDV, IL-2 and INF-γ gene expression, and lysozyme production) has been observed with increasing inclusion rate of dietary nucleotides. YCW and nucleotides are able to stimulate the innate immunity of broilers and help birds to furnish faster and stronger immune responses under pathogen challenge conditions.

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 enterocytes during intestinal development, maturation, and repair after damage caused by stress or pathogens .NT lead to increase intestinal villi correspond to increase surface area of the intestine and higher activities of digestive enzymes, therefore, increased nutrient absorption and improved digestibility 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 fed diets with 1% glutamine supplementation had heavier intestinal relative weights and longer intestinal villi. They enhance intestinal growth and barrier-related gene expression, besides the diversity and richness of the intestinal microbiota.Supplementing nucleotides to mycotoxin-challenged chickens was found to repair DNA damage in immune cells, that are greatly sensitive to mycotoxin action. Adenine, Uracil and Cytidine signiﬁcantly increases activity of brush border enzymes (Alkaline phosphatase and Aminopeptidase).

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 Eicosapentanoic and Docosahexanoic acid.The degree of unsaturation was higher in the nucleotides group and Atherogenic index was positively impacted by the nucleotide supplementation.The physical and 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 .

EFFECT OF NUCLEOTIDES DURING DISEASE CHALLENGE

Alteration in nutrients available for fermentation in the cecum can lead to alterations in the microbial population and metabolites that may impact gut growth and development .This is especially important when the impact of *Eimeria* on the microbiota is considered where growth of other bacteria such as *Lactobacillus* and *Bifidobacterium* is suppressed through change in the intestinal environment (e.g., increased flow of mucus in the ceca), predisposing the bird to necrotic enteritis through proliferation of mucolytic *Clostridium perfringens .*The upregulation of IL-4 in the ileum of birds receiving nucleotides may indicate that yeast nucleotides could have anti-inﬂammatory properties and perhaps inhibit any further inﬂammation caused by *C. perfringens.* Nucleotide supplementation alleviates the pathology associated with the *C. perfringens* challenge in broilers, 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. Nucleotide 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.

EFFECT OF NUCLEOTIDES ON IMMUNE RESPONSE TO VACCINES

Intestinal development, intestinal barrier-related gene expression, intestinal microbiota, and infectious bronchitis virus (IBV) antibody titer of specified pathogen-free (SPF) hens were examined in relation to the effects of dietary yeast nucleotides 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 nucleotides can strengthen intestinal development, the expression of genes in relation to barriers and the diversity and richness of the intestinal microbiota.

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.

IL-18 is considered an important mediator of Th1 response and together with IL-12 induces cell-mediated immunity following microbial infections.Activation of PRRs by PAMPs mediates the production of cytokines necessary for innate immune system activation .Therefore, upregulation of IL-18 in ileum of birds fed with nucleotides and YCW might be associated with increased expression of TLRs and MMR observed in the ileum of these birds. Dietary nucleotides may favour the balance of T cell differentiation to Th-2 cells, which are imperative in production of anti-inﬂammatory cytokines and suppression of pro-inﬂammatory cytokines.Interleukin-4 is an anti-inﬂammatory mediator which inhibits the production of pro-inﬂammatory cytokines such as IFNγ and IL-12 .Therefore, the upregulation of IL-4 in the ileum of birds receiving nucleotides may indicate that yeast nucleotides could have anti-inﬂammatory properties and perhaps inhibit any further inﬂammation caused 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. Upregulation of TLRs in the ileum is associated with an enhanced gastroepithelial barrier against pathogenic bacteria. The upregulation of toll-like receptors by the nucleotide-containing diet might be related to the influence of these products in the development and proliferation of tissues with rapid cell turnover such as enterocytes and lymphocytes .

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 identifying and characterizing 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.

REFERENCES

Will be given on request