The role of dietary nucleotides in single-stomached animals

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Abstract
The transition from liquid to solid feed during weaning results in morphological, histological and microbial changes in the young animal’s intestinal tract and often is associated with diarrhoea. The ban of in-feed antibiotics in pig production in the European Union has led to increasing interest in alternatives to overcome weaning-associated problems. Among others, nucleotides may have the potential to alleviate health impairments due to weaning. Nucleotides are natural components of the non-protein fraction of milk and have important effects on the maintenance of health in young animals. Nucleotides and their related metabolic products play key roles in many biological processes and become essential dietary components when endogenous supply is insufficient for normal function. The present review summarises nucleotide composition of milk from different species, the biology of nucleotides and possible effects of dietary nucleotides on intestinal morphology and function, intestinal microbiota, immune function, nutrient metabolism, hepatic morphology and function as well as growth performance. Special attention is given to data available for pigs, and suggestions are made for inclusion of nucleotides in the diet to benefit piglets’ health and reduce the consequences accompanying early weaning.

Key words: Gastrointestinal tract: Single-stomached animals: Nucleotides: Pigs

Introduction
Until 2006, in-feed antibiotics were used to overcome weaning-associated problems in pig production. Nowadays, an interest in antibiotic-free animal production has been established worldwide due to the risk of antibiotic resistance in humans(1). As weaning is accompanied by morphological, histological and microbial changes in the gastrointestinal tract of young mammals(2,3), nutritional strategies are being developed to aid immature animals to overcome weaning-associated problems and withstand infectious diseases. Among others, dietary nucleotides are one group of bioactive agents which may have the potential to diminish challenges related to weaning(4). Nucleotides are naturally present in all foods of animal and vegetable origin(5) but in lower concentrations than in mammalian milk(6). Soluble nucleotides have been determined in milk from various mammals, contributing to as much as 20 % of its non-protein fraction(7). The nucleotide pattern in milk is species-specific, and concentrations vary with stage of lactation. Nucleotides and their metabolic products play key roles in many biological processes, for example, as monomeric units of nucleic acids, in transferring chemical energy, in biosynthetic pathways, as biological regulators and as coenzyme components(8). Although nucleotides are synthesised endogenously, there is also evidence that dietary supplementation with nucleotides may exert beneficial effects both in humans and animals (for reviews, see Carver & Walker(9) and Taylor-Pickard(10)). In animals, including pigs and rodents, positive effects on small-intestinal growth and development(11), on intestinal hyperaemia(12), on stimulation of systemic immunity(13) and on hepatic composition(14) have been described. In addition, a protective role in the prevention of diarrhoea in pigs has been reported by Martinez-Puig et al.(15). Accordingly, Gil et al.(16) described in human subjects beneficial effects of supplemental soluble nucleotides on the composition of the faecal microbiota, as measured by an increase in bifidobacteria and lactobacilli. Inconsistent results, however, have been found for small-intestinal disaccharidase activity as influenced by nucleotide supplementation(17,18). However, there are few data on the animals’ requirement for exogenous nucleotides, and most of the studies have investigated effects of dietary nucleotides on the immune function in rodents, whereas only limited information exists on the possible role of nucleotides in promoting intestinal health or in stimulating the development of the immune system in other species. The present review summarises data published on nucleotide composition of various food and feed ingredients with special emphasis on milk originating from different species. Furthermore, possible effects of dietary

Abbreviations: CMP, cytosine 5′-monophosphate; IMP, inosine 5′-monophosphate; UMP, uridine 5′-monophosphate.

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nucleotides on intestinal morphology and function, intestinal microflora, immune function as well as on growth performance are described with special focus on pigs, but, where applicable, data from human subjects and rodents are also included. Special attention is given to data describing the potential role of dietary nucleotides to improve piglets’ health and development.

**Biochemical and functional characteristics of nucleotides**

Nucleotides are low-molecular-weight intracellular compounds that participate in numerous biochemical processes. They are composed of a nitrogenous base (pyrimidine or purine) linked to a pentose (ribose or deoxyribose) sugar (= nucleoside) to which one, two or three phosphate groups are attached. The pyrimidines comprise cytosine (C), uridine (U) or thymine (T), as well as orotic acid, which is present in the milk of ruminants (19), and which is produced as an intermediate product during the synthesis of uridine 5'-monophosphate (UMP). The purines comprise adenine (A), guanine (G) and hypoxanthine (I). The prefix *d* is added when the sugar of the nucleotide consists of deoxyribose. The capital letters MP, DP and TP indicate mono-, di- and tri-phosphate esters, respectively (8). The purine and pyrimidine bases are hydrophobic and relatively insoluble in water at the near-neutral pH of the cell. At acid or alkaline pH, the bases become charged and relatively insoluble in water increases (20). Nucleotides and nucleic acids are constantly being formed and degraded in all tissues, especially in tissues with a rapid turnover rate such as cells in the immune system, intestinal mucosa, skin, and progenitors of leucocytes or erythrocytes (7). For example, according to an in vitro study on intestinal explants from suckling piglets, the jejunal mucosae may be capable of nucleotidase (21) and endonucleases, phosphodiesterases, and nucleoside phosphorylases are the major enzymes involved in the transport process (Fig. 1). They originate from the brush-border epithelium (33), pancreas (35) and bile (36). The duodenum has the most developed absorptive capacity for nucleosides (57). Differences in the efficiency of uptake among nucleosides have been reported, with guanosine being taken up most rapidly (27). More than 90% of dietary and endogenous nucleosides and bases are transported into the enterocytes (7,38). Nucleoside transport into the enterocyte occurs by facilitated diffusion and by specific Na⁺-dependent carrier-mediated mechanisms (57). Several enterocyte degradation products of dietary and endogenous nucleotides and nucleosides, mainly nitrogenous bases, such as adenine, cytosine, guanine, hypoxanthine, uracil or thymine, enter the hepatic portal vein. These molecules are carried to the hepatocytes for synthesis of nucleotides, thus requiring exogenously supplied bases that can be utilised by the salvage pathway (24–26). For example, the intestinal mucosa, the haematopoietic cells of the bone marrow, leucocytes, erythrocytes and lymphocytes are incapable of de novo synthesis (27), and thus utilise the salvage pathway, suggesting that an exogenous supply of nucleotides via the diet might be important for these cells (80).

The absorption, transport and use of exogenous nucleotides and nucleosides are complex processes, which have mainly been investigated in vitro by using mucosal exposures of the ileum and ex vivo by exerted gut sacs of rabbits and rats (28–30) or in cultured cell lines (30,31). Under physiological conditions, nucleotides have a limited capacity to pass through cell membranes (27). This may be due to the absence of a nucleotide transport system. Nucleotides also have a high negatively charged phosphate group that prevents them from being absorbed (27). Except for small amounts, dietary nucleotides need to be enzymatically hydrolysed before absorption, because only nucleotides and bases can be absorbed in the small intestine (32). Endonucleases, phosphodiesterases, and nucleoside phosphorylases are the major enzymes involved in this transport process (Fig. 1). They originate from the brush-border epithelium (33,34), pancreas (35) and bile (36). The duodenum has the most developed absorptive capacity for nucleosides (57). Differences in the efficiency of uptake among nucleosides have been reported, with guanosine being taken up most rapidly (27). More than 90% of dietary and endogenous nucleosides and bases are transported into the enterocytes (7,38). Nucleoside transport into the enterocyte occurs by facilitated diffusion and by specific Na⁺-dependent carrier-mediated mechanisms (57). Several enterocyte degradation products of dietary and endogenous nucleotides and nucleosides, mainly nitrogenous bases, such as adenine, cytosine, guanine, hypoxanthine, uracil or thymine, enter the hepatic portal vein. These molecules are carried to the hepatocytes for synthesis of nucleotides, thus requiring exogenously supplied bases that can be utilised by the salvage pathway (24–26). For example, the intestinal mucosa, the haematopoietic cells of the bone marrow, leucocytes, erythrocytes and lymphocytes are incapable of de novo synthesis (27), and thus utilise the salvage pathway, suggesting that an exogenous supply of nucleotides via the diet might be important for these cells (80).

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**Fig. 1.** Digestion and absorption of nucleoproteins and their related products (adapted from Quan & Uauy (26)).
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the S-phase(40). Additionally, Paubert-Braquet arrest of T-cells in the G-phase, preventing a response to the cell cycle, for example, their withdrawal leads to the influences on immune function(44). Although nucleotides including glucagon and adrenaline, which cannot signal transduction, such as transferring the action of hormones, namely RNA and DNA, as they provide their monomeric units. The concentration of ribonucleotides is relatively constant in all cells, whereas the concentration of deoxyribonucleotides varies during the cell cycle(59). Furthermore, nucleotides may influence the cell cycle, for example, their withdrawal leads to the arrest of T-cells in the G-phase, preventing a response to various immunological signals that occur by transition to the S-phase(40). Additionally, Paubert-Braquet et al.(41) reported a decrease in phagocytic activity, lymphokine production and/or inhibited lymphocyte maturation due to nucleotide removal. Furthermore, nucleotides, mainly the adenine nucleotides (ATP, ADP, AMP), are the most important molecules for the transfer of chemical energy from energy-yielding reactions to energy-requiring reactions(7,8). Special forms of nucleotides activate as co-enzymes such as NAD, NADP, FAD and CoA, and play key roles as activated intermediates in the synthesis of lipids, carbohydrates and protein(7). Nucleotides participate in biological regulation, particularly as cyclic AMP which is a second messenger used for intracellular signal transduction, such as transferring the action of hormones including glucagon and adrenaline, which cannot pass the cell membrane(42). Further properties of cyclic AMP include the activation of protein kinase and regulating the effects of adrenaline and glucagon(43). It also serves as a regulator of the passage of Ca²⁺ through ion channels(42).

Besides their role in biochemical processes, nucleotides are eventually involved in biological reactions as well. Several in vivo studies suggest that dietary nucleotides may promote the development of the gastrointestinal ultrastructure(17), modulate the intestinal microbiota(16) and have influences on immune function(44). Although nucleotides are synthesised endogenously, it has been suggested that dietary supplementation with nucleotides may exert beneficial effects on small-intestinal growth and development, lipid metabolism and hepatic function (for a review, see Carver & Walker(59)). Conditions under which nucleotides may become essential include certain disease states, periods of limited nutrient intake or rapid growth, and the presence of regulatory or developmental factors which may interfere with full expression of the endogenous synthetic capacity(7). Under such conditions, dietary nucleotides may spare the cost of de novo synthesis and/or the salvage pathway, and thus may help to optimise the function of tissues requiring high concentrations of nucleotides (for example, intestinal mucosa). Furthermore, there is evidence that some cells, for example, erythrocytes, possess no or limited capacity for the de novo synthesis of nucleotides(59). Additionally, the intestinal mucosa, as well as the haematopoietic cells of the bone marrow, for example, leucocyte proliferation, preferentially utilise preformed pyrimidine and purine bases(20). These cells require exogenously supplied nucleotides that can be utilised via the salvage pathway rather than forming bases by de novo synthesis. For these cells, an exogenous supply of nucleotides may be essential for optimal function. There is sufficient de novo synthesis of purines and pyrimidines by the liver to meet its own requirements and the liver delivers bases to other tissues, for example, the intestine with high demands due to rapid turnover rates(20). The allocation of purines and pyrimidines via de novo synthesis in the liver is linked to high energy expenditure. In the absence of dietary nucleotides, the de novo pathway for purine and pyrimidine synthesis is activated(26). However, supplementing nucleotides may suppress the metabolic costly de novo synthesis, thereby activating the salvage pathway(24,25,45). Thus, the term ‘semi-essential nutrient’ has been introduced to describe the role of dietary nucleotides in nutrition of newborn, adult or ill mammals when endogenous supply is insufficient for normal body function, even though their absence from the diet does not necessarily lead to classical clinical aetiopathology(46).

Contents of nucleotides in milk and other food and feed ingredients

Colostrum and milk are the only nutrient supply in early life, and provide young animals with a source of nourishment which is ideally adapted to species-specific digestive and metabolic requirements at every stage of early life. Protection of the young animal against microbes is ensured via the intake of maternal immunoglobulins, lactoferin, lysozymes, lactoperoxidase and oligosaccharides as well as leucocytes present in colostrum and milk (for a review, see Xu(47)). Furthermore, milk lipids have the potential to suppress inflammatory reactions in the piglet’s gastrointestinal tract due to their antiviral, antibacterial and antiprotozoal activity(48). Colostrum and milk assure sufficient supply with enzymes needed for digestion due to low production rates of endogenous enzymes in early life(49). Furthermore, gastrointestinal functions may be promoted by the presence of bioactive components, for example, polyamines(50), hormones and growth factors(47), as well as nucleotides(51) that have the potential to stimulate cell division and differentiation in the small intestine, by, for example, increased mitosis rates and lower
apoptosis rates\(^{(11)}\). A higher production rate of digestive enzymes in the epithelial cells may occur, thereby leading to an increased digestive and absorptive capacity of the intestine. Furthermore, according to Hartmann & Holmes\(^{(49)}\), nucleotides present in sows' colostrum and milk belong to biologically active compounds that have the potential to influence the behaviour of piglets such as stimulating voluntary feed intake\(^{(52–55)}\).

The nucleotide concentration in milk of mammals is species-specific and the concentration of most nucleotides changes during the lactation period (Table 1). When comparing colostrum and milk of different species, colostrum shows generally higher amounts of nucleotides, but there are species-specific differences in composition. For example, orotic acid, an intermediate product of UMP synthesis, is present in the colostrum and milk of ruminants but not of single-stomached animals (Table 1)\(^{(19)}\).

The composition of sows' milk, in terms of total milk solids, crude protein (\(N \times 6.38\)) and 5'-monophosphate nucleotide contents, during lactation is shown in Table 2. High concentrations of total milk solids, crude protein and UMP can be found at day 0 of lactation, followed by declining levels during the first week of lactation, while contents of AMP and GMP increase. From day 14 of lactation, the content of total milk solids, crude protein and all nucleotides decreases compared with the first 7d of lactation. After day 21 of lactation, UMP (122.8 \(\mumol/100\)ml) dominates in sows' milk, whereas concentrations of GMP, AMP, cytosine 5'-monophosphate (CMP) and inosine 5'-monophosphate (IMP) average 6.0, 4.3, 2.3 and <1.0 \(\mumol/100\)ml, respectively.

Nucleotides, particularly IMP, are mainly found in protein-rich food ingredients\(^{(5)}\), such as organ meats, poultry and seafood\(^{(5,39,56)}\). Yeast protein sources, such as baker's or brewer's yeast and yeast extract, are ingredients that also show a relatively high concentration of nucleotides\(^{(57–59)}\). However, feed ingredients commonly used in animal nutrition are not routinely analysed for nucleotide content, resulting in limited information for many feedstuffs. The few data that are available show that, in comparison with colostrum and milk, there are rather low concentrations of individual nucleotides in feedstuffs such as barley, soya protein concentrate and soyabean meal (Table 3)\(^{(60)}\). For example, the concentration of UMP in milk amounts to 122.8 \(\mumol/100\)ml on lactation day 21 (Table 2), whereas barley contains no UMP and soyabean meal only 0.9 mg/100 g (Table 3). Furthermore, AMP, CMP, GMP and IMP concentrations vary in feedstuffs between non-detectable levels (for example, GMP content of casein) and 1.9 mg/100 g (AMP content of dried whey). Higher concentrations can be found for AMP in spray-dried erythrocytes (4.4 mg/100 g), for CMP in dried whey (27.0 mg/100 g) and in fish meal (2.6 mg/100 g) as well as for IMP in fish meal (3.5 mg/100 g).

### Biological effects of dietary nucleotides

**Physiological, morphological and metabolic effects**

**Hepatic morphology and function.** Several studies with rodents and cats are available focusing on potential effects of supplemental dietary nucleotides and/or nucleosides on blood parameters\(^{(14)}\), on hepatic blood flow\(^{(61)}\) and on liver morphology\(^{(62)}\). Novak et al.\(^{(14)}\) reported increased hepatic cholesterol and lipid P concentration as well as

### Table 1. Comparison of the nucleotide composition (\(\mumol/100\)ml) of colostrum and milk from different species (adapted from Gil & Sanchez-Medina\(^{(122)}\))

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Human*</th>
<th>Sow†</th>
<th>Sheep†</th>
<th>Goat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of lactation</td>
<td>2</td>
<td>30</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>AMP</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>CMP</td>
<td>6</td>
<td>2</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>IMP</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>UMP</td>
<td>2</td>
<td>1</td>
<td>103</td>
<td>12</td>
</tr>
<tr>
<td>Orotic acid</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

AMP, adenosine 5'-monophosphate; CMP, cytosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; IMP, inosine 5'-monophosphate; Nd, not detected. *Analysed with HPLC. †Analysed with enzymic methods.

### Table 2. Concentration of total milk solids, crude protein (nitrogen \(\times 6.38\)) and 5'-monophosphate nucleotides in colostrum and milk from sows at different lactation stages (adapted from Mateo et al.\(^{(86)}\))

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total milk solids (%)</td>
<td>26.7</td>
<td>23.4</td>
<td>19.4</td>
<td>18.2</td>
<td>18.8</td>
<td>19.2</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>16.6</td>
<td>7.8</td>
<td>6.2</td>
<td>5.5</td>
<td>5.7</td>
<td>6.3</td>
</tr>
<tr>
<td>5'-Monophosphate nucleotides ((\mumol/100)ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>4.0</td>
<td>11.3</td>
<td>12.8</td>
<td>6.8</td>
<td>4.3</td>
<td>3.0</td>
</tr>
<tr>
<td>CMP</td>
<td>1.5</td>
<td>7.1</td>
<td>7.1</td>
<td>3.5</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>GMP</td>
<td>5.4</td>
<td>14.7</td>
<td>14.0</td>
<td>10.2</td>
<td>6.0</td>
<td>7.1</td>
</tr>
<tr>
<td>IMP</td>
<td>1.4</td>
<td>1.4</td>
<td>0.9</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>UMP</td>
<td>555.6</td>
<td>305.6</td>
<td>263.1</td>
<td>144.0</td>
<td>122.8</td>
<td>104.0</td>
</tr>
</tbody>
</table>

AMP, adenosine 5'-monophosphate; CMP, cytosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; IMP, inosine 5'-monophosphate; UMP, uridine 5'-monophosphate.
decreased liver weight and glycogen content when feeding nucleotide-free diets to weanling mice, in comparison with mice receiving nucleotide-enriched diets. They concluded that dietary nucleotide supplementation in general may affect hepatic growth and composition in weanling mice. However, these differences were more pronounced if the diet was supplemented with AMP alone rather than with a mixture of different nucleotides including AMP, CMP, GMP, IMP and UMP. Furthermore, the effects of added extracellular nucleotides or nucleosides, and their mixture, on primary monolayer cultures of normal hepatocytes and cultures of hepatoma cells (AH130) on DNA and RNA syntheses was studied by Ohyanagi et al. (63), who found that a mixture of nucleotides and nucleosides at a concentration of 335 mg/dl (3350 mg/l) inhibited the syntheses of DNA and RNA by hepatocytes and hepatoma cells. Also, they found that excessive concentrations of nucleotide and nucleoside mixtures reduce the proliferation of both hepatocytes and hepatoma cells. However, Ohyanagi et al. (63) assumed that the addition of mixtures of nucleosides and nucleotides at lower levels (3-5 mg/dl; 35 mg/l) would enhance the growth of hepatocytes, particularly during liver regeneration, but on the other hand they may also enhance the proliferation of tumour cells in the liver. These findings were confirmed in studies with rats (62) and rabbits (64) that were diagnosed with liver injury. These findings were confirmed in experiments with hepatocyte cultures which showed an enhanced mitochondrial phosphorylative activity in the remnant liver, when fed a nucleoside–nucleotide mixture. Similarly, in hepatectomised rabbits, a continuous intravenous infusion of a nucleoside–nucleotide mixture led to an increased mitochondrial phosphorylative activity and elevated DNA content of the remnant liver (66).

Lipid metabolism. A study with human infants revealed no differences in HDL-cholesterol levels when a group receiving a nucleotide-enriched diet was compared with a breast-fed and a control group (milk formula-fed without nucleotide supplementation) (67). However, the authors found lower VLDL-cholesterol concentrations and increased HDL concentrations in the nucleotide-fed and breast-fed groups than in the control group. These findings suggest that nucleotides may play a role in lipoprotein synthesis (68). Moreover, nucleotides eventually interfere with the formation of LDL-cholesterol by affecting apoprotein synthesis (67). Similarly, orotic acid, an intermediate product of UMP synthesis, may induce fatty liver in rats by blocking VLDL secretion due to an inhibition of hepatic apo B synthesis (69–71).

Feeding a nucleotide-supplemented formula to rats has been associated with higher plasma levels of long-chain PUFA (72,73) and, according to the results of a study by Jimenez et al. (74), supplementation of nucleotides (AMP, CMP, GMP, IMP, UMP) to diets of rats enhanced the concentrations (mainly of the n-6 series) of long-chain PUFA of erythrocyte membranes compared with the control group. The authors, therefore, suggest that dietary nucleotides may have the potential to modify the conversion of essential fatty acids into their long-chain derivates. Ramirez et al. (72) obtained an increase in the prostaglandin thromboxane (TXA₂), and a trend towards higher prostaglandin 6-keto-PGF₁α levels when a semi-purified standard diet for weanling rats was supplemented with 50 mg/100 g each of the nucleotides AMP, CMP, GMP, IMP and UMP. These results coincided with an increased plasma concentration of arachidonic acid, a precursor for the production of the eicosanoids TXA₂ and PGI₂. The authors concluded that dietary purines and pyrimidines positively affect PUFA composition and therefore eicosanoid synthesis.

Dietary nucleotides may promote faster recoveries from nodular cirrhosis of the liver artificially induced by thioacetamide. For example, the addition of dietary nucleotides (AMP, CMP, GMP, IMP, UMP) following thioacetamide...
administration induced a decrease in the percentage area of fibrous septae in the liver of rats\(^{(75)}\). Furthermore, the authors found a reduced total area of fibrosis in animals with liver cirrhosis after feeding the nucleotide-enriched diet for 2 weeks. In a study by Fontana et al.\(^{(76)}\), rats with experimental cirrhosis induced by carbon tetrachloride (CCl\(_4\)) showed lower saturated (mainly stearic acid), monounsaturated and n-6 long-chain PUFA (mainly arachidonic acid) concentrations as well as a reduced unsaturation index in plasma. Administration of a diet supplemented with nucleotides (AMP, GMP, CMP, IMP, UMP) to CCl\(_4\)-treated rats led to a microsomal fatty acid profile closer to the group that received the semi-purified diet without CCl\(_4\) treatment. Furthermore, a high level of (n - 6) PUFA, particularly arachidonic acid, was observed in liver microsomal phospholipids of the rats fed the nucleotide-supplemented diet. The authors concluded that nucleotides are important for the recovery of liver function following hepatic damage.

**Intestinal morphology and function.** In the intestine, exogenous nucleotides are important for rapidly dividing mucosal cells, due to absent\(^{(24)}\) or limited\(^{(25)}\) de novo nucleotide synthesis. Some studies in single-stomached animals have focused on the effect of supplemental commercial products containing pure nucleotides or nucleotide-enriched diets on the intestinal ultrastructure\(^{(15)}\), on intestinal cell proliferation\(^{(77)}\), but also on intestinal enzyme activities\(^{(78)}\), and on recovery from diarrhoea\(^{(79)}\). For example, intestinal cell proliferation could be increased by adding a nucleoside–nucleotide mixture to a diet for rats\(^{(90)}\) or by supplementing pigs' diets with a commercial product containing nucleotides\(^{(11)}\). Under *in vitro* conditions, exogenous nucleotides (AMP, CMP, GMP, IMP, UMP) did not affect human colon tumour cell line (Caco-2 cell) proliferation, although significantly higher maltase and sucrase activities could be found\(^{(77)}\). In the same study, however, an enhancement of cell proliferation and maltase activity could be observed in a normal small-intestinal crypt cell line (intestinal epithelial cell-6) of rats. Under *in vivo* conditions with early-weaned rats, these authors reported higher small-intestinal sucrase activities, and they concluded that dietary nucleotides may enhance enterocyte proliferation and/or maturation both under *in vitro* and *in vivo* conditions. However, N Sauer, M Eklund, S Roth and R Mosethin (unpublished results) could not confirm these findings in a study with piglets fed a commercial product containing nucleotides, since α-amylase, maltase, lactase and leucine aminopeptidase activities in ileal digesta on days 13 and 20 after weaning were not affected. Furthermore, Lee et al.\(^{(144)}\) found an increase in gastric pepsin and jejunal alkaline phosphatase activity, associated with a decrease in ileal aminopeptidase, sucrase and maltase activity, following the supplementation of the diet for weaned pigs with 0-1% of a commercially available nucleotide product. Moreover, a study with rats revealed that withdrawal of dietary nucleotides led to a decrease in the content and specific activity of alkaline phosphatase, leucine aminopeptidase, maltase, sucrase and lactase in the villous tip\(^{(78)}\). These authors hypothesised that dietary nucleotides may affect the maturation status of the small-intestinal epithelium, since the aforementioned enzymes are maturation markers of intestinal cells.

The addition of a commercial nucleotide-containing product to the diet of weaning piglets may influence intestinal morphology, as indicated by a higher mitosis and lower apoptosis rate\(^{(11)}\) and a decrease in villous atrophy after weaning\(^{(15)}\). For example, Shen et al.\(^{(81)}\) found a higher villous height and villous height: crypt depth ratio in a yeast-supplemented group compared with an unsupplemented group. Furthermore, Godlewski et al.\(^{(82)}\) showed an increase in the rate of apoptosis as well as lower DNA damage and lower mitosis apoptosis index in pigs fed a diet supplemented with a commercial product containing nucleotides in comparison with the unsupplemented control treatment. Similarly, adding nucleosides, the degradation products of nucleotides, to the diets of rats enhanced mucosal protein and intestinal DNA content\(^{(17)}\). On the other hand, studies by van der Peet-Schwering et al.\(^{(53)}\) did not indicate an increased villous length and crypt depth when adding a commercial product based on yeast culture to diets for piglets.

There are further contradictory results with regard to possible effects of dietary nucleotides on nutrient digestibilities. It can be speculated if these differences can be attributed to differences in origin and dietary level of nucleotides, to variations in the adaptation period of the administered nucleotides before the start of the experiment and/or to differences in the age of the experimental animals. For example, in a study by Kornegay et al.\(^{(83)}\) with piglets (aged 25–29 d) there was no effect on apparent digestibilities of DM, N, neutral-detergent fibre and acid-detergent fibre, as well as on P, when 0.75% of a commercial product based on yeast culture to diets for piglets.

On the other hand, van Heugten et al.\(^{(55)}\) observed lower digestibilities of DM, crude protein, crude ash and crude fibre in piglets (aged 24–38 d) fed a diet supplemented with 1000 parts per million of a commercial product containing nucleotides. However, Shen et al.\(^{(81)}\) reported higher apparent digestibilities of DM, crude protein and net energy in piglets (aged 21–42 d) fed a diet supplemented with 5 g/kg of a yeast culture product compared with the control group. On the other hand, van Heugten et al.\(^{(53)}\) observed lower digestibilities of DM, fat and net energy in the pre-starter phase, and also a decrease in DM, fat, P and net energy digestibility in the starter phase of piglets (aged 17–45 d) which were fed a live yeast product (1.6 × 10\(^{7}\) colony-forming units *Saccharomyces cerevisiae*/g feed) in comparison with the control treatment. After recovery from diarrhoea, rats fed a nucleotide-enriched diet showed an intestinal histology and ultrastructure close to those of the normal uninfected control.
The authors suggested that dietary nucleotides may be important nutrients for intestinal repair following infection. Accordingly, Nunez et al.\(^{85}\) reported in weanling rats faster intestinal recovery after chronic diarrhoea, as indicated by higher intestinal DNA contents and disaccharidase activities when adding nucleotides (AMP, CMP, GMP, IMP, UMP) to a basal diet with lactose as the main carbohydrate compared with a basal diet containing glucose polymers as the main dietary carbohydrate source. Additionally, Arnaud et al.\(^{79}\) reported that nucleotide supplementation (AMP, CMP, GMP, IMP, UMP) of diets of rats with lactose-induced diarrhoea resulted in faster recoveries for the nucleotide-supplemented group than for the control group. The authors concluded that dietary nucleotides are able to promote earlier restoration of the ileal mitochondrial function after chronic diarrhoea. With regard to weaning-associated diseases in pigs, few studies have been performed investigating the effect of supplemental nucleotides on the disposition to diarrhoea. Martinez-Puig et al.\(^{115}\) observed faster recovery from diarrhoea when a commercial product containing nucleotides was included in a diet of weaning piglets. On the other hand, according to Bekaert et al.\(^{86}\), there was no effect on the incidence of diarrhoea in pigs fed a diet supplemented with a yeast culture (Saccharomyces cerevisiae).

With regard to the use of dietary nucleotides in weaned pigs, possible effects on Fe absorption might be of importance, as Fe is a mineral which frequently becomes deficient in swine diets (for a review, see National Research Council\(^{87}\)). The hepatic Fe stores of the newborn piglet are not sufficient to meet the pig’s Fe need for rapid growth and increase in blood volume\(^{88}\). Consequently, the use of exogenous sources of Fe to prevent Fe deficiency in neonatal pigs has been well documented\(^{89,90}\). However, the bioavailability of Fe from different sources may vary considerably\(^{91,92}\) and, according to Rincker et al.\(^{98}\), is not only dependent on the Fe status of the animal but also on dietary Fe as well as on various nutritional and non-nutritional substances within the diet. Dietary nucleotides, particularly the degradation product of IMP, inosine, might play a role in facilitating intestinal Fe absorption\(^{93}\). Cheney & Finch\(^{95}\) found an improved Fe absorption in rats following intravenous inosine administration. Furthermore, Faelli & Esposito\(^{94}\) investigated the effect of inosine supplementation on Fe absorption in the everted gut of rats. The authors confirmed an increase in Fe absorption and suggested that nucleotides in milk may affect the gut microflora indirectly by increasing Fe absorption in the small intestine.

### Immunological effects

Various studies have focused on the effect of supplemental dietary nucleotides on the immune function in human subjects\(^{95}\) and pigs\(^{96}\); however, most studies have been performed in rodents\(^{115}\). For example, feeding nucleotide-supplemented diets to rodents has been associated with an increase in graft v. host disease mortality\(^{97}\), a rejection of allogenic grafts\(^{98,99}\) and a delayed cutaneous hypersensitivity\(^{100}\). However, other studies with rodents have revealed faster recovery of malnutrition- and starvation-induced immunosuppression\(^{101,102}\), an increase in antigen-induced lymphoproliferation\(^{103}\) as well as higher natural killer cell activity and IL-2 production\(^{103}\). Additionally, resistance to a challenge with Candida albicans\(^{104}\) and Staphylococcus aureus\(^{105}\) by activating humoral and cell-mediated immunity has been observed in mice fed diets enriched with nucleotides originating from yeast and RNA, respectively. The results of several studies pertaining to possible effects of dietary nucleotides on immune function in mice are summarised in Table 4.

Dietary nucleotides may also affect immune function in pigs. For example, Godlewski et al.\(^{82}\) observed higher autophagy capacity in the mid-jejunum of pigs fed a diet supplemented with a commercial nucleotide product compared with the control group. Additionally, Domeneghini et al.\(^{111}\) reported differences in the growth and maturation of the intestinal mucosa by adding 0·05 % nucleotides to a weanling piglet diet, especially as indicated by higher percentages of macrophages in the intestine and higher intra-epithelial lymphocytes. Due to close and intimate contact with the epithelial cells and the environment, higher numbers of intra-epithelial lymphocytes, components of the intra-epithelial defensive system, play an important role in mucosal immunity. They possess a number of pathogen-specific and non-specific functions enabling them to play a critical role in host defences at mucosal surfaces. For example, higher intra-epithelial lymphocytes have been shown to possess pathogen-specific cytotoxic T-cell activity\(^{106}\), and to be capable of mediating delayed-type hypersensitivity responses\(^{107}\). Moreover, they also secrete various cytokines such as interferon-\(\gamma\), TNF-\(\alpha\) and IL-2\(^{108,109}\), which may play an important role in immune modulation at the mucosal surfaces. Furthermore, Lee et al.\(^{114}\) demonstrated an immunomodulating effect as indicated by increased levels of plasma immunoglobulins (IgA, IgM) in weaned pigs when fed a commercial product containing nucleotides at 0·1 %.

A study by Cameron et al.\(^{96}\) revealed that feeding nucleotides to early-weaned piglets may enhance T-cell functions in delayed hypersensitivity to antigenic challenge in the intestine and in vitro lymphocyte proliferation response to concanavalin A, a mitogen which stimulates T-lymphocytes. The duration of the nucleotide supplementation seems to be critical for exhibiting effects on cell functions and cell proliferation, as the immuno-enhancing effects described above require at least 2 weeks of administration to the pigs\(^{96}\). However, a higher mitotic activity in the ileum could be shown when feeding a combination of 0·05 % nucleotides and 0·5 % glutamine. Thus, the inclusion of nucleotides and glutamine to a weanling...
piglet diet appears to have a positive effect on the maturation of the ileal mucosa by supporting the development of lymphocytes and thereby providing an immune-reactive intestine. Furthermore, Salobir et al.\(^{110}\) found, upon feeding a diet supplemented with different levels of PUFA to growing pigs, that a commercial product of nucleotides was included in the assay. On the other hand, Sauer et al.\(^{113}\) obtained no differences in the growth responses of *Lactobacillus reuteri* (strain DSM 6134) when adding single nucleotides to a minimal medium. This stimulatory effect was even more pronounced when a mixture of these monophosphate nucleotides was included in the assay. On the other hand, Sauer et al.\(^{113}\) obtained no differences in the growth responses of *Lactobacillus reuteri* (strain DSM 2146) and *E. faecalis* (DSM 6134) when adding single nucleotides (AMP, CMP, GMP, IMP, UMP) to a minimal medium. However, the authors found diverse growth responses for different *Escherichia coli* strains (*E. coli* DSM 2840, *E. coli* PS 37, *E. coli* PS 79) upon the addition of single nucleotides to a minimal medium, depending on the type of nucleotide as well as on the concentration used, but also on the specific bacterial strain tested. These results suggest that bacteria might be able to use nucleosides and/or nucleotides for their proliferation, although this has to be confirmed by further *in vitro* studies by expanding the range of bacterial strains under investigation.

**Microbial effects**

Few *in vitro* studies have been performed pertaining to possible effects of supplemental nucleotides or nucleosides on the growth of single bacterial strains; however, results are rather controversial. For example, an *in vitro* study of Tanaka & Mutai\(^{111}\) showed that nucleosides (adenine, guanine, xanthine, uracil) in combination with pyruvic acid and riboflavin in a minimal culture medium promote the growth of bifidobacteria. Accordingly, Uauy\(^{77}\) summarised that Gil\(^{112}\) obtained a slight but significant stimulation of bifidobacterial *in vitro* growth upon the addition of single nucleotides (AMP, CMP, GMP, IMP, UMP) to a minimal medium. This stimulatory effect was even more pronounced when a mixture of these monophosphate nucleotides was included in the assay. On the other hand, Sauer et al.\(^{113}\) obtained no differences in the growth responses of *Lactobacillus reuteri* (strain DSM 2016), *L. amylovorus* (DSM 20513), *Enterococcus faecium* (DSM 2146) and *E. faecalis* (DSM 6134) when adding single nucleotides (AMP, CMP, GMP, IMP, UMP) to a minimal medium. However, the authors found diverse growth responses for different *Escherichia coli* strains (*E. coli* DSM 2840, *E. coli* PS 37, *E. coli* PS 79) upon the addition of single nucleotides to a minimal medium, depending on the type of nucleotide as well as on the concentration used, but also on the specific bacterial strain tested. These results suggest that bacteria might be able to use nucleosides and/or nucleotides for their proliferation, although this has to be confirmed by further *in vitro* studies by expanding the range of bacterial strains under investigation.

Roselli et al.\(^{114}\) evaluated the potential of several natural substances (for example, yeast extract) to protect against enterotoxigenic *Escherichia coli* (ETEC)
K88-induced membrane damage by using intestinal pig epithelial cells-1. The authors showed that a commercial product containing nucleotides could protect the cells against an increase in membrane permeability caused by enterotoxigenic Escherichia coli. Additionally, results from an in vitro study with human subjects suggest that dietary nucleotides may modify the composition of the intestinal microflora by promoting beneficial bacteria including bifidobacteria and lactobacilli (16). Young infants fed a formula containing dietary nucleotides (AMP, CMP, GMP, IMP, UMP) had higher percentages of faecal bifidobacteria and lactobacilli and lower percentages of Gram-negative enterobacteria than formula-fed infants without supplementation (16). High numbers of intestinal bifidobacteria and lactobacilli are required, in particular around the time of weaning during the transition from liquid to solid feeding. For example, bifidobacteria may lower the pH of the colonic content due to their capacity to hydrolyse sugars to lactic acid which, in turn, may suppress the proliferation of acid-susceptible pathogenic bacteria. Bifidobacteria also may inhibit the growth of enterobacteria responsible for diarrhoeal diseases (for a review, see Braun (115)). However, only few studies are available pertaining to the effect of dietary nucleotides on the composition and activity of the intestinal microflora of young pigs. For example, Andrés-Elias et al. (4) concluded that nucleotides appear to modulate the intestinal ecology in weaning piglets by stabilising the microbiota in the ileum, when testing nucleotides (commercially available product) as possible alternatives to antimicrobial growth promoters. However, Mathew et al. (52) could not show any differences in total ileal numbers of Escherichia coli, lactobacilli and streptococci as well as in microbial metabolites (SCFA composition), when feeding yeast cultures (Saccharomyces cerevisiae) to weaning piglets. Similarly, N Sauer, M Eklund, S Roth and R Mosethin (unpublished results) failed to observe a response in ileal counts of lactobacilli, bifidobacteria, enterococci and eubacteria as well as in the level of microbial metabolites, including SCFA and lactic acids, when feeding a diet to weaning piglets supplemented with a commercial product containing nucleotides. Also, no changes in the PCR-denaturing gradient gel electrophoresis fingerprint analysis of total bacterial community profile could be shown when adding nucleotides (yeast culture) to diets of pigs (55). Results of studies pertaining to the effect of nucleotides, given either as single nucleotides or originating from yeast products, on the composition and activity of the intestinal microbiota of humans and pigs are still equivocal.

Zootechnical effects

Several studies have been conducted focusing on the growth-promoting effect of dietary nucleotides, supplemented as yeast products to pig diets (Table 5). In several studies dietary nucleotides as a yeast product were added to the diets of pigs of different ages but failed to improve growth performance (4, 11, 53, 55, 83, 86, 96, 116, 117). Similarly, a study by Sijben et al. (118) showed that dietary yeast nucleotides (Saccharomyces cerevisiae) did not affect energy intake, heat production and energy retention of barrows. On the other hand, supplementation of yeast products to diets of piglets resulted in similar improvements in growth performance as compared with antimicrobial growth promoters (55, 81). Similarly, Mathew et al. (52) observed a trend towards an increased growth rate in pigs fed a diet supplemented with live yeast (Saccharomyces cerevisiae) from weaning until age 41 d. Furthermore, the addition of nucleotide products alone or in combination with glutamine to diets of weaning piglets has revealed contradictory results. Domeneghini et al. (111) observed no difference in body weight and feed intake, whereas in a study by Yi et al. (119) a combination of nucleotides (RNA) and glutamine improved average daily gain and feed intake. Further studies investigated sows’ and piglets’ performance upon supplementation of sows’ diets with yeast products. Jurgens et al. (120) and Moore et al. (121) found higher growth performance in piglets when adding live yeast (Saccharomyces cerevisiae) and a commercial product containing Saccharomyces cerevisiae, respectively, to sows’ diets.

Conclusions

There is increasing evidence that dietary nucleotides may affect different aspects of body function and health in single-stomached animals, including intestinal morphology and function, immune response, the composition of the intestinal microbiota, liver function and morphology as well as growth performance. Although nucleotides can be synthesised de novo, several tissues, including those with a rapid turnover rate such as the intestine, may benefit from an exogenous supply to decrease energy expenditures. In times of disease states, periods of limited nutrient intake or rapid growth, such as the weaning period, nucleotides can become semi-essential nutrients, as in general nucleotides are present at very low levels in animal diets. Furthermore, due to the ban of in-feed antibiotics, efficient and feasible alternatives have gained increasing attention to improve the health of animals, to increase the potential to withstand infectious diseases and to secure growth performance. For dietary nucleotides, beneficial effects have been proven for a faster intestinal recovery after challenge with, for example, diarrhoea, and also for a faster immune response in several disease situations, for example, malnutrition. A decreased permeability of the lamina propria and an increased stabilisation of the intestinal microbial ecosystem by promoting beneficial bacteria may further improve animal health. Additionally, there is evidence that IMP may facilitate Fe absorption, which is of special interest in pigs as Fe...
has been acknowledged as a mineral which frequently becomes deficient in pig nutrition.

However, results of studies pertaining to biological effects of nucleotides in single-stomached animals are equivocal. In most studies, commercially available yeast products have been assayed both under in vitro and in vivo conditions. Since these yeast culture products contain variable amounts of nucleotides in addition to viable cells, cell wall components, and parts of the medium on which the yeast cells were grown, it is difficult to assign the observed biological effects exclusively to specific nucleotides present in these products. Studies in

<table>
<thead>
<tr>
<th>Effect</th>
<th>Animal</th>
<th>Amount</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily gain – Pig</td>
<td>2-0 %</td>
<td>&lt; 35 kg</td>
<td>S. c. culture</td>
<td>Bowman &amp; Veum (1973)</td>
</tr>
<tr>
<td>Average daily feed intake – Pig</td>
<td>1-5 %</td>
<td>&gt; 34 kg + antibiotics</td>
<td></td>
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</tr>
<tr>
<td>Gain:feed ratio – Pig</td>
<td>1-5 %</td>
<td>2-0 %, 2-5 %</td>
<td>S. c. culture</td>
<td>Veum &amp; Bowman (1973)</td>
</tr>
<tr>
<td>Average daily gain – Pig</td>
<td>2-5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance – Pig</td>
<td>1-5/2</td>
<td>0-2-5 % (23–72 d):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average daily gain – Pig</td>
<td>0-75 %</td>
<td></td>
<td>Yeast culture</td>
<td>Kornegay et al. (1995)</td>
</tr>
<tr>
<td>Average daily feed intake – Pig</td>
<td>500 g/</td>
<td>1000 g/t</td>
<td>Cp</td>
<td>Bekaert et al. (1996)</td>
</tr>
<tr>
<td>Average daily gain – Pig</td>
<td>0-1</td>
<td>or 0-2 % (gestation diet)</td>
<td>Live S. c. cells</td>
<td>Jurgens et al. (1997)</td>
</tr>
<tr>
<td>Sows’ body weight – Sow</td>
<td>0-15</td>
<td>or 0-3 % (lactation diet)</td>
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<td></td>
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<tr>
<td>Birth Weight –</td>
<td>0-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average daily gain post-weaning – Pig</td>
<td>0-05 %</td>
<td></td>
<td></td>
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<tr>
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<td>2-5 %</td>
<td>(days 1–14), 2-5 % (days 15–28)</td>
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<td>Average daily feed intake – Pig</td>
<td>5 %</td>
<td>(days 1–130)</td>
<td></td>
<td></td>
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<tr>
<td>Average daily gain – Pig</td>
<td>2 g/kg</td>
<td></td>
<td>S. c.</td>
<td>Bontempo et al. (2006)</td>
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<td>Average daily gain – Pig</td>
<td>1500 mg/</td>
<td></td>
<td>Cp</td>
<td>Andres-Elias al. (2007)</td>
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<td>Average daily gain – Pig</td>
<td>1000 ppm, 2000 ppm</td>
<td></td>
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<td>Martinez-Puig et al. (2007)</td>
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<td>Average daily feed intake – Pig</td>
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<td>Moore et al. (2007)</td>
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<td>Sows’ body weight – Sow</td>
<td>2 %</td>
<td></td>
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<tr>
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<td>Yeast culture</td>
<td>Van der Peet-Schwering et al. (2007)</td>
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<td>Average daily gain – Pig</td>
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<td>average daily gain</td>
<td></td>
<td>Shen et al. (2009)</td>
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<tr>
<td>Average daily gain – Pig</td>
<td>5 and 10 g/kg</td>
<td>average daily feed intake</td>
<td></td>
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<tr>
<td>Average daily gain – Pig</td>
<td>2-5 g/kg, 5-0 g/kg, 10 g/kg, 20 g/kg</td>
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which the biological effects of purified sources of nucleotides, alone or in combination, were assayed are scarce. Therefore, further research should be directed to the evaluation of the efficiency of purified nucleotides, including the effects of varying concentrations and different combinations of nucleotides. In addition, future research should also focus on the potential associated effects of nucleotides if supplied in combination with other bioactive substances, for example, glutamine.

Finally, advanced knowledge about the contents of nucleotides present in commonly used feed components would facilitate the development of feeding strategies to meet animals’ requirements for nucleotides, in particular in young animals suffering from various kinds of stress.

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