Administration of nucleotides in poultry's diet: Effect on the lipid composition of the *Pectoralis major* muscle

B. CHIOFALO[1]*, V. LO PRESTI[2], A. AGAZZI[3], G. INVERNIZZI[3], V. CHIOFALO[1, 2] and L. LIOTTA[1]

[1]Faculty of Veterinary Medicine/Department of Morphology, Physiology, Biochemistry and Animal Production, University of Messina, Polo Universitario Annunziata, 98168 - Messina, Italy; [2]Consortium of Meat Research, Polo Universitario Annunziata, 98168 - Messina, Italy; [3]Faculty of Veterinary Medicine/Department of Veterinary Sciences and Technologies for Food Safety, University of Milan, Via Celoria, 10, 20133 - Milan, Italy.
***Corresponding author: biagina.chiofalo@unime.it**

There are conflicting reports regarding the effectiveness of dietary nucleotides to influence the lipid metabolism. Many studies focussed the attention on the effects of nucleotides in human diet, whereas, there are few information in animal nutrition. Aiming to a better knowledge of the effects of nucleotide supplementation in poultry's diet, the lipid and the fatty acid levels of the meat of forty male broiler chickens, ROSS 508 genotype, were studied. The birds were divided into two homogeneous groups of twenty each one: Control (C) and Nucleotides (N); the N group received, from the birth (24 hours of age) to the slaughtering age (52 days), the basal diet supplemented with 0.1% of a Nucleotide pool containing AMP, GMP, CMP and UMP in similar quantities. On the individual samples of *Pectoralis major* muscle the lipid content and the fatty acids composition were determined; the quality indices were calculated. The results, expressed in g/100 g of edible part (total lipid) and in percentage of the total fatty acid identified (fatty acids), were subjected to ANOVA. The nucleotides influenced significantly (P<0.001) the lipid content, showing the higher values (mean ± SE) in the N group (1.9 g/100 g ±0.05) than in the C group (1.66 g/100 g ±0.05), probably in relation to the physiological effect of nucleotides to stimulate the alfa-lipoprotein synthesis during the neonatal period. No significant differences were observed for the total PUFA (N: 21.38% vs. C: 21.96%; SE=0.44; P=0.363) and the n6 (N: 14.00% vs. C: 13.61%; SE=0.21; P=0.188) content as linoleic (N: 12.19% vs. C: 11.93%; SE=0.16; P=0.283) and arachidonic (N: 3.51% vs. C: 3.41%; SE=0.13; P=0.610) acids, whereas the nucleotides have significantly influenced the n3 content (N: 3.27% vs. C: 4.40%; SE=0.12; P<0.001) as linolenic (N: 0.66% vs. C: 0.55%; SE=0.01; P<0.001), eicosapentanoic (N: 0.43% vs. C: 0.62%; SE=0.02; P<0.001) and docosahexanoic (N: 1.18% vs. C: 2.13%; SE=0.06; P<0.001) acids. As regard the quality indices, the nucleotide supplementation influenced the Atherogenic index (N: 0.43 vs. C: 0.47; SE=0.01; P=0.001) but not the Thrombogenic index (N: 0.91 vs. C: 0.89; SE=0.01; P=0.272). Results suggest that there has been no induction of PUFA accumulation in *Pectoralis major* muscle owing to dietary supplementation with nucleotides in accordance to Gibson' et al. (2005) observations.

Keywords: poultry; nucleotides; meat; lipid composition.

Introduction

Nucleotides are low-molecular-weight intracellular compounds made up of three components: (1) a nitrogenous heterocyclic base derivative of either a pyrimidine or purine; (2) a pentose (deoxyribose or ribose), and (3) one or more phosphate groups (Stryer, 1988). Nucleotides participate in many

biochemical processes that are essential to cellular metabolism: as nucleic acids, in biosynthetic pathways, in transferring chemical energy, as co-enzyme components as well as biological regulators (Consgrove, 1998).

Among the numerous metabolic processes, nucleotides modulate the lipid metabolism, particularly the long-chain polyunsaturated fatty acid and the lipoprotein synthesis (Fontana et al., 1999). With regards to polyunsaturated fatty acids, Gil et al. (1988) suggested that in the neonatal period, dietary supplementation with nucleotides stimulates the conversion of essential fatty acids, linoleic and linolenic acids, to their longer superior homologous fatty acids (arachidonic, eicosapentanoic and docosahexanoic acids); different mechanisms have been proposed to explain this increment: i) the enhanced conversion could be attributed to the increased intestinal or hepatic synthesis of delta-5-desaturase, as a result of increased protein synthesis; in fact, nucleotides are known to facilitate protein synthesis by increasing the availability of precursors of RNA synthesis (Gil et al., 1986; Garcia-Molina et al., 1991); ii) nucleotides may produces changes in the intestinal microflora that may affect long-chain polyunsaturated fatty acids levels, because bacteria possess necessary enzymes for fatty acid elongation and desaturation (Consgrove, 1998); iii) nucleotides may modulate chain elongation and desaturation in the enterocyte or in the hepatocyte (Consgrove, 1998) causing an increase of phospholipid synthesis in the liver (Garcia-Molina et al., 1991). More recently, there have been conflicting reports regarding the effectiveness of dietary nucleotides to regulate tissue desaturase and hence stimulate accumulation of both n6 and n3 long chain polyunsaturated fatty acids in newborns (Gibson et al., 2005).

Although many studies have focussed the attention on the effects of nucleotides supplementation as one of the most important aspect of research in clinical nutrition and functional food development for humans, there are few information in animal nutrition even if, the researches pertaining to nucleotide administration in rats (Yamamoto et al., 1997), piglets (Domeneghini et al., 2004) and broilers (Riolo et al., 2006) have shown rather consistent and encouraging beneficial results in health management thanks to their potential effect of growth promoter. In consideration of the recent proposal of the European Parliament and Council (COM (2002) 153 – C5 – 0143/2002 – 2002/0073 COD to reduce strictly the use of chemical additives in animal nutrition, the aim of this study was to evaluate the effect of nucleotide supplementation in poultry's diet on the lipid composition of the meat.

Materials and methods

The research was carried out on 10000 male broiler chickens, ROSS 508 genotype, divided into two groups homogeneous for body weight (49.2 ± 6.2 g) called Control (C) and Nucleotides (N), housed separately in two environmentally-controlled (20/4 hr light/dark cycle) floor pens with straw litter and free access to feed and water; the N group received, from the birth (24 hours of age) to the slaughtering age (52 days), the basal diet (*Table 1*) supplemented with 0.1% of a Nucleotide pool (Prosol S.p.A., Madone, BG, Italy) containing adenosine, guanosine, cytidine and uridine 5'-monophosphates (AMP, GMP, CMP and UMP) in similar quantities. After 52 days of feeding with the experimental diet, twenty animals from each group were selected at random and sacrificed by electrically stunned; the *Pectoralis major* muscles were removed, vacuum packed and quickly frozen and stored at -20°C until lyophilized.

Table 1 Chemical composition of the basal diet (as fed)

	1 – 10 days	11 – 21 days	22 – 40 days	41 – 52 days
Dry Matter (%)	88	88	88	88
Crude Protein (%)	23	21	21.8	20.5
Ether Extract (%)	7	6	7.4	7.6
Crude Fiber (%)	2.9	2.6	2.6	2.5
Ash (%)	7	6.5	6.7	6.2

On the individual lyophilised samples of breasts, the lipids were extracted by Soxhlet, using a mixture of chloroform/methanol (2:1, v/v) according to Baroli et al. (2000); the fat content was expressed in g/100 g of edible part.

Fatty acids methyl esters of the intramuscular fat were prepared by direct transesterification with 1% sulphuric acid : methanol of weighted portion (15 mg) of the total lipids (Christie, 1993) and analysed using an Agilent Technologies 6890N (U.S.A) gas cromatograph operated with a split/splitless injector, a Gerstel autosampler MPS2 (Germany), a flame ionization detector and fused silica capillary column OMEGAWAX 250 (Supelco, U.S.A.), 30m x 0.25mm I.D., 0.25 μm film thickness. Column temperature was programmed: initial isotherm of 160 °C (6 min.), increment of 3°C/min and final isotherm of 250°C (30 min.). Temperature of the injector and detector: 250°C. Injection volume: 1.0 μL. Carrier gas: helium (1 mL/min). Split ratio: 1:50. Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from Supelco (U.S.A.). Peak areas were acquired and calculated by Chemstation software (Agilent, U.S.A.) and expressed in percentage of the total fatty acid identified. On the basis of the fatty acid identified, the quality indices were calculated using the equations proposed by Ulbricht and Southgate (1991). The results were subjected to ANOVA (SAS, 2001) using the following model: $y_{ik} = \mu + \alpha_i + e_{ik}$; where y_{ik} = dependent variable; μ = overall mean, α_i = diet (Control, Nucleotides), e_{ik} = residual error.

Results and discussion

The nucleotides influenced significantly (P<0.001) the lipid content, showing higher values (mean ± SE) in the N group (1.9 g/100 g ±0.05) than in the C group (1.66 g/100 g ±0.05), probably in relation to the physiologic effect of nucleotides to stimulate the α-lipoprotein synthesis in the neonatal period (Sanchez-Poza et al., 1996).

Concerning the acidic composition (*Figure 1)*, the treated group showed significantly (P<0.0001) lower value of the saturated fatty acids but significantly (P=0.0002) higher values of the monounsaturated fatty acids. No significant differences were observed for the total polyunsaturated fatty acids (N: 21.38% vs. C: 21.96%; SE=0.44; P=0.3627), even if, slightly higher levels for the n6 PUFAs (N: 14.00% vs. C: 13.61%; SE=0.21; P=0.1876) and significantly lower percentages for the n3 PUFAs (N: 3.27% vs. C: 4.40%; SE=0.12; P<0.0001) were observed in the treated group.

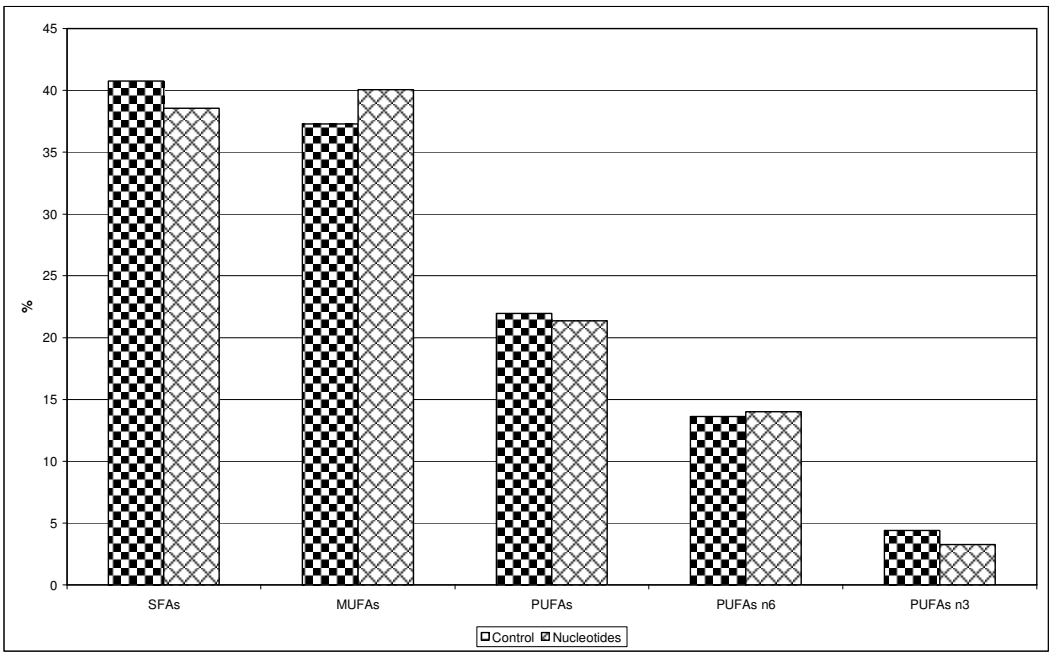


Figure 1 Effect of the dietary nucleotides on the acidic class of *Pectoralis major* muscle

An hypothesis could be that the nucleotides have stimulated the elongation and desaturation enzymes for the metabolic conversion of acidic products as reported by Schlimme et al. (2000), but only in the direction of n6 family thanks to the higher content of linoleic acid than that of α-linolenic acid.

With regards to the essential fatty acids and their longer superior homologous fatty acids (*Table 2*), the linoleic ($C_{18:2n6}$) and arachidonic ($C_{20:4n6}$) acids showed similar percentages, whereas the nucleotide supplementation has significantly influenced the percentages of the α-linolenic ($C_{18:3n3}$), eicosapentanoic ($C_{20:5n3}$) and docosahexanoic ($C_{22:6n3}$) acids in the meat.

These findings suggest that there has been no induction of long chain polyunsaturated fatty acid accumulation in *Pectoralis major* muscle owing to dietary supplementation with nucleotides in accordance to Gibson' et al. (2005) observations.

Table 2 Effect of the dietary nucleotides on the percentages of some polyunsaturated fatty acids of nutritional interest (mean ± SE)

PUFAs*	Control	Nucleotides	SE	*P*
$C_{18:2n6}$	11.94	12.19	0.16	0.2826
$C_{18:3n3}$	0.55	0.66	0.01	<0.0001
$C_{20:4n6}$	3.41	3.51	0.13	0.6096
$C_{20:5n3}$	0.62	0.43	0.02	<0.0001
$C_{22:6n3}$	2.13	1.18	0.06	<0.0001

*Percentage of the total fatty acid methyl esters identified

The UFAs/SFAs ratio was significantly higher in the treated group according to Gil et al. (1988), but the PUFAs/SFAs ratio showed no significant differences between the groups (*Table 3*).

Regarding the quality indices (*Table 3*), no significant difference was observed for the Thrombogenic index, whereas the supplementation has positively influenced the Atherogenic index suggesting that dietary nucleotide may protect against the early development of atherosclerosis (Cosgrove, 1998).

Table 3 Effect of the dietary nucleotide on the UFAs/SFAs and PUFAs/SFAs ratios and on the quality indices (mean ± SE)

	Control	Nucleotides	SE	*P*
UFAs/SFAs	1.53	1.59	0.01	0.0016
PUFAs/SFAs	0.54	0.55	0.01	0.4486
Atherogenic index	0.47	0.44	0.01	0.0012
Thrombogenic index	0.89	0.91	0.01	0.2715

From a nutritional point of view, the Nucleotide supplementation has determined an improvement of the dietetic characteristics of the meat, testified by the lower Atherogenic index as well as by the higher unsaturation degree, this latter in relation to the higher monounsaturated fatty acid content. In fact, recently (Williams et al., 1999), it has been shown that MUFas have beneficial effects on blood cholesterol and other health related outcomes in human (Williams et al., 1999). Moreover, in spite of the cholesterol-lowering response to polyunsaturated fatty acids that is greater than that to monounsaturated fatty acids, there has been caution in recommending high dietary polyunsaturated fatty acid in human, because of potentially adverse health effects of their lipoperoxidation products (Williams, 2000).

References

BAROLI, D., ARLATI, M., BIANCHINI, S., PIGNATELLI, P. and GUIDOBONO CAVALCHINI, L. (2000). Qualità nutrizionale della carne di broiler italiana. *Avicoltura* **5**: 60-65.

CONSGROVE M. (1998). Nucleotides. *Nutrition* **14**:748-751.

CHRISTIE W.W. (1993). Preparation of Ester Derivatives of fatty acids for chromatographic analysis, in: *Advanced in Lipid Methodology-Two* (The Oily Press LTD, ed.), pp 69-111, Dundee, Scotland.

DOMENEGHINI, C., DI GIANCAMILLO, A., SAVOINI, G., PARATTE, R., BONTEMPO, V. and DELL'ORTO, V. (2004). Structural patterns of swine ileal mucosa following L-glutamine and nucleotide administration durino the weaning period. An histochemical and histometrical study. *Histol. Histopathol. 19: 49-58.*

FONTANA, L., MOREIRA, E., TORRES, M.I., PERIAGO, J.L., SANCHEZ DE MEDINA, F. and GIL, A. (1999). Effects of dietary polyunsaturated fatty acids and nucleotides on tissue fatty acid profile of rats with carbon tetrachloride-induced liver damage. *Clinical Nutrition* **18**: 93-101.

GARCIA-MOLINA, V., ANGUILERA, J.A., GIL, A. and SANCHEZ-POZO, A. (1991). Plasma lipoproteins in suckling rats: Effect of dietary nucleotides. *J. Clin. Nutr. Gastroenterol*. **6**: 184-190.

GIL, A., PITA, M., MARTINEZ, A., MOLINA, J.A. and SANCHEZ-MEDINA, F. (1986). Effect of dietary nucleotides on the plasma fatty acids in at-term neonates. *Hum. Nutr.: Clin. Nutr.* **40c**: 185-195.

GIL, A., LOZANO, E., DE-LUCCHI, C., MALDONADO, J., MOLINA, J.A. and PITA, M. (1988). Changes in the fatty acid profiles of plasma lipid fractions induced by dietary nucleotides in infants born at term. *Eur. J. Clin. Nutr.* **42**: 473-481

GIBSON, R.A., HAWKES, J.S. and MAKRIDES, M. (2005). Dietary nucleotides do not alter erythrocyte long-chain polyunsaturated fatty acids in formula-fed term infants. *Lipids* **40**: 631-634.

RIOLO, E.B., INVERNIZZI, G., D'ALESSANDRO, E., RIPAMONTI, B., PICCOLO, D. and CHIOFALO V. (2006). Administration of nucleotides in broiler diet. Proceedings of the LX S.I.S.Vet. Congress, Terrasini (PA, Italy), 27-30 September. *In press*.

SANCHEZ-POZO, A., PITA, ML., MARTINEZ, A., MOLINA, JA., SANCHEZ DE MEDINA, F. and GIL, A. (1996). Effect of dietary nucleotides upon lipoprotein pattern of newborn. *Nutr Res* **6**:763-77.

SAS (2001). *User's Guide Version 8.2 Statistical Analysis System Inst.* Cary, NC, U.S.A..

SCHLIMME, E., MARTIN, D. and MEISEIL, H. (2000). Nucleosides and nucleotide: natural bioactive substances in milk and colostrums. *British Journal of Nutrition* **84**: S59-S68.

STRYER L. (1988). *Biochemistry* (WH Freeman & Co, 3rd ed.), 601, New York, U.S.A..

ULBRICHT, T.L.V. and SOUTHGATE, D.A.T. (1991). Coronary heart disease: seven dietary factors. *The Lancet* **338**: 985-992.

YAMAMOTO, S., WANG, M., ADJEI, A.A. and AMEHO C. (1997). Role of the nucleosides and nucleotides in the immune system, gut reparation after injury and brain function. *Nutrition* **13**: 372-374.

WILLIAMS, C.M. (2000). Dietary fatty acids and human health. *Ann. Zootech*. **49**: 165-180.

WILLIAMS, C.M., FRANCIS-KNAPPER, J.A., WEBB, D., BROOKES, C.A., ZAMPELAS, A., TREDGER, J.A., WRIGHT, J., CALDER, P.C., YAQOOB, P., ROCHE, H. and GIBNEY M.J. (1999). Cholesterol reduction using manufactured foods high in monounsaturated fatty acids, a randomised cross-over study. *Brit. J. Nutr*. **81**: 439-446.

Acknowledgement

Authors wish to thank Prosol S.p.A. (Italy) for providing the nucleotides. Research financed by MIUR-P.O.N. n.12864 (Azienda LEOCATA Mangimi S.p.A., Modica-RG, Italy).