Nucleotides in Broiler Chicken Diet: Effect on Breast Muscles Quality

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Abstract

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The study evaluated the effects of nucleotide dietary supplementation on the physical and nutritional characteristics of the *Pectoralis major* muscle of male broiler chickens (n = 60 000), divided into two homogeneous groups: Control (C) and Nucleotides (N). The animals of the two groups, from the birth (24 h of age) to the slaughtering age (52 days), received the same diet, supplemented (N) or not (C) with 0.1% of a Nucleotide pool. At the slaughtering, on a sample of 130 animals per group, randomly selected, the physical and nutritional characteristics of *Pectoralis major* muscle were determined. The meat of the N group showed significantly higher redness and Hue values, lower shear force values, higher lipid and ash percentages and iron content. Moreover, nucleotides significantly increased monounsaturated acids and linolenic acid and decreased eicosapentanoic and docosahexanoic acids. The unsaturation degree was higher in the Nucleotides group and Atherogenic index was positively influenced by the nucleotide supplementation. Nucleotide dietary supplementation improved the physical and nutritional characteristics of the *Pectoralis major* muscle of broiler chickens.

Keywords: dietary nucleotide; meat; broiler chicken

Biotechnology provides new and unprecedented opportunities to improve the productivity of animals through increased growth, carcass quality, and reproduction, improved nutrition and feed utilisation, improved quality and safety of food, improved health and welfare of animals, and reduced waste through more efficient utilisation of resources (Bonneau & Laarveld 1999). In this context, the advances in human medical biotechnology form an important basis for the research and development in animal biotechnology. In the application of biotechnology for the nutrition of farm animals, some nutrients, called nucleotides, improve feed characteristics through the gut and immune systems development and function in young animals (Gardiner *et al.* 1995); they represent an active and fertile area of research.

Nucleotides are low-molecular-weight intracellular compounds made up of three components: (1) a nitrogenous heterocyclic base derivative of

either pyrimidine or purine; (2) a pentose (deoxyribose or ribose), and (3) one or more phosphate groups (Stryer 1988). Nucleotides participate in many intracellular biochemical processes (Lerner & Shamir 2000) 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 (Cosgrove 1998).

Nucleotides are common components of the diet and the body provides mechanisms for their absorption and incorporation into tissues (Sanchez-Pozo & Gil 2002). However, during the periods of rapid growth, certain disease states, limited nutrient intake or disturbed endogenous synthesis of nucleotides (Lerner & Shamir 2000), their availability could limit the maturation of fast dividing tissues with a low biosynthetic capacity, such as the intestine (Leleiko *et al.* 1983; Van Buren & Rudolph 1997).

Regarding chickens, dramatic changes occur in the development of the small intestinal mucosa after hatching, including enterocyte maturation, intensive cryptogenesis and villous growth (Geyra *et al.* 2001). This intestinal development influences the growth rate, since intestinal maturation plays a rate determining role in providing the substrates for the growth (Smith *et al.* 1990).

Although many studies have focussed the attention on the effects of nucleotides supplementation as one of the most important aspects of research in clinical nutrition and functional food development for humans (Sanchez-Pozo & Gil 2002) thanks to their capacity to induce intestinal anabolic effects expressed by increased mucosal protein, DNA, villous height, and brush-order enzyme activities (Uauy *et al.* 1990), there is little information on animal nutrition even though, the researches pertaining to nucleotide administration in rats (Yamamoto *et al.* 1997), piglets (Domeneghini *et al.* 2004; Martinez-Puig *et al.* 2007), and broiler chickens (Chiofalo *et al.* 2006; Riolo *et al.* 2006; Esteve-Garcia *et al.* 2007) have shown rather consistent and encouraging beneficial results in the health management thanks to their potential effects as growth promoters.

Among the numerous metabolic processes, nucleotides modulate the lipid metabolism, particularly the long-chain polyunsaturated fatty acids 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 Δ-5-desaturase, as a result of increased protein synthesis; in fact, nucleotides are known to facilitate protein synthesis by increasing the availability of the precursors of RNA synthesis (Gil *et al.* 1986; Garcia-Molina *et al.* 1991); (*ii*) nucleotides may produce changes in the intestinal microflora that may affect long-chain polyunsaturated fatty acids levels, because bacteria possess enzymes necessary for fatty acid elongation and desaturation (Cosgrove 1998); (*iii*) nucleotides may modulate chain elongation and desaturation in the enterocyte or in the hepatocyte (Cosgrove 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 the accumulation of both n-6 and n-3 long chain polyunsaturated fatty acids in newborns (Gibson *et al.* 2005).

In consideration of the recent proposal of the European Parliament and Council to reduce strictly the use of chemical additives in animal nutrition (2004) and considering the scarce preferences of the consumers in industrialised countries to eat meats for their saturated fat content (Clauss 1991), the aim of this study was to evaluate the effect of a nucleotide pool supplemented in poultry diet on the physical and nutritional characteristics of the *Pectoralis major* muscle.

MATERIAL AND METHODS

Birds and diets. A total of 60 000 male broiler chickens Ross 508 genotype (Aviagen™, Newbridge, Midlothian, UK), one-day-old broiler chickens (mean weight 49.2 ± 6.2 g) were used in the feeding trial that lasted until the birds reached 52 days of age. In total, 2-floor pens (surface area 1800 m² per pen) were used, each containing 30 000 broiler chickens, called Control (C) and Nucleotides (N).

The birds were given *ad libitum* access to feed and water. A lighting schedule of 20L:4D was imposed

Table 1. Nutrient composition of basal diet in the different growing phases*

g/100g as fed	Growing phase (days)			
	1–10	11–21	22–40	41–52
Dry Matter	88	88	88	88
Crude Protein	25	23.5	21.4	21.4
Ether Extract	7	7.4	9.8	9.8
Crude fibre	2.7	2.8	2.9	2.9
Ash	6.4	6.1	6.0	5.7
Methionine	0.7	0.6	0.5	0.48

*basal diet provided per kg: vitamin A (12 000 IU), vitamin D_3 (5000 IU), α-tocopherol (80 mg), Cu (25 mg)

throughout the experimental period. Ambient temperature was gradually decreased from 32°C on day 1 to 20°C at the end of the experiment.

On day 1, birds were vaccinated against infectious bronchitis, Newcastle and Marek's diseases, whereas the Gumboro vaccination took place on day 18.

All the animals received a basal diet represented by a commercial complete feed containing: cereal grains, oilseed products and by-products (including soyabean meal), oils, fats and minerals in different proportions in relation to the age of the animals; the nutrient concentrations of the basal diet (Table 1) met the requirements according to the NRC (1994). The N group received, from the birth (24 h of age) to the slaughtering age (52 days), the basal diet supplemented with 0.1% of a nucleotide pool (Prosol S.p.A., Madone, Bergamo, Italy) containing adenosine, guanosine, cytidine, and uridine 5'-monophosphates (AMP, GMP, CMP, and UMP) in similar quantities, extracted from yeasts (*Saccharomyces cerevisiae*) through enzymatic hydrolysis.

The fatty acid composition of the diet administered in the finishing period (from day 41 to 52) was analysed by GC-FID (Agilent Technologies 6890N, Palo Alto, USA) with a split/splitless injector, a flame ionisation detector, and fused silica capillary column OMEGAWAX 250 (Supelco, Bellefonte, USA), 30 m × 0.25 mm *i.d.*, 0.25 μm film thickness. The column temperature was programmed: initial temperature of 100°C, increment of 4°C/min up to 200°C, and increment of 1.5°C/min up to the final temperature of 220°C. The temperature of the injector and detector were 250°C, injection volume 1.0 μl, carrier gas helium (1 ml/min), and split ratio 1:50. The identification of fatty acids was made by comparing the relative retention times of FAME peaks of the samples with the standards from Supelco (Bellefonte, USA). The chromatogram peak areas were acquired and calculated by Chemstation software (Agilent, Palo Alto, USA) and expressed in percentages of the identified total fatty acid methyl esters. The results are reported in Table 2.

Collection of animals and meat sampling. On 130 animals for each group, randomly selected, the final body weight was determined and used to calculate the body weight gain (final body weight – initial body weight/experimental period (52 days); moreover, at the end of the trial the feed intake was calculated to determine the feed conversion ratio (feed intake/body weight gain). Mortality and litter quality were evaluated, too. The litter score was visually determined by 4 experienced technicians, using a scale of 1 (very wet litter) to 10 (very dry litter) (Nollet *et al.* 2007).

Table 2. Fatty acid composition* (%) of the finishing phase (days 41 to days 52) of broiler chicken basal diet (mean ± SD)

Item	Mean	SD
ΣSFA[1]	40.30	1.35
ΣMUFA[2]	39.16	0.48
ΣPUFA[3]	20.54	0.66
ΣPUFA n-6	18.24	0.79
ΣPUFA n-3	2.30	0.20

*percentages of the total identified fatty acid methyl esters

[1]ΣSFA = $C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$ + $C_{20:0}$ + $C_{22:0}$

[2]ΣMUFA = $C_{16:1}$ + $C_{17:1}$ + $C_{18:1n9}$ + $C_{18:1n7}$ + $C_{20:1n9}$ + $C_{20:1n7}$

[3]ΣPUFA = $C_{18:2n6}$ + $C_{18:3n3}$ + $C_{20:2n6}$ + $C_{20:3n6}$ + $C_{20:4n6}$ + $C_{20:3n3}$ + $C_{20:5n3}$ + $C_{22:4n6}$ + $C_{22:5n3}$ + $C_{22:6n3}$

On day 52 of age, all birds were slaughtered after a 12-h feed deprivation period in identical conditions following the recommendations of European Union concerning animal care (European Union Directive 86/609/EEC 1986), using electrical stunning at 205 V to 225 V during 3 s, and were bled, scalded in a vertical bath at a temperature of 82.8°C/5 min, defeated, eviscerated, and rinsed. From each group, 130 carcasses (a total of 260 carcasses of the two groups) were randomly selected on the slaughter line, according to the slaughterhouse criteria, and stored at 4°C. On 24 h *post-mortem*, the carcasses were deboned and the breast muscles (*Pectoralis major*) without skin and adipose tissues were removed from each carcass, vacuum packed and chilled at 4°C until the analytical procedures.

Meat instrumental quality. The pH at 24 min *post mortem* (pH_{24}) was measured on each *Pectoralis major* muscle with a portable pH meter (HI9023, Hanna Instruments, Padova, Italy) equipped with a pH glass piercing electrode (Hamilton Double Pore^{TM}, Reno, USA). The pH meter was standardised by a 2-point method against standard buffers of pH 4.0 and pH 7.0.

The CIE system (CIE 1978) colour profile of lightness (L^*), redness (a^*), and yellowness (b^*) was measured by a reflectance colorimeter using the illuminant source D65. The instrumental colour measurements were performed using a desktop photometer (Desk-Top Spectral scanner, DV Tecnologie d'Avanguardia, Padova, Italy), calibrated throughout the study using a standard white ceramic reference. The colour was measured in single on the cranial, medial surface (bone side) in an area free of obvious colour defects (bruises, discolorations, haemorrhages, full blood vessels, picking damage, or any other conditions which might have affected a uniform colour reading). Hue (*H*) were calculated according to the following formula (Fraqueza *et al*. 2006): $H = \tan^{-1}(b/a)$.

The cooking losses were determined on the individual half breast samples, cooked by immersing the individual bags in 80°C water bath for several minutes until the internal temperature reached 75°C. After cooking, the bags were tempered at room temperature before opening to drain the liquid. The cooking yield was calculated by dividing the cooked weight by the prefreezing weight and multiplying by 100 (Liu *et al*. 2004).

Shear force values were determined using an analyser equipped with a Warner Bratzler shear cell (INSTRON 5542, Burlington, Canada). A 10-mm (diameter) core was removed from the thickest part of each cooked fillet. Each sample was sheared with blades at the right angle to the fibres using a 50-kg load cell and crosshead speed of 200 mm/minute. The shear force values are reported as kilograms of shear per cm^2 of sample.

Meat chemical, fatty acid, and mineral composition. Moisture, fat, protein, collagen, and salt contents in each sample were determined, according to AOAC (2007) methods, by using Near Infrared Spectroscopy in Transmittance (FoodScan^{TM} Meat Analyser; FOSS, Hilleroed, Denmark).

For the analysis of the acidic composition of intramuscular fats (Perfiled *et al*. 2002) on the individual lyophilised samples of meat, lipids were extracted by Bligh and Dyer (1959) method using a mixture of chloroform/methanol (2:1, v/v) and fatty acids methyl esters of the intramuscular fat were prepared by direct transesterification with sulphuric acid/methanol (1:9, v/v) of a weighed portion (15 mg) of the total lipids (Christie 1993) and analysed using the HRGC (Agilent Technologies 6890N, Palo Alto, USA) with a split/splitless injector, a flame ionisation detector, and fused silica capillary column OMEGAWAX 250 (Supelco, Bellefonte, USA), 30 m × 0.25 mm *i.d.*, 0.25 μm film thickness. The 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 were 250°C, injection volume: 1.0 μl, carrier gas helium (1 ml/min), and split ratio 1:50. The identification of fatty acids was made by comparing the relative retention times of FAME peaks of the samples with the standards from Supelco (Bellefonte, USA). Chromatogram peak areas were acquired and calculated by Chemstation software (Agilent, Palo Alto, USA) and expressed in percentages of the identified total fatty acid methyl esters.

On the basis of the fatty acid identified, the quality indices were calculated using the equations proposed by Ulbricht and Southgate (1991).

Phosphorus (P) and iron (Fe) contents were determined by using atomic absorption spectrophotometer (Cosgrove 1998). An aliquot of 0.8 g of the meat sample was mixed with HNO_3/H_2O_2 (4:1) and mineralised in a microwave oven using Teflon PFA reactors, equipped with a system of pressure and temperature regulation (by means of a vessel acting as a sensor). The same process was followed with a blank solution, prepared only with HNO_3/H_2O_2 (4:1). After mineralisation, the

samples and blank solutions were made up to 25 ml with ultrapure water for iron analysis, and then diluted 1:4 for the analysis of phosphorous. All the glassware was treated with a diluted solution of HNO_3 (0.1%) to prevent contamination.

The concentrations of phosphorus and iron were determined by two different atomic absorption methods:

– graphite furnace atomic absorption spectrometry (GFAAS) for phosphorus determination;
– air-acetylene flame atomic absorption spectrometry (FAAS) for iron determination.

The atomic absorption spectroscopic determination of phosphorus was carried out with a Perkin Elmer AAnalyst 700 (Shelton, USA) spectrophotometer, equipped with a single-element hollow cathode lamp (current 35 mA), WinLab32 software for electronic processing of the results, a Perkin Elmer AS 800 Autosampler (Shelton, USA) and Perkin Elmer Graphite Tubes, using a background correction mode. Phosphorus standard solutions were prepared starting from the concentration of 1 mg/ml in a blank reagent solution. The analytical concentrations, used to construct the calibration curve (5, 15, and 30 mg/l), were obtained by auto-dilution with an auto-sampler. The calibration curve parameters: correlation coefficient 0.991, slope 0.0485, intercept 0.01016. A matrix modifier solution, consisting of a mixture of Pd (20 µl) and $CaCO_3$ (5 µl), was added to each sample, reagent blank solution and standards were used to construct the calibration curve.

The atomic absorption spectroscopic determination of iron was carried out with a Perkin Elmer AAnalyst 700 spectrophotometer (Shelton, USA), equipped with a single-element hollow cathode lamp (current: 30 mA), WinLab32 software for electronic

processing of the results, and a Perkin Elmer AS 90 plus Autosampler (Shelton, USA). For atomisation, air/acetylene flame was used. Iron standard solutions were prepared by serial dilution of a primary solution of 1 mg/ml in a blank reagent solution to obtain the analytical concentrations (0.1, 0.2, and 0.5 mg/l) used for the calibration curve. The calibration curve parameters: correlation coefficient 0.999, slope 0.031, intercept 0.00032.

Statistical analysis. The data were analysed by ANOVA using the GLM procedure of SAS (2001) with a model that included the treatments effects (Control and Nucleotides) and experimental error. Individual animals were considered as experimental units. The means were separated by means of the Least Significant Difference (*LSD*) test when a significant ($P < 0.05$) treatment was observed.

RESULTS AND DISCUSSION

The N group showed a significantly higher final body weight (3300 g vs 3159 g; $P < 0.001$) and body weight gain (62.60 g/day vs 59.54 g/day; $P < 0.01$) in accordance with Esteve-Garcia *et al.* (2007) and Tipa (2002). No significant differences were observed for the feed conversion ratio (Nucleotides 2.24 kg/kg vs Control 2.34 kg/kg), litter score (Nucleotides 5.6 vs Control 5.5; $P = 0.733$), and mortality (Nucleotides 3.25 vs Control 3.20%).

Meat physical characteristics

No significant difference between the treatments was observed with pH, lightness, and cooking loss (Table 3) in muscle, whereas a significant increase

Table 3. Analysis of variance, least-squares means and SEM of the physical characteristics of the *Pectoralis major* muscle of broiler chickens fed diets supplemented or not with a nucleotide pool

Item	Control	Nucleotides	SEM	*P*-values*
pH_{24}	5.75	5.77	0.219	0.300
Lightness (*L**)	59.78	50.50	4.159	0.503
Redness (*a**)	10.82	11.58	1.712	< 0.001
Yellowness (*b**)	12.54	11.18	2.491	0.091
Hue (*H*)	0.86	0.77	0.092	< 0.001
Cooking loss of ground meat (%)	15.88	16.38	3.833	0.62
Shear value of ground meat (kg/cm^2)	1.18	0.97	0.217	< 0.001

*ANOVA of average data in relation to the treatments effects (Nucleotides vs Control)

of the redness and a significant decrease of the Hue values in the N group were observed. These results are in agreement with those by Zhang *et al.* (2008) that showed, in broiler chickens fed with increasing doses of inosinic acid, a reduction of the lightness and yellowness values and an increase of the redness value which contributed to the amelioration of the meat colour. These results could be related to a better intestinal absorption of iron as observed in rats (Faelli & Esposito 1970), as a factor that improves iron bioavailability (Lerner & Shamir 2000; Mateo & Stein 2007). Table 3 shows the effect of dietary supplementation with Nucleotide pool on shear force value. The breast muscle of the N group had significantly lower shear force values in comparison with the C group, these results could have been caused by the effect of dietary supplementation with Nucleotide pool on collagen fibril and on calpain enzymatic activity (Zhang *et al.* 2008) and by a higher ether extract content of the N group (Table 4).

Nutritional characteristics of the meat

The chemical composition of the *Pectoralis major* muscles is reported in Table 4. The results, expressed in g/100 g of edible part, are in agreement with those obtained by others (Castellini *et al.* 2006; Cavani *et al.* 2009; INRAN 2010).

No significant difference was observed for protein and energy contens between the groups. Whereas, nucleotides administration influenced significantly (*P* < 0.001) the ether extract content, showing higher values in the N group than in the C group;

this could be related to the physiologic effect of nucleotides to stimulate the plasmatic α-lipoprotein synthesis (Schlimme *et al.* 2000; Mateo & Stein 2007) and to increase the haematic levels of high density lipoprotein during the neonatal period (Sanchez-Pozo *et al.* 1996).

Moreover, the significantly (*P* < 0.001) higher value of ash observed in the N group could have a twofold explanation: (*i*) the chemical structure of nucleotides where are, among the three components of the structure, one or more phosphate groups and (*ii*) nucleotides stimulate the iron absorption at the intestinal level (Cosgrove 1998) through the conversion of purine nucleotides (AMP GMP) to inosine, hypoxanthine, and uric acid which increase the absorption of iron. In fact, the iron content of the meat shows a significantly (*P* < 0.05) higher value in the nucleotides group (Table 4).

As regards the phosphorus content (Table 4), the analysis performed on muscle tissue showed a slightly higher mean value in the nucleotides group, even though the difference is not significant. This could be due to a higher accumulation of phosphorus, as hydroxyapatite, in the skeletal structure compared with the accumulation in the muscle tissue (Al-Masri *et al.* 1995) as also confirmed by the significantly (*P* < 0.001) higher value of the final body weight (52 days) of the animals of the N group.

Concerning the fatty acid composition (Table 5), dietary nucleotides caused a significant decrease of palmitic acid ($C_{16:0}$) and a significant increase of oleic acid ($C_{18:1}$). With regard to the essential fatty acids and their longer superior homologous fatty

Table 4. Analysis of variance, least-squares means and SEM of the nutritional characteristics of the *Pectoralis major* muscle of broiler chickens fed diets supplemented or not with a nucleotide pool

Item	Control	Nucleotides	SEM	*P*-values*
Moisture (g/100 g)	75.14	74.59	0.07	0.062
Protein (g/100 g)	22.19	22.19	0.04	0.993
Collagen (g/100 g)	1.58	1.56	0.02	0.542
Ether extract (g/100 g)	1.66	1.96	0.02	< 0.001
Ash (g/100 g)	1.01	1.26	0.01	< 0.001
Salt (g/100 g)	2.04	2.01	0.03	0.326
Energy (kj/kg)	4332	4445	3.45	0.602
Iron (mg/100 g)	0.387	0.467	0.001	0.025
Phosphorus (mg/100 g)	200.73	207.46	1.72	0.116

*ANOVA of average data in relation to the treatments effects (Nucleotides vs Control)

Table 5. Analysis of variance, least-squares means and SEM of some polyunsaturated fatty acids (%)* of nutritional interest in the *Pectoralis major* muscle of broiler chickens fed diets supplemented or not with a nucleotide pool

Item	Control	Nucleotides	SEM	*P*-values**
$C_{16:0}$	22.75	21.83	1.18	0.004
$C_{18:1n9}$	28.78	30.95	2.14	< 0.001
$C_{18:2n6}$	11.94	12.19	0.16	0.283
$C_{18:3n3}$	0.55	0.66	0.01	< 0.001
$C_{20:4n6}$	3.41	3.51	0.13	0.610
$C_{20:5n3}$	0.62	0.43	0.02	< 0.001
$C_{22:6n3}$	2.13	1.18	0.06	< 0.001

*percentages of the total identified fatty acid methyl esters

**ANOVA of average data in relation to the treatments effects (Nucleotides vs Control)

acids (Table 5), a significant increase ($P < 0.001$) of the percentage of α-linolenic acid ($C_{18:3n3}$), and a significant decrease ($P < 0.001$) of eicosapentanoic ($C_{20:5n3}$) and docosahexanoic ($C_{22:6n3}$) acids were observed in the N group; no significant difference was observed between the groups for linoleic ($C_{18:2n6}$) and arachidonic ($C_{20:4n6}$) acids.

The acidic classes (Table 6), showed a significantly ($P < 0.001$) lower values of the sum of saturated fatty acids ($C_{13:0} + C_{14:0} + C_{15:0} + C_{16:0iso} + C_{16:0} + C_{17:0} + C_{18:0iso} + C_{18:0} + C_{20:0} + C_{22:0}$) and significantly ($P = 0.0002$) higher values of the sum of monounsaturated fatty acids ($C_{16:1} + C_{17:1} + C_{18:1\,n9} + C_{18:1n7} + C_{20:1n9} + C_{20:1n7}$) in the treated group; in par-

ticular, it has been showed that MUFA have beneficial effects on blood cholesterol and other health related outcomes in humans (WILLIAMS *et al.* 1999).

No significant differences (Table 6) were observed for the sum of the polyunsaturated fatty acids ($C_{16:4n1} + C_{18:2\,n6} + C_{18:3n3} + C_{20:2n6} + C_{20:3n6} + C_{20:4n6} + C_{20:3n3} + C_{20:5n3} + C_{22:4n6} + C_{22:5n6} + C_{22:5n3} + C_{22:6n3}$) and for the n-6 PUFA, whereas, significantly lower percentages for the n-3 PUFA ($P < 0.001$) were observed in the N group. These results are in accordance with those observed by BOZA *et al*. (1992) in weanling rats fed nucleotides.

A hypothesis could be considered that nucleotide supplementation stimulated the intestinal or he-

Table 6. Analysis of variance, least-squares means and SEM of the fatty acid composition* (%) in the *Pectoralis major* muscle of broiler chickens fed diets supplemented or not with a nucleotide pool

Item	Control	Nucleotides	SEM	*P*-values**
Σ SFA[1]	40.75	38.55	0.21	< 0.001
Σ MUFA[2]	37.29	40.07	0.50	< 0.001
Σ PUFA[3]	21.96	21.38	0.44	0.363
Σ PUFA n-3	4.40	3.27	0.12	< 0.001
Σ PUFA n-6	13.61	14.00	0.21	0.188
UFA/SFA	1.53	1.59	0.01	0.002
PUFA/SFA	0.54	0.55	0.01	0.449
Atherogenic index	0.47	0.44	0.01	0.001
Thrombogenic index	0.89	0.91	0.01	0.272

*percentages of the total identified fatty acid methyl esters

**ANOVA of average data in relation to the treatments effects (Nucleotides vs Control)

[1]Σ SFA = $C_{13:0} + C_{14:0} + C_{15:0} + C_{16:0iso} + C_{16:0} + C_{17:0} + C_{18:0iso} + C_{18:0} + C_{20:0} + C_{22:0}$

[2]Σ MUFA = $C_{16:1} + C_{17:1} + C_{18:1\,n9} + C_{18:1\,n7} + C_{20:1n9} + C_{20:1n7}$

[3]Σ PUFA = $C_{16:4n1} + C_{18:2\,n6} + C_{18:3n3} + C_{20:2n6} + C_{20:3n6} + C_{20:4n6} + C_{20:3n3} + C_{20:5n3} + C_{22:4n6} + C_{22:5n6} + C_{22:5n3} + C_{22:6n3}$

patic synthesis of the elongation enzymes and all the desaturase enzymes (Δ9-,Δ6-, Δ5-desaturase) as a result of increased protein synthesis (Gil *et al.* 1986; Garcia-Molina *et al.* 1991) for the metabolic conversion of acidic products as reported by Schlimme *et al.* (2000) but: (*i*) in relation to the individual fatty acid levels and (*ii*) in relation to the affinity of the desaturase enzymes with the fatty acid classes, the effect of stimulation was remarkable only for the Δ-9-desaturase.

These findings suggest that no induction occurred of long chain polyunsaturated fatty acid accumulation in *Pectoralis major* muscle owing to the dietary supplementation with nucleotides in accordance to Gibson *et al.* (2005) observations. This could mean that the effectiveness of the nucleotide supplementation in relation to the lipid metabolism depends on the dietary EFA levels.

From the nutritional point of view, the decrease of the polyunsaturated fatty acids, because of potentially adverse health effects of their lipoperoxidation products, is in line with the recommendation for human nutrition (Williams *et al.* 1999).

The UFA/SFA ratio (Table 6) was significantly higher ($P = 0.002$) in the group treated according to Gil *et al.* (1988) but the PUFA/SFA ratio showed no significant differences between the groups.

Regarding the quality indices (Table 6), which are important indices for the nutritional evaluation of fat, no significant difference was observed for the Thrombogenic index, whereas the supplementation positively influenced the Atherogenic index resulting in a significant decrease ($P \leq 0.01$) of this index and suggesting that dietary nucleotide may protect against the early development of atherosclerosis (Cosgrove 1998).

CONCLUSION

During the early stage of life the Nucleotides supplementation in broiler chickens diet modulated the UFA synthesis, promoting the growth and health of the animals. From the nutritional point of view, the *Pectoralis major* muscle of the broiler chickens that received, from the birth to the slaughtering age, the basal diet supplemented with 0.1% of a nucleotide pool showed better physical and nutritional characteristics. In fact, the nucleotide administration improved the tenderness and the Hue value of the meat and also increased the lipid content and the iron level. Moreover, the fatty acid composition of the *Pectoralis major* muscle of the N group showed an increase of the percentage of the monounsaturated fatty acids and, consequently, an increase of the unsaturation degree and a decrease of the atherogenic index with beneficial effects for the human nutrition. In this regard, it has been shown (Williams *et al.* 1999) that MUFAs have beneficial effects on blood cholesterol and other health related outcomes in humans (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, precaution should be taken against recommending high dietary polyunsaturated fatty acids for humans, because of the potentially adverse health effects of their lipoperoxidation products (Williams 2000).

In conclusion, the results showed that the effectiveness of nucleotide supplementation in relation to the lipid metabolism depends on the dietary EFA levels.

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