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Effects of dietary ω-3 fatty acids content on productive performances and meat quality of Martina Franca donkey foals

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Titolo
Effetti di una dieta contenente acidi grassi ω-3 sulle performance produttive e sulla qualità della carne di puledri asinini di razza Martina Franca

Key words
PUFA, ω3, donkey, meat quality

Parole chiave
PUFA, ω3, asino, qualità della carne

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Lavoro eseguito con fondi Murst 60%

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Summary
To investigate the effect of diets containing fatty acids of the ω-3 series on productive traits, on carcass cut composition, on meat chemical composition as well as on meat fatty acid composition, eight Martina Franca donkey foals were utilized. The animals, weaned at 8 months of age, were divided into two groups of 4 subjects each, homogeneous for age and live weight. The first group (control) was given wheat straw and a complete feed non supplemented with ω-3; the second (trial) was given wheat straw and a ω-3 supplemented complete feed. All the animals were slaughtered after 138 days of trial. From the collected data, it emerges that the utilization of ω-3 enriched feed, even though with different levels of statistical significance ($P \leq 0.05$ and/or $P \leq 0.01$), determines differences in the content of fat, meat and bone of shoulder and loin and in the meat ashes percent. As regards the meat fatty acids distribution, no remarkable differences are observed as to the saturated series, whereas the unsaturated one ($P \leq 0.05$) seems to increase the content of $C_{18:3\omega3}$ (ALA - α-linoleic), of $C_{22:5\omega3}$ (DPA – docosapentenoic acid), of $C_{22:6\omega3}$ (DHA – docosahesanoic acid) and of UFA, PUFA and total ω-3 acids.

Riassunto
Per valutare l'incidenza sugli aspetti produttivi, sulla composizione in tagli di carcassa, su quella chimica delle carni ed acidica del loro grasso, di razioni contenenti acidi grassi della serie ω-3, sono stati utilizzati 8 puledri di asini di Martina Franca, svezzati a 8 mesi di vita, suddivisi in due gruppi di 4 soggetti ciascuno omogenei per età e peso vivo. Il primo gruppo (controllo) è stato alimentato con paglia di frumento e mangime completo privo di ω-3; il secondo (sperimentale) è stato allevato con paglia e mangime contenente ω-3. Tutti gli animali sono stati macellati dopo 138 giorni di prova. Dai dati raccolti, l'impiego di mangime contenente ω-3, anche se con diverso livello di validità statistica ($P \leq 0{,}05$ e/o $P \leq 0{,}01$), determina differenze nel contenuto in grasso, carne ed osso della spalla e della lombata e nella percentuale di ceneri della carne. Per quanto concerne la distribuzione acidica del grasso della carne non si osservano differenze degne di nota a carico della serie satura, mentre quella insatura sembra ($P \leq 0{,}05$) aumentare il contenuto di $C_{18:3\omega3}$ (ALA – α-linolenico), di $C_{22:5\omega3}$ (DPA – acido docosapentenoico), di $C_{22:6\omega3}$ (DHA – acido docosaesaenoico) e degli UFA, PUFA e degli ω-3 totali.

Introduction

In the last 50 years, the total per capita consumption of meat in Italy has passed from 20.5 kg in 1955 to 91.1 kg in 2002 (1). Horse meat consumption has sensibly increased in the 1997-2001 quinquennium (2). In Apulia, where the reared horse population reaches 7,500 animals approx., 78,000 horses are slaughtered yearly, corresponding to the 34% of the horses slaughtered every year in Italy, the total amount of which is equal to 137,644 animals (3). It stands clear that the regional requirements are above all safisfied with both imported carcasses and live horses for slaughter.
In 2005, 90,010 horses were imported in Italy, equal to the 65.44% of the whole requirement, the 1.10% of which was represented by asses, mules and hinnies (3). The Martina Franca ass, an autochthonous dying breed (4), like similar realities (5), could be reared to produce quality meat thus satisfying the requirements of the modern consumer who, more and more attentive to the relation nutrition-health, more and more prefers food – and therefore meat – poor in fat and cholesterol, but rich in polyunsaturated fatty acids. The interest towards the latter and, in particular, the ω-3 ones depends on the role they play against neoplastic (6, 7) ad cardiovascular (8-12) diseases.

Table 1 - Feed composition (% DM)

	Control	Group ω-3
Wheat middlings	22	22
Alfalfa meal dehy.	20	20
Wheat straw	11	11
Barley	17	17
Carob pulp wo seeds desiccated	3.5	3.5
Beet pulp dehy.	4.7	4.7
Sugar cane melasses	2.5	2.5
Soybean meal solv. extd.	9	9
Sunflower flour	3	3
Coconut oil	1.5	-
Plus ω3 oil	-	1.5
Dicalcium phosphate	2.5	2.5
Calcium carbonate	0.8	0.8
Bentonite	0.3	0.3
Sodium-chloride	0.5	0.5
Sodium dicarbonate	0.2	0.2
L-lysine	0.5	0.5
Irradiated yeast	0.4	0.4
Magnesium carbonate	0.1	0.1
DL-methionine	0.2	0.2
L-threonine	0.1	0.1
Vit.-min. Suppement:	0.2	0.2
- Vit. A (U.I.)	12500	12500
- Vit. D_3 (“)	1300	1300
- Vit. E (mg)	16	16
- Vit. B_1 (“)	3	3
- Vit. PP (“)	150	150
- Vit. B_{12} (“)	0.008	0.008
- Choline chloride (“)	75	75
- Cobalt (“)	7.5	7.5
- Iron (“)	30	30
- Iodine (“)	1.5	1.5
- Manganese (“)	130	130
- Cupper (“)	6	6
- Selenium (“)	0.08	0.08
- Zinc (“)	65	65

The quality of the carcass and meat depends by several factors, such as genotype, rearing system, feeding, sex, slaughter techniques and physiological conditions (13), even though some authors assert that the meat fatty acid composition might depend on the carcass adiposity degree (14-17). In horses, the meat fatty acid composition can be improved within the limits allowed by the animal genotype, since the alimentary ones are basically deposited unchanged in the adipocytes as triglycerides. To support this thesis, several authors evidenced that it is possible to improve both meat quality by increasing the content of fatty acids (PUFA) and fat composition through feeding (18-26).
The literature we consulted reports interesting news on the quality of horse meat (17, 27-30, 31-35), but is rather poor as to asses (36, 37). On the grounMds of what exposed, it seemed to us interesting to deepen our knowledge on this subject.
This work aimed at studying the influence of complete feeds supplemented with oil plus ω-3 on productive performances of Martina Franca breed donkey foals, on the cut percent of their carcasses, on the chemical composition of meat and the acid distribution of fats extracted from them. To complete the research, it seemed interesting, due to the lack of news in literature, to evaluate the effect of such a treatment on the cholesterol content of the meat, on account of the action exercized by lipids on the diet and on the plasma level of cholesterol (38).

Table 2 - Fatty acid composition plus-ω-3 oil and coconut oil (%)

	Plus ω-3 oil	Coconut oil
C14:0	1.54	16.80
C16:0	14.81	13.50
C18:0	5.45	4.00
C16:1ω-7	1.68	-
C18:1ω-9	23.12	11.05
C18:2?6	14.56	2.00
C18:3ω3 - ALA	19.63	-
C20:5ω-3 - (EPA)	1.90	-
C22:6ω-3 - (DHA)	7.08	-
ω6	15.24	2.00
ω3	28.61	-
SFA	23.12	84,11
MUFA	27.23	11.05
PUFA	45.71	2.00
Other fatty acids	3.74	2.84

Table 3 - Chemical diet composition (%DM)

	Concentrate	Straw
Dry matter	82.15	89.80
Crude protein	14.28	3.30
Ether extract	3.42	1.57
NDF	29.83	89.80
Crude cellulose	5.59	7.87
Digestible energy (MJ/kg DM)	12.05	1.60
Lysine	0.82	0.23

Table 4 - Performances of the fattening foals

		Control	Group ω-3	ESD (6 = DF)
Foals	n.	4	4	
Days of trial	d	138	138	-
Initial live weight	kg	133.50	132.00	4.813
Final live weight	kg	207.50	207.75	5.127
Weight gain (180-304 die)	g/d	536.23	548.91	0.025

Materials and methods

The research, carried on at the experimental farm "V. Ricchioni" of the Department of Animal Production of the University of Bari, aimed at assessing the effects of a diet enriched with fatty acids of the ω-3 series on the productive traits, the carcass cut composition, the chemical and acid composition of meat from donkey foals of Martina Franca breed.

For this purpose, eight male whole donkey foals of Martina Franca breed, weaned at 8 months and with an average live weight of 133 kg, separared into two groups of 4 animals each, homogeneous for age and weight, were reared receiving diets differing only in the kinf of lipid supplementation (Table 1).

The first group (control) was fed on a diet containg wheat straw and a complete feed supplemented with 1.5% cocoanut oil; the second, the experimental one (ω-3 group) received the same straw and a complete feed supplemented with 1.5% oil plus ω-3 (a mixture of fish and linseed oils) (39) (Table 2).

The feeds in both the adaptation and fattening stages were given twice a day at 7.00 a.m. and 5 p.m., with a progressive quantity up to the consumption of 2-3% d.m. of the animal liveweight. In the experimetal phase, the animals registered a consumption of 4.195 g d.m./head/day corresponding to

Table 5 - Daily intake and conversion index

		Control	Group ω-3	ESD (6 = DF)
Mean daily intakes				
Feed	g	5099.50	5100.00	0.022
Dry matter	g	4194.50	4195.00	0.018
DE	MJ/Kg	49.89	49.90	0.652
Conversion index				
Feed./gain		9.51	9.51	0.012
DM/gain		7.82	7.64	0.584
DE/gain		93.04	90.90	2.180

Table 6 - Slaughtering data

		Control	Group ω-3	ESD (6 = DF)
Foals	n	4	4	
Fasting body weight	Kg	195.75[a]	189.25[b]	1.674
Carcass weight	Kg	113.12	112.75	1.719
Yield hot	%	57.79	59.58	0.021
Reconstituted right half carcass	%	49.01	48.62	1.949
Carcass length	cm	130.50	135.25	1.274
Pelvic limb length	cm	49.87	53.87	1.062
Largest pelvic limb width	cm	37.50	35.50	1.001
Warm dressing-out	%	53.95	54.29	0.650
	% Empty body weight			
Hide		7.19[b]	7.68[a]	0.276
Empty gastrointestinal-tract		7.04[b]	9.77[a]	0.835
Head		5.21	5.41	0.109
Liver		1.49	1.66	0.096
Spleen		0.65	0.74	0.014
Kidney		0.17	0.18	0.008
Limbs distal part		2.53	2.69	0.157
Heart, lungs and trachea		1.96	1.99	0.063
	% Empty body weight			
Neck		7.98	8.69	0.676
Proximal thoracic limb		11.55	10.94	0.507
Steaks		8.68	8.74	0.100
Distal thoracic limb		5.17	4.26	0.465
Brisket		17.67	17.60	1.343
Abdominal region		13.78	13.17	1.55
Lumbar region		12.97	13.20	0.372
Proximal pelvic limb		30.20	29.79	0.583
Distal pelvic limb		4.26	4.50	0.229

the quantity suggested by Lewis (40).
This manner of diet supply was necessary since horses don't regulate so much the assumption of food on the repletion state of the gastro-enteric tract as on the organoleptic characteristics of the diet (41). The feeds were analyzed according to the Italian Association of Animal Production (ASPA) recommendations (42) (Table 3) and the nutritive value was evaluated according to NRC recommendations (43).
At slaughter, carried out after 138 days of trial, according to local habits, but keeeping as near as possible to ASPA recommendations (44), the data in Table 5 were collected. The fat, lean and bone per cent composition was assessed on the shoulder, leg and loin cuts (Table 6). Afterwards, on a muscle homogeneous sample taken from these cuts the chemical composition was determined, whereas on the *longissimus dorsi* and *quadriceps femoris* (qf) the content in cholesterol and the chemical composition of the meat as well as of the feeds were carried out following ASPA procedures (45). The acid distribution of their fat, after methylation, was determined by a gas-chromatography system, equipped with capillary colums of 60 m in silicated glass with stationary phase in 100% cyanoprile (46). The cholesterol present in the meat was determined by high-resolution liquid chromatography HPLC (47). Moreover, the ratio PCL/PCE (plasma cholesterol lowering/plasma cholesterol elevating) (48) and the atherogenicity and thrombogenicity indexes were calculated (49).
The data collected were subjected to analysis of variance according to

Table 7 - In vivo measurements

		Control	Group ω-3	ESD (6 = DF)
Foals	n.	4	4	
Withers height	cm	116.50	110.75	3.259
Croup height	cm	124.00	122.50	1.779
Thorax height	cm	50.00	52.50	1.581
Thorax breadth	cm	69.50	67.50	1.316
Croup length	cm	46.25	44.00	1.677
Croup breadth	cm	26.75	28.50	1.513
Thorax circumference	cm	36.50	38.00	1.779
Shin circumference	cm	125.00	126.75	2.596
Withers height	cm	19.00	19.00	-

Table 8 - Composition of some carcass cuts

		Control	Group ω-3	ESD (6 = DF)
Proximal thoracic limb				
- lean	%	72.14[A]	65.59[B]	1.462
- fat	%	6.73[B]	9.61[A]	0.275
- bone	%	20.85[b]	24.43[a]	1.527
Lumbar region				
- lean	%	62.45[A]	56.49[B]	0.579
- fat	%	11.00[B]	16.11[A]	1.201
- bone	%	26.78	27.35	0.988
Distal pelvic limb				
- lean	%	69.15	67.87	2.282
- fat	%	15.472	17.639	2.514
- bone	%	14.498	15.729	1.518

On the same row A, B: P<0,01; a, b: P<0,05

Table 9 - Chemical composition and meat quality (% on edible substance)

	Control	Group ω-3	ESD (6 = DF)
Samples	4	4	
Moisture	71.83	71.74	0.781
Protein	21.80	22.09	0.235
Crude fat	4.54	3.99	0.624
Ash	0.99[b]	1.24[a]	0.101
N-free extract	0.84	0.94	0.074
Cholesterol (mg/100 g)			
– *longissimus lumborum*	57.2	50.7	0.046
– *quadriceps femoris*	58.1	50.3	0.039

Table 10 - Saturated fatty acid composition of the intramuscolar fat (%)

	Control	Group ω-3	ESD (6 = DF)
$C_{6:0}$	0.30	0.10	0.057
$C_{8:0}$	0.10	-	-
$C_{10:0}$	0.20	0.10	0.021
$C_{12:0}$	1.02	-	-
$C_{14:0}$	4.02	2.42	0.079
$C_{15:0}$	0.15	0.15	0.008
$C_{16:0}$	29.57	28.86	0.184
$C_{17:0}$	0.32	0.35	0.010
$C_{18:0}$	4.65	4.70	0.193
$C_{20:0}$	0.05	0.05	0.018
$C_{22:0}$	1.00	1.00	0.006
$C_{24:0}$	0.10	0.10	0.971
SFA	41.48	37.83	0.042

GLM procedures (50); the T Student's test was used to determine significant differences in mean values.

Results and discussions

The research evidenced that the donkey foals from both groups did not present valid differences as regards the daily gains. In fact, the last oscillated around 540 g with a slighter prevalence of group B (548.91 g/d *vs* 536.23 g/d of control group) (Table 4).

The best conversion indexes were obtained with the diet enriched with ω-3 (7.64 kg of d.m./kg *vs* 7.82 kg of control group), trend reflected on energy (DE), where a better conversion of ω-3 group (90.90 Mj/kg *vs* 93.04 MJ/kg, of control group) was evidenced (Table 5).

The somatic measurements, taken before slaughter (Table 7), offered conformation data not far from the standards of Martina Franca breed, even considering that they were very young animals, rejected by the selection programmes of breed committee.

At the end of the trial, the element that differentiated the two groups was the weight loss after fasting. In fact, whereas the mean live weight was similar for the two groups, the body weight after fasting was higher in the control group (195.75 kg *vs* 189.25 kg) ($P \leq 0.05$). As regards the warm dressing-out we detected more favourable values for group B (59.58% *vs* 57.79%), even without any statistical support (Table 6).

The conformation of the carcass and its cutting did not present any significative differences except for the reduced per cent incidence of

skin ($P \leq 0.05$) (7.19% *vs* 7.68% in favour of control group) and a reduced incidence ($P \leq 0.05$) of the gastro-intestinal tract in the animals of ω-3 group (Table 6).
The single cuts of the carcass showed nearly always data a little higher for ω-3 group, but without any statistical relevance (Table 6).
After dissection (Table 8), we noticed that the fat of shoulder and loin was in per cent higher in the control group than in the experimental one (72.14 *vs* 65.59%) and (62.45% *vs* 56.49%) ($P \leq 0.01$), respectively.
The different diets did not significantly influence the chemical composition of meat; in fact, the meat of donkey foals from the experimental group was only slightly richer in ashes ($P>0.05$) and poorer in cholesterol (Table 9). Such values are in line with what reported by Catalano and Quartarelli (27) and Pinto et al. (26).
As concerns the fatty acid distribution, the kind of feed didn't seem to have any influence at the level of saturated fatty acids (Table 10); on the contrary, the unsaturated ones seemed to depend significantly from the diet (Table 11). In fact, the meat fat of the ω-3 group animals was significantly richer ($P > 0.05$) in CLA (2.45% *vs* 1.45%), UFA (58.52% *vs* 61.72%), PUFA (12.95% *vs* 16.10%), ω-3 (3.51% *vs* 1.77%) and with a more favourable ratio ω-6/ω-3 (6.10% *vs* 3.36%),

Table 11 - Unsaturated fatty acid composition (% on total) and qualitative indexes of the intramuscolar fat

	Control	Group ω-3	ESD (6 = DF)
$C_{14:1}$	0.55	0,55	0.058
$C_{15:1}$	0.08	0.08	0.024
$C_{16:1}$	6.97	6.85	0.844
$C_{17:1}$	0.55	0.55	0.071
$C_{18:1\ ?7}$	1.97	2.01	0.241
$C_{18:1\ ?9}$	35.04	35.15	0.073
$C_{18:2\ ?6}$	9.79	10.45	0.094
$C_{18:3\ ?3}$ – ALA	1.25[b]	2.45[a]	0.022
$C_{18:3\ ?6}$	0.35	0.37	0.241
$C_{20:1?9}$	0.09	0.10	0.754
$C_{20:2\ ?6}$	0.35	0.44	0.241
$C_{20:3\ ?3}$	0.10	0.10	0.489
$C_{20:3\ ?6}$	0.05	0.18	0.041
$C_{20:4\ ?6}$	0.15	0.36	0.058
$C_{20:4\ ?3}$	0.22	0.54	1.008
$C_{20:5\ ?3}$ (EPA)	0.10	0.16	0.042
$C_{22:1\ ?9}$	0.19	0.25	0.145
$C_{22:4\ ?6}$	0.05[b]	0.45[a]	0.028
$C_{22:5\ ?3}$ (DPA)	0.07[b]	0.35[a]	0.053
$C_{22:6\ ?3}$ (DHA)	0.35	0.45	0.041
$C_{24:1\ ?9}$	0.25	0.36	0.041
UFA	58.52[b]	62.17[a]	1.026
MUFA	45.25	45.26	0.175
PUFA	13.02[b]	16.55[a]	1.820
ω6	10.74	12.25	2.089
ω3	1.87[b]	3.51[a]	0.791
ω6/ω3	5.74[a]	3.49[b]	0.185
Unsatured/Satured	1.41	1.64	0.101
Index of atherogenicity (AI)	0.81	0.63	0.149
Index of thrombogenicity (TI)	1.10	0.89	0.103
Saturated/Polynsaturated	3.19	2.29	0.316
PCL/PCE	1.03	1.25	0.220

UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids;
AI = atherogenicity index = (lauric + 4 x myristic + palmitic) / (ω6 PUFA + ω3 PUFA + MUFA); Ulbricht and Southgate (1991).
TI = thrombogenicity index = (myristic + palmitic + stearic) / [0.5 x ω6 PUFA + 3 x ω3 PUFA + 0.5 x MUFA + (ω3 PUFA /ω6 PUFA)]; Ulbricht and Southgate (1991).
PCL/PCE = (plasma cholesterol lowering)/(plasma cholesterol elevanting)

datum nearer to the value of 4 as recommended by the Human Nutrition Society (52, 53), since near this value positive effects for human health are evidenced (54). As to EPA, the relevant values, even without any statistical value, seemed to be more favourable to group B (0.10% *vs* 0.16%) as well as for DHA ($P \leq 0.05$) (0.05% *vs* 0.45%). It is worth stressing that, for the consumer's health, these two ω-3 fatty acids have a different influence on triglyceride plasma levels: EPA determines a reduction of triglyceride plasma rate, DHA reduces HDL cholesterol level in plasma (55, 56). Moreover, EPA competes with the arachidonic acid, thus influencing the platelet activity (57) and thrombogenesis (58-61).

Conclusions

The research, under our experimental conditions, evidenced that the use of ω-3 fatty acids in diets for meat donkey foals, even not influencing in a significative manner the productive traits, the half side cut composition, the meat chemical and/or saturated fatty acid composition, significantly influenced and improved the content of polyunsaturated fatty acids such as C18:3ω3 (ALA - α-linoleic), C22:5ω3 (DPA – docosapentaenoic acid), C22:6ω3 (DHA – docosahexaenoic acid) and UFA, PUFA, ω-3 per cent and the ratio ω-6/ω-3, thus following the lines suggested by Sinopoulos (62) and (53) for an appropriate human nutrition.

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