

1 Title: Horse meat consumption affects iron status, lipid profile and fatty acid
2 composition of red blood cells in healthy volunteers

3
4 Cristian Del Bo¹, Paolo Simonetti¹, Claudio Gardana¹, Patrizia Riso¹, Giorgio Lucchini², Salvatore
5 Ciappellano¹

6
7 ¹Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Sezione Nutrizione, Università
8 degli Studi di Milano, 20133 Milan, Italia.

9 ²Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli
10 Studi di Milano, 20133 Milan, Italia.

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13 Correspondence concerning this article should be addressed to Salvatore Ciappellano, Dipartimento di
14 Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Sezione Nutrizione, Università degli Studi di
15 Milano, Via Celoria 2, 20133, Milano, Italy. Tel.: +39-02-503-16725; fax: +39-02-503-16721. E-mail
16 address: salvatore.ciappellano@unimi.it

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28 Horse meat, compared to others, is high in iron, low in fat and cholesterol and a good source of
29 polyunsaturated fatty acids such as linoleic and α -linolenic acid. The aim of the study was to
30 investigate the effect of moderate consumption of horse meat on iron status, lipid profile and fatty acid
31 composition of red blood cells in healthy male subjects. Fifty-two subjects were randomly assigned to
32 two groups of 26 subjects each: a test group consuming two portions of 175 g/week of horse meat, and
33 a control group that continued their usual diet abstaining from eating horse meat during the 90 days
34 trial. At the onset of the study and after 90 days, blood samples were collected for analysis. Horse meat
35 consumption significantly ($P \leq 0.05$) reduced serum levels of total and LDL cholesterol (-6.2% and -
36 9.1% respectively) and transferrin (-4.6%). Total n-3, LCPUFA n-3 and DHA content in erythrocytes
37 increased ($P \leq 0.05$) by about 7.8%, 8% and 11% respectively. In conclusion, the regular consumption
38 of horse meat may contribute to the dietary intake of n-3 PUFA and may improve lipid profile and iron
39 status in healthy subjects.

40

41 *Keywords:* horse meat, iron status, lipid profile, red blood cells, fatty acid composition, healthy
42 subjects.

43

44 *Abbreviations:* BHT, 2,6-di-tert-butyl-4-methylphenol; BMI, body mass index; C, control group; CRI,
45 collision reaction interface; CVDs, cardiovascular diseases; DHA, docosahexanoic acid; EPA,
46 eicosapentanoic acid; GC, gas chromatography; HDL, high density lipoprotein; HM, test group; ICP-
47 MS, inductively coupled plasma mass spectrometry; LC-PUFA, long chain polyunsaturated fatty acid;
48 LDL, low density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid;
49 RBCs, red blood cells; SFA, saturated fatty acid.

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54 **Introduction**

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56 Several studies have correlated the high consumption of red meat with an increased risk for CVDs and
57 colon cancer (Kelemen *et al.*, 2005; Kontogianni *et al.*,2008; Iqbal *et al.*, 2008; Zyriax *et al.*, 2008;
58 Cross *et al.*, 2007; Wei *et al.*, 2004). Nowadays, dietary recommendations provided by the Italian
59 National Research Institute for Food and Nutrition (INRAN), the American Heart Association (AHA)
60 and the United States Department of Agriculture (USDA) Center for Nutrition Policy and Promotion,
61 and the World Cancer Research Funds suggest limiting the intake of red meat to less than 500g cooked
62 weight per week and to encourage the intake of white meat (including chicken, game and turkey)
63 because they are low in fat and cholesterol. This message is particularly important for Mediterranean
64 populations whose traditional diet does not include a high intake of red meat.

65 Recent research has emphasized the nutritional value of horse meat. Compared to other meats, it is very
66 low in fat and cholesterol (about 20% less) and it is a good source of PUFA and low in SFA, indicating
67 that the consumption may be beneficial for health (Lanza *et al.*, 2009; Lee *et al.*, 2007). In addition, the
68 horse meat is very high in iron, one portion (175 g) providing about one-third of daily recommended
69 dietary intake. Insufficient iron intake results in the deficiency condition anemia, impaired cognitive
70 performance and reduced immune function (Geissler & Singh, 2011).

71 The aim of the present study was to investigate the effect of regular moderate consumption (twice per
72 week) of horse meat on iron status (hemoglobin, sideremia, transferrin, transferrin saturation and
73 ferritin), serum cholesterol, triglyceride levels and on fatty acid composition of red blood cells in free-
74 living healthy subjects. To our knowledge, this is the first study that attempts to evaluate the effect of
75 regular horse meat consumption on the above markers in humans.

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79 **Materials and Methods**

80 *Chemicals and dietary ingredients*

81 Omegawax test mix standard, Cholesterol, 6-ketocholestanol, BHT, sodium chloride were from Sigma-
82 Aldrich (St.Louis, MO, USA). Chloroform, acetyl chloride, potassium carbonate, toluene, methanol,
83 acetonitrile and hexane were purchased from Merck (Darmstadt, Germany). Water was obtained from a
84 Milli-Q apparatus (Millipore, Milford, MA). Horse meat was provided by NabaCarni Spa (Rezzato,
85 BS, Italy); pork and beef meat were purchased from local market.

86

87 *Experimental design*

88 Fifty-two male healthy and free-living volunteers (ages 20-50 years, BMI 24.4 ± 3.8 kg/m²), were
89 enrolled from the staff and student population of the University of Milan. Only male subjects were
90 considered for the study in order to exclude bias related with changes in iron status and other
91 parameters that occur in premenopausal women due to the hormonal fluctuation during the various
92 phases of the menstrual cycle.

93 The selection of the volunteers was performed based on the assessment of anthropometric
94 characteristics, clinical and biochemical parameters concerning iron status and lipid profile. Exclusion
95 criteria included: smoking (>10 cigarettes/day), hypertension (diastolic pressure ≥ 90 mm Hg and
96 systolic ≥ 140 mm Hg), high serum cholesterol (≥ 5.9 mmol/L; 230 mg/dL), high triglycerides (\geq
97 2.3mmol/L; 200 mg/dL), obesity (BMI > 30). A medical history questionnaire was used to exclude
98 diseases such as diabetes, renal insufficiency, dysfunctions related to iron metabolism, known food
99 allergies or other diseases. The selection of the volunteers was performed also by considering their
100 eating behaviors evaluated by means of a questionnaire about food intake and preferences, specifically
101 evaluating the frequency of fish and meat, but also nuts and seeds, and pulses, intake targeting horse
102 and beef products in order to have a homogeneous group of volunteers for meat consumption habits.

103 Volunteers who did not eat meat and those who followed specific diets were excluded. Additionally
104 subjects were excluded from the study if they were taking drugs, supplements or medications. The
105 experimental protocol was conducted according to the guidelines reported in the Declaration of
106 Helsinki and all the procedures involving subjects were approved by the Ethics Committee of the
107 University of Milan (March 26th 2009 - all.4). A written informed consent was signed by each
108 participant. The randomized parallel dietary intervention study was carried out for a period of 90 days.
109 Subjects were randomly assigned into two groups of 26 subjects each: a test group (HM) that replaced
110 two portions of red meat (beef and pork) with two portions of 175 g/week of horse meat, and a control
111 group (C) in which volunteers abstained from the consumption of horse meat maintaining their eating
112 meat habits (in particular beef and pork). Throughout the 90 days period, subjects kept a daily dietary
113 record and a short interview was randomly conducted in order to check diet compliance. From dietary
114 records we observed an energy intake of 2450 kcal, and a consumption of 90 g of proteins and 93 g of
115 fats. The difference within the subjects was about 20% for the energy, proteins and fats in according to
116 Turrini et al, (2008). Every 2 weeks, volunteers came to the laboratory to deliver the dietary records
117 and to receive four portions of horse meat, under vacuum skin-pack, that were consumed at home;
118 barbecued and roast cooking were not permitted. During the experimental period none of the volunteers
119 has deserted the study or changed eating habits. The HM group introduced about 5 mg of iron and 500
120 mg of PUFA per week more than C group. At the beginning and after 90 days, venous blood samples
121 were collected for the analysis.

122

123 *Proximate analysis*

124 Proximate analysis was conducted using AOAC official method (AOAC 942.05 and AOAC 934.01,
125 2006).

126

127

128 *Iron determination in food*

129 Iron (Fe^{2+}) was measured by ICP-MS (Varian 820 ICP-MS) as ^{56}Fe . Typical interferences (like ArO^+)
130 were removed by using CRI with an H_2 flow of $70 \text{ mL}/\text{min}^{-1}$ and Fe^{2+} was determined in quadruplicate
131 (Chen *et al.*, 2005).

132 *Cholesterol determination in food*

133 Meat samples (0.5 g) were placed into a 125 mL flask followed by the addition of 500 μL of 6-
134 ketocholestanol as internal standard (IS 1mg/mL), 4 mL of absolute ethanol and 1 mL of 60%
135 potassium hydroxide in water. The mixtures were vortexed and treated at 50°C for 1 h till the complete
136 digestion of the samples. Samples were then cooled to room temperature (20°C) prior to the addition of
137 5 mL of water and 5 mL of hexane for the first extraction. Then, at least four extractions with hexane
138 were carried out and the supernatants were brought to a final volume of 20 mL. An aliquot (3 mL) of
139 sample was evaporated under nitrogen and dissolved with 200 μL of the mobile phase for the HPLC
140 injection (20 μL). The chromatographic system consisted of an Alliance mod. 2695 (Waters, Milford,
141 MA, US) coupled to a photodiode array detector mod. 2998 (Waters Milford, MA, USA). A $3\mu\text{m}$ C18
142 Luna HST column (100x2 mm, Phenomenex, Torrence, CA, USA) was used for the separation at a
143 flow-rate of $0.35 \text{ mL}/\text{min}$. The column was maintained at 50°C and the separation was performed in
144 isocratic mode. The mobile phase was a mixture of acetonitrile:methanol (50:50 v/v). For peak
145 identification, chromatograms were acquired in the range of 195-320 nm and integrated at 205 nm. The
146 primary stock solutions of cholesterol and IS (1 mg/mL) were prepared in methanol and diluted to give
147 working solutions in the range of 10-500 $\mu\text{g}/\text{mL}$.

148

149 *Red blood cells separation*

150 Blood samples were collected into a vacutainer containing silicon, and plasma was separated by
151 centrifugation at 1400xg for 15 min at 4°C. The buffy layer of white blood cells was removed by using
152 a pipette and the red blood cells were washed twice with an equal volume of a physiologic solution (0.9
153 % NaCl, w/v). An aliquot (0.5 g) of red blood cells was stored at -80°C until analysis.

154

155 *Iron status and lipid profile in humans*

156 Iron status and lipid profile were determined in serum following standard laboratory techniques.
157 Hemoglobin was measured by using a CELLTAC MEK-6400 analyser (Nihon Kohden Corporation,
158 Tokyo, Japan). Ferritin was determined by using an AIA-600 analyser (Tosoh Bioscience, San
159 Francisco CA-USA). Sideremia, transferrin, triglycerides, total cholesterol and HDL cholesterol were
160 measured by using an ILAB-600 analyser (Instrumentation Laboratory, Lexington, MA-USA).
161 Transferrin Saturation was calculated by the formula (Serum Iron / Total Iron Binding Capacity) x
162 100%; LDL cholesterol was calculated using the Friedewald's method.

163

164 *Analysis of fatty acid composition of meats and phospholipids of red blood cells*

165 Fatty acids extraction from red blood cells (RBC) and meats was performed in accordance to the
166 method previously described (Simonetti *et al.*, 2002).

167 The GC analysis was performed as described by Ackman (1989). Fatty acid retention times were
168 obtained by injecting the Omegawax test mix as standard.

169

170 *Statistical analysis*

171 Sample size was calculated to include 20 subjects per group in order to detect significant differences in
172 the fatty acid composition of red blood cells and iron status with a power of 80%. STATISTICA
173 software (Statsoft Inc, Tulsa, OK) was used for statistical analysis of data. One-way ANOVA was

174 applied to compare the nutritional composition of horse, beef and pork meat. Two-way ANOVA was
175 applied to compare the effect of the diet (HM vs C group) and time (time 0 vs time 90 days) on serum
176 levels of triglycerides, cholesterol, iron status and fatty acid composition of red blood cells. Differences
177 were considered significant at $P \leq 0.05$; post-hoc analysis of differences between treatments was
178 assessed by the Least Significant Difference (LSD) test with $P \leq 0.05$ as level of statistical significance.
179 All data are presented as mean and standard deviation (SD).

180

181 **Results**

182 *Proximate composition and fatty acid profile of horse, pork and beef meat*

183 In **Table 1** the proximate and nutritional composition, for 100 g of product, of three different cuts of
184 meat (rump from horse and beef and loin from pork) is presented. Data are reported as mean \pm SD.
185 Horse meat is characteristically high in iron (3.81 ± 0.46 mg/100g) and low in cholesterol (53.3 ± 1.20
186 mg/100g) with respect to beef and pork. The content of lipids (2.2 ± 0.00 g/100g) is similar to beef and
187 pork. Horse meat is high in PUFAs (655.8 ± 3.2 mg/100g) that represent 30% of the total lipid content
188 with respect to beef (505.6 ± 68.9 mg/100g; 19%) and pork (348.9 ± 5.9 mg/100g; 13%). The content
189 of n-3 is significantly higher ($P \leq 0.05$) in the horse meat (217.4 ± 5.7 mg/100g) with respect to beef
190 (61.3 ± 14.1 mg/100g) and pork (21.5 ± 0.3 mg/100g). Among the n-3 fatty acids, the content of alpha-
191 linolenic acid (18:3n3) and docosapentaenoic acid (22:5n3) in the horse meat (167.8 ± 5.2 mg/100g and
192 31.2 ± 0.2 mg/100g, respectively) is considerably higher ($P \leq 0.05$) with respect to beef and pork. A
193 significant difference ($P \leq 0.05$) was also observed for the ratio n-3/n-6 that in horse meat is much
194 higher (0.49 ± 0.03) with respect to beef and pork (0.13 ± 0.03 and 0.07 ± 0.04 , respectively).

195

196 *Effect of horse meat consumption on serum iron status*

197 The concentrations of sideremia, transferrin, transferrin saturation and ferritin are reported in **Table 2**.
198 At the beginning of the study, the serum iron status did not differ significantly between HM and C
199 groups. Although during the experimental period the biochemical parameters were within the normal
200 range, the consumption of horse meat significantly ($P < 0.001$) reduced the serum levels of transferrin
201 with respect to baseline and C group at the onset and at the end of the study. Sideremia and transferrin
202 saturation tended to increase in the HM group with respect to C group.

203

204 *Effect of horse meat consumption on serum lipid profile*

205 The levels of total cholesterol, HDL and LDL cholesterol, triglycerides, before and after 90 days are
206 reported in **Table 3**. At the beginning of the study, the serum lipid profile did not differ significantly
207 between diet groups. During the experimental period all the biochemical parameters were within the
208 normal range and were identical in the HM and C groups. However, compared to baseline the serum
209 concentrations of total cholesterol, LDL-cholesterol and the ratio of total cholesterol/HDL-cholesterol
210 significantly declined after the intervention study in the HM group compared to C group. In particular,
211 total cholesterol concentration was 0.29 mmol/L (6.2%) lower ($P < 0.02$); LDL-cholesterol
212 concentration was 0.26 mmol/L (9.1%) lower ($P < 0.01$); the ratio total cholesterol/HDL-cholesterol
213 was 9.5% lower after the intervention. No significant effect was detected on triglyceride and HDL
214 cholesterol levels.

215

216 *Effect of horse meat consumption on fatty acids composition of red blood cells*

217 The mean percent of fatty acid composition of red blood cells in the HM and C groups after 90 days is
218 presented in **Table 4**.

219 At the end of the study, the consumption of horse meat significantly changed the fatty acids
220 composition of red blood cells. In particular, the content of PUFA and n-6 was significantly reduced (P

221 < 0.01) in the HM group by about 4.4% and 7.2% respectively, with respect to baseline and C group.
222 Among n-6 fatty acids, linoleic acid (18:2n6), dihomo-gamma-linolenic acid (20:3n6) and arachidonic
223 acid (20:4n6) significantly decreased ($P < 0.01$) after horse meat consumption by about 7.8%, 8.4% and
224 6.2% respectively. On the contrary, SFA and n-3 significantly increased ($P < 0.05$) in the HM group by
225 about 3.0% and 7.8% respectively after 90 days of horse meat consumption, while no significant
226 changes occurred in the C group. Docosahexaenoic acid showed a significant increase (from $4.92 \pm$
227 1.04% to $5.46 \pm 0.89\%$; $P < 0.05$) in the HM group after 90 days consumption of horse meat. When
228 considering the long chain PUFA (LCPUFA; $C \geq 20$, double bonds ≥ 3), a significant modulation was
229 observed only for the content of LCPUFA-n3, which increased significantly ($P < 0.05$) in the HM
230 group by about 8% with respect to C group. Horse meat consumption induced a statistically significant
231 increase in the n-3/n-6 ratio (15.3%; $P < 0.01$) and the omega-3 index (defined as the summation of
232 EPA and DHA content) (7.5%; $P < 0.05$). No difference was observed for the ratio LC n-3/n-6.

233

234 **Discussion**

235 Cardiovascular diseases are one of the most leading causes of morbidity and mortality in the Western
236 world (Mottillo *et al.*, 2010). Modifiable risk factors include: smoke, alcohol consumption, physical
237 activity and diet (Yusuf *et al.*, 2004). Evidence shows that the Mediterranean diet is the best way to
238 prevent many diseases. It is characterized by high consumption of fruits, vegetables, a moderate
239 consumption of white meat (poultry, rabbit, etc.) and a limited consumption of red meat to a few times
240 per month because of the high content in cholesterol and saturated fats (Sparling & Anderson, 2009).
241 In recent years, red meat is leaner and lower in fat content than that produced in the past (Martuzzi *et*
242 *al.*, 2001). Among red meats, horse meat is very interesting from a nutritional point of view because it
243 is low in cholesterol, rich in iron and PUFA such as α -linolenic (18:3 n3). For these reasons the

244 consumption of horse meat could have beneficial effects on health than that of other red meats (Badiani
245 *et al.*, 1997; Lee *et al.*, 2007).

246 The objective of the present study was to investigate whether the replacement of red meat with two
247 portions of 175 g/week of horse meat for 90 days, can improve iron status, lipid profile and fatty acid
248 composition of red blood cells in free-living healthy male subjects.

249 The consumption of two portions per week of horse meat provided 3 g of SFA, 2 g of MUFA, 186 mg
250 of cholesterol. In addition horse meat provided a greater amount of PUFA (2.3 g) and iron (13.34 mg)
251 with respect to beef (1.8 g of PUFA and 8.1 mg of iron) and pork (1.2 g of PUFA and 6.1 mg of iron)
252 while the energy intake was comparable between meats (372.4 kcal for horse meat, 388.2 kcal for beef
253 and 424.4 kcal for pork). The results of the study have shown that the consumption of horse meat can
254 significantly affect iron status, in particular the levels of transferrin. Transferrin is an iron- binding
255 blood glycoprotein that controls the level of free iron in the blood; when the amount of iron is high, the
256 body reduces the production of transferrin so that less of the iron is taken up and transported (Geissler
257 & Singh, 2011). Two portions per week of horse meat significantly reduced ($P < 0.001$) the serum
258 levels of transferrin in the HM group (- 4.5%) with respect to C group. No modulation was observed
259 for the concentration of hemoglobin and serum ferritin (intracellular protein that binds and sequesters
260 intracellular iron not utilized in the functional iron pool) while an improvement, even if not significant,
261 was observed for the sideremia (the amount of circulating iron that is bound to transferrin) and the
262 percentage of transferrin saturation (the percentage of total iron binding capacity that is occupied by
263 serum iron). These results are in accordance with data reported in literature, in which the consumption
264 of meat (beef, pork, poultry) has been associated with a positive effect on iron status (Worthington-
265 Roberts *et al.*, 1988; Leggett *et al.*, 1990; Brussaard *et al.*, 1997; Ortega *et al.*, 1998). However, very
266 few dietary intervention studies evidenced an improvement of the iron status in healthy/unhealthy
267 subjects (Navas-Carretero *et al.*, 2009; Tetens *et al.*, 2007). Most of the evidence reported, involves

268 anemic individuals in which iron was supplemented with tablets or fortified food (Navas-Carretero *et*
269 *al.*, 2009; Szymlek-Gay *et al.*, 2009; Zhu & Haas, 1998).

270 Several studies suggest that the fat content of red meat might be an important risk factor for the
271 development of CVDs. However, it is extremely important to evaluate not only the contribution of fat
272 intake from meat consumption but also from the total diet. To our knowledge there is not enough
273 evidence able to demonstrate that consuming lean red meat, low in total fat and cholesterol can increase
274 the risk factors for CVDs. In fact, no negative effect on blood lipids was observed in different human
275 intervention studies after the consumption of lean red meat (Hodgson *et al.*, 2007; Beauchesne-
276 Rondeau *et al.*, 2003; O'Dea *et al.*, 1990). In our experimental conditions, the replacement of two
277 portions of red meat with horse meat, seems to improve the cholesterol profile of healthy human
278 volunteers. The consumption of horse meat reduced the serum concentrations of total cholesterol (-
279 6.2%), LDL-cholesterol (-9.1%) and improved the ratio of total cholesterol/HDL-cholesterol (-9.5%) in
280 the HM group with respect to C group. Similar results have been observed by other authors that have
281 demonstrated a reduction in total and LDL-cholesterol in hypercholesterolemic men after a 26 day
282 intervention study with lean beef, lean fish or poultry (Beauchesne-Rondeau *et al.*, 2003).

283 Long-chain (LC) n-3 PUFA play also an important role in the modulation of lipid and HDL-
284 cholesterol profile. However, most of the beneficial evidence on HDL-cholesterol and lipid profiles has
285 been observed after administration of high doses of LC n-3 PUFA (>450 mg/day) and fish related to
286 their high content of EPA and DHA. Milte *et al.*, (2008) reported that 3g tuna fish oil per day
287 (providing 780 mg DHA and 180 mg EPA) reduced the levels of triglycerides (-23%) and increased the
288 level of HDL-cholesterol (+4.4%) in healthy subjects after a 12-week intervention study. Lindqvist *et*
289 *al.*, (2009) demonstrated that 6-weeks of a herring-rich diet significantly reduced the plasma levels of
290 triglycerides (-22%) and improved the level of HDL-cholesterol (+4%) in healthy but overweight men.
291 Similar results were also obtained after regular consumption for 12 weeks, of n-3 fatty acid-enriched

292 pork. Coates *et al.*, (2009) demonstrated that the weekly consumption of 200g of pork meat (providing
293 1.3g PUFA per week) significantly reduced the serum levels of triglycerides. In our experimental
294 conditions, no significant difference was observed in serum concentration of triglycerides and HDL-
295 cholesterol. The lack of the effect after a 12 week horse meat intervention may be attributed to different
296 factors such as: physiologic concentration of the levels of lipids and cholesterol of the volunteers or
297 their healthy status.

298 Several studies reported that balance of omega-3 fatty acids in the plasma are a good indicator
299 of cardiac health. Different studies have shown that n-3 enriched food can increase the percentage of n-
300 3 PUFA incorporated into red blood cell membranes. Coates *et al.*, (2009) demonstrated that a regular
301 consumption of n-3 fatty acid-enriched pork increased the erythrocyte DHA levels (+15%) in healthy
302 adult volunteers. McAfee *et al.*, (2011) shown that consumption of three portions (200g) per week of
303 grass-fed animals (beef and lamb), may provide valuable amount of LC n-3 PUFA and modulate the
304 fatty acid composition of plasma and platelets by improving LC n-3 PUFA status. In the present study,
305 the consumption for 12 weeks of two portions/week of horse meat (providing 2.3 g per week of n-3
306 PUFA) was able to increase significantly ($P < 0.05$) the content of n-3 fatty acids (9.6%), and in
307 particular the content of DHA acid (+13.6%) with respect to linoleic acid and n-6 fatty acids. This
308 could be attributable to a preferential uptake of n-3 PUFA or a different metabolism that convert PUFA
309 to longer and more unsaturated species. These processing activities seem particularly higher for n-3
310 than n-6 PUFA (Di Nunzio *et al.*, 2010). This evidence suggests that the low content of EPA and DHA
311 into erythrocytes is an important but independent risk factor for the development of CVDs. In this
312 regard Harris & von Schacky, (2004) have defined an Omega-3 index as a sum of EPA + DHA
313 contained within phospholipids of erythrocyte membranes; a low index ($\leq 4\%$) is associated with a
314 high risk of mortality (von Schacky, 2010). In the present study the consumption of horse meat
315 significantly ($P < 0.05$) increased the Omega-3 index by about 7.5%. ~~In addition, an improvement of~~

316 ~~the ratio n-3/n-6 (+15.3%) and a reduction of the total content of n-6 PUFA(-6.7%) was observed in~~
317 ~~HM with respect to C group.~~

318 **Conclusions**

319

320 The present study shows that the horse meat is an important source of omega 3 and iron. With respect
321 to other meats, it is very low of SFA but rich of PUFA. A regular consumption of horse meat may
322 contribute to reduce total and LDL cholesterol, and improve omega 3 index, docosahexaneic acid
323 (22:3n6) and iron status. Therefore, a regular consumption of horse meat with respect to other red
324 meats, may improve the fatty acid profile of red blood cells and in particular modulate PUFA, the most
325 useful to maintain nutritional status and the protective effects.

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332

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336 paper.
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451 **Table 1- Nutritional composition of horse, pork and beef meat.**

	Horse	Beef	Pork
Water, %	72.70 ± 0.10	74.85 ± 0.07	71.55 ± 0.07
Ash, %	1.20 ± 0.00	1.10 ± 0.00	1.15 ± 0.07
Protein, %	21.71 ± 0.10	21.65 ± 0.35	24.35 ± 0.07
Lipids, %	2.18 ± 0.00	2.70 ± 0.10	2.65 ± 0.07
Cholesterol, mg/100g	53.30 ± 1.20 ^b	65.50 ± 2.00 ^a	67.30 ± 2.10 ^a
Iron, mg/100g	3.81 ± 0.46 ^a	2.32 ± 0.31 ^b	1.73 ± 0.43 ^b
<i>Fatty acids, mg/100g</i>			
14:0	45.2 ± 1.2 ^a	53.5 ± 10.7 ^a	34.6 ± 1.8 ^b
16:0	576.2 ± 2.1	673.8 ± 55.5	611.4 ± 10.4
18:0	274.6 ± 14.5 ^b	621.4 ± 26.8 ^a	452.4 ± 73.9 ^c
20:0	6.8 ± 0.4	9.0 ± 2.7	7.0 ± 0.4
22:0	2.9 ± 1.0 ^b	4.6 ± 2.1 ^a	2.2 ± 0.0 ^b
24:0	3.7 ± 0.1 ^a	2.8 ± 1.2 ^a	1.3 ± 0.2 ^b
16:1n9	7.4 ± 0.5	6.4 ± 0.8	7.3 ± 0.4
16:1n7	77.8 ± 0.9	59.5 ± 2.1	69.6 ± 1.8
18:1n9	478.9 ± 18.4 ^b	693.5 ± 37.7 ^b	1016.4 ± 46.4 ^a
18:1n7	39.1 ± 1.6 ^c	263.5 ± 0.4 ^a	82.9 ± 9.1 ^b
20:1n9	8.6 ± 0.5 ^c	4.1 ± 1.0 ^b	14.4 ± 1.1 ^a
24:1n9	3.0 ± 0.5	2.4 ± 2.2	1.5 ± 0.2
18:2n6	371.8 ± 5.3 ^a	319.8 ± 31.2 ^b	255.2 ± 2.1 ^b
18:3n6	0.6 ± 0.2	1.7 ± 0.7	1.8 ± 0.2
20:2n6	6.8 ± 0.0 ^a	7.1 ± 0.8 ^b	9.9 ± 0.1 ^b
20:3n6	9.8 ± 0.8 ^b	17.7 ± 2.9 ^c	6.7 ± 0.4 ^a
20:4n6	47.9 ± 2.7 ^a	88.6 ± 16.8 ^b	47.3 ± 4.3 ^a
22:4n6	1.6 ± 0.2 ^a	9.4 ± 2.4 ^c	6.4 ± 0.4 ^b
18:3n3	167.8 ± 5.2 ^a	19.8 ± 0.5 ^b	7.5 ± 0.0 ^b
20:4n3	1.9 ± 0.0	1.9 ± 0.9	0.7 ± 0.2
20:5n3	11.0 ± 0.5 ^a	12.4 ± 3.6 ^a	5.3 ± 0.4 ^b
22:5n3	31.2 ± 0.2 ^a	23.0 ± 7.2 ^b	6.0 ± 0.6 ^c
22:6n3	5.5 ± 0.2 ^a	4.3 ± 1.8 ^a	2.0 ± 0.2 ^b
Total SFA	909.4 ± 16.2 ^b	1365.0 ± 33.4 ^a	1109.0 ± 62.3 ^b
Total MUFA	614.8 ± 19.4 ^b	829.4 ± 35.5 ^b	1192.1 ± 56.4 ^a
Total PUFA	655.8 ± 3.2 ^a	505.6 ± 68.9 ^b	348.9 ± 5.9 ^b
n -3	217.4 ± 5.7 ^a	61.3 ± 14.1 ^b	21.5 ± 0.3 ^c
n -6	438.4 ± 8.8 ^b	444.3 ± 54.9 ^a	327.3 ± 6.3 ^c
n3/n6	0.49 ± 0.03 ^a	0.13 ± 0.03 ^b	0.07 ± 0.04 ^b

452 SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, n-6= Omega 6 fatty
 453 acids, n-3= Omega 3 fatty acids. Data are reported as mean \pm SD. Data with different letters are significantly different, $P \leq$
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Table 2- Change in serum iron status between horse meat and control groups after 90 days of consumption of horse or control meat.

Iron status	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Hemoglobin, g/dL	15.1 \pm 0.8	14.8 \pm 1.1	14.9 \pm 0.9	14.7 \pm 0.8
Sideremia, μ g/ dL	99.1 \pm 29.5	104.2 \pm 32.9	113.2 \pm 32.0	107.0 \pm 27.4
Transferrin, mg/ dL	262.6 \pm 31.0 ^a	250.5 \pm 30.0 ^b	265.0 \pm 33.0 ^a	260.0 \pm 31.7 ^a
Transferrin saturation, %	27.2 \pm 9.6	29.7 \pm 9.9	30.6 \pm 10.0	29.4 \pm 8.6
Ferritin, ng/mL	113.2 \pm 79.9	112.5 \pm 72.2	119.6 \pm 68.4	117.8 \pm 73.8

460 HM group = horse meat group
 461 C group = control group
 462 Data are reported as mean \pm SD. Data with different letters are significantly different.
 463 ^b Different from HM group at baseline and C group (before and after 90 days), $P \leq 0.001$

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Table 3- Change in serum lipid profile between horse meat and control groups after 90 days of consumption of horse or control meat.

Lipid profile	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Triglycerides, mmol/L	0.94 \pm 0.40	0.92 \pm 0.43	0.97 \pm 0.40	0.96 \pm 0.40
Total cholesterol, mmol/L	4.74 \pm 0.92 ^a	4.45 \pm 0.82 ^b	4.66 \pm 0.65 ^a	4.53 \pm 0.49 ^a
HDL-cholesterol, mmol/L	1.28 \pm 0.29	1.32 \pm 0.23	1.32 \pm 0.32	1.35 \pm 0.27
LDL-cholesterol, mmol/L	2.88 \pm 0.73 ^a	2.62 \pm 0.66 ^b	2.78 \pm 0.59 ^a	2.64 \pm 0.47 ^a
Total Cholesterol/HDL	3.80 \pm 0.85 ^c	3.44 \pm 0.70 ^d	3.64 \pm 0.73 ^c	3.46 \pm 0.66 ^c

469 HM group = horse meat group
 470 C group = control group
 471 Data are reported as mean \pm SD. Data with different letters are significantly different.
 472 ^b Different from HM group at baseline, $P \leq 0.05$
 473 ^d Different from HM group at baseline, $P \leq 0.001$

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Table 4- Change in fatty acid composition of red blood cells between horse meat and control groups after 90 days of consumption of horse or control meat.

%	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Total SFA,	47.22±1.19 ^b	48.61±1.88 ^a	47.40±1.29 ^b	47.58±3.18 ^b
Total MUFA	19.32±1.27	19.43±1.33	19.63±1.35	19.47±1.66
Total PUFA	33.01±1.71 ^c	31.57±1.51 ^d	32.49±1.74 ^c	32.56±2.44 ^c
SFA/PUFA	1.44±0.10 ^d	1.55±0.13 ^c	1.46±0.11 ^d	1.47±0.20 ^d
18:2n6	11.45±1.17 ^c	10.56±1.43 ^d	11.24±1.38 ^c	10.88±1.84 ^c
20:3n6	2.02±0.42 ^c	1.85±0.42 ^d	1.79±0.34 ^d	1.73±0.30 ^d
20:4n6	11.04±1.62 ^a	10.36±1.12 ^b	10.73±1.27 ^a	11.02±1.93 ^a
22:4n6	1.81±0.45	1.66±0.37	1.81±0.44	1.94±0.78
Total n-6	26.31±2.40 ^c	24.42±1.77 ^d	25.68±2.28 ^c	25.57±2.94 ^c
18:3n3	0.06±0.02	0.06±0.03	0.06±0.03	0.04±0.03
20:5n3	0.39±0.12	0.39±0.14	0.43±0.24	0.43±0.17
22:5n3	1.32±0.18	1.25±0.16	1.32±0.24	1.33±0.26
22:6n3	4.92±1.04 ^d	5.46±0.89 ^c	5.08±0.90 ^d	5.18±0.96 ^d
Total n-3	6.63±1.11 ^b	7.15±0.96 ^a	6.90±1.16 ^b	6.99±1.04 ^b
n-3/ n-6	0.26±0.06 ^d	0.30±0.06 ^c	0.27±0.07 ^c	0.28±0.07 ^c
n-3 index	6.24±1.04 ^a	6.71±0.96 ^b	6.41±1.00 ^a	6.51±0.95 ^a
LCPUFA n-6	14.86±1.91	14.33±1.74	13.86±1.29	14.70±2.60
LCPUFA n -3	6.56±1.13 ^a	7.09±0.97 ^b	6.86±1.13 ^a	6.94±1.04 ^a
LC n-3/n-6	0.45±0.13	0.50±0.09	0.50±0.10	0.49±0.12

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SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, LCPUFA= Long Chain Polyunsaturated fatty acids (C≥20, double bonds ≥3). Data are reported as means ± SD. Data with different letters are significantly different.