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# LEARNING ABILITY OF DAY-OLD PARTRIDGES (*Alectoris rufa rufa*) HATCHED FROM EGGS LAYED BY HENS FED WITH DIFFERENT n 3 LEVELS

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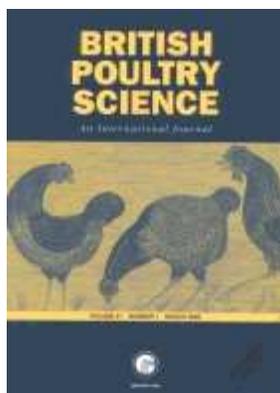
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**LEARNING ABILITY OF DAY-OLD PARTRIDGES (*Alectoris rufa rufa*) HATCHED FROM EGGS LAYED BY HENS FED WITH DIFFERENT n 3 LEVELS**

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## SHORT COMMUNICATION

**LEARNING ABILITY OF 1-D-OLD PARTRIDGES (*Alectoris rufa*) FROM  
EGGS LAID BY HENS FED WITH DIFFERENT n-3 FATTY ACID  
CONCENTRATIONS**

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**Running head : n-3 DIET AND LEARNING ABILITY**

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3 **Abstract.** 1. The diets of commercial strains of laying partridge are usually lower in  
4 PUFA and n-3 fatty acids than the diets of wild partridges. The aim of this experiment  
5 was to examine the effects of three different PUFA and n-3 concentrations in partridge  
6 laying diets.  
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10 2. Offspring learning ability (passive avoidance test of 1-d-old chicks) was used to  
11 assess the effect of three different maternal diets (144 chicks were tested for each diet).  
12 A negative experience, allowing the bird to peck a bead bathed in a bitter liquid (methyl  
13 anthranilate - MA), was used for this purpose. The adults had been fed one of three  
14 different diets with n-3 contents of 0.48, 4.04 or 7.60 g/kg.  
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20 3. There was better memory retention in the offspring of adults fed the intermediate n-3  
21 content compared to those fed the lower content. Discrimination ratio (DR) of the  
22 latency time toward the wrong (red) bead was less for the lower n-3 content (0.48) than  
23 for the middle n-3 PUFA content (0.43). DR of the number of pecks toward the wrong  
24 beads was greater for the lower n-3 content (0.51) than for the middle n-3 PUFA  
25 content (0.71).  
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31 4. The partridges fed the diet containing the lowest concentration of n-3 and PUFA  
32 were unable to express the expected behavioural score (neural embryo development  
33 index) given the genetic characteristics of the animals.  
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### INTRODUCTION

37  
38  
39 Essential fatty acids contribute to the structure of all tissues and are involved in cell  
40 membrane synthesis. Long-chain polyunsaturated fatty acids (l.c. PUFA) are  
41 particularly represented in the brain, retina and other neural tissues and they seem to be  
42 involved in the functional activity of the brain. Lipids are about 50-60% of human brain  
43 dry matter of which approximately 35% are l.c. PUFA. Arachidonic acid (AA) and  
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3 docosahexaenoic acid (DHA) are the main constituents of the l.c. PUFA in the brain.  
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5 These fatty acids have two different main origins: a) through biosynthesis from their  
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7 precursors, primarily linoleic acid and  $\alpha$ -linolenic acid (LNA); b) directly from dietary  
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9 sources (Wainwright, 1999).

10  
11 DHA, a 22-carbon fatty acid with 6 double bonds, the last of which in the n-3  
12  
13 position, is considered essential for neuronal plasticity and development (Itokazu,  
14  
15 2000). Docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and LNA are n-3  
16  
17 fatty acids involved in the same processes and they can also be used as DHA precursors.  
18  
19 An inadequate PUFA content in the diet, particularly of n-3 and n-6 fatty acids, may  
20  
21 cause imperfect development of the tissues and their functionality might be impaired or  
22  
23 reduced. Some kinds of algae and fish industry by-products (mainly oil) are foods  
24  
25 particularly rich in PUFA. The algae are the primary producers and fish that eat the  
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27 algae store these fatty acids in the liver. These two different sources have a different  
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29 PUFA concentration and bio-availability in the digestive tract of different animal  
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31 species. Other conventional foods such as soybean and other oil seeds, some species of  
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33 fresh forages such as alfalfa, and unconventional food such as snails, worms and  
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35 insects, also contain high concentrations of n-3 PUFA (Bagliacca *et al.*, 2000).

36  
37 In bird species, the only source of PUFA for the embryo is the yolk. For this  
38  
39 reason, the yolk fatty acid composition is essential for the optimal development of the  
40  
41 neural embryo-tissues. Many studies have demonstrated the possibility of increasing the  
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43 n-3 yolk content by dietary alteration (Cherian *et al.*, 1991). Learning ability and  
44  
45 memory performance have been studied on several mammalian species, such as  
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47 humans, pigs, mice and rats (Helland *et al.*, 2003) but few studies have been carried out  
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49 in avian species (Bagliacca *et al.*, 2000). The ingredients of commercial laying diets  
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3 greatly differ from the diet ingredients selected by partridge in their natural environment  
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5 (Bagliacca *et al.*, 2000).  
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7 The aim of the present study was to examine the effect of three different n-3  
8 PUFA diet contents supplied to red partridge laying hens (*Alectoris rufa rufa*), on CNS  
9 functionality by means of the learning ability of their offspring (1-d-old chicks).  
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12

## 13 METHODS

### 14 Animals and diets

15  
16 Eggs laid by three different parent groups (called 480, 4040 and 7600) were used for the  
17 experiment. Each group was composed of 48 red partridge pairs, two and three years  
18 old. Pairs were distributed at random in the groups and in the cages. The cages (45 x 80  
19 x 35 cm: w x d x h, with a mesh floor of 1 x 1 cm, wire cross section 2.0 mm), each held  
20 one pair of birds, were equipped with one feeder (20x7x15 cm, with a capacity of 1.2 kg  
21 approximately) and were kept outdoors in a fenced area. An artificially-extended  
22 photoperiod was used (natural + artificial 16L:8D; 35 lux light intensity), eggs were  
23 collected daily, stored (14°C, RH 70%) for one week and incubated each week.  
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25 Each parent group was fed with a different diet starting from 30 d before the laying  
26 period began. The only treatment difference was the diet of the breeding pairs.  
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34 The diets, formulated for partridge laying hens, contained two different  
35 commercial fat sources: diet 480, 30 g/kg palm oil (as fatty acid calcium salts); diet  
36 4040, 15 g/kg palm oil (as fatty acid calcium salts), 15 g/kg oil-mixture (linseed oil 600  
37 g/kg and fish oil 400 g/kg, micro-encapsulated); diet 7600 30 g/kg oil-mixture  
38 (composed as described above). Diet ingredients and their fatty acid compositions, are  
39 shown in Table 1.  
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3 The third, 7<sup>th</sup> and 11<sup>th</sup> hatches were used to test learning ability and memory  
4 retention and a total of 144 chicks (48 chicks for each group) were chosen at random  
5 from each hatch for this purpose.  
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7

### 8 **Passive avoidance test**

9  
10 A passive avoidance task (PAL) was used to assess brain development through the  
11 learning ability and memory retention of the 1-d-old chicks (Andrew, 1991). Sociable  
12 birds cannot be tested alone because they show signs of distress if placed alone in a  
13 strange environment and the presence of more than one bird in the same box seems not  
14 to affect the test results (Andrew, 1991). For this reason, three partridge chicks (just  
15 hatched) coming from three different parent groups were placed together in one shaving  
16 floor cardboard holding box (26 x 21 cm). The boxes were continuously warmed by 100  
17 W red bulbs (30 cm high) and the room was kept at a constant temperature of 27°C. To  
18 recognise chicks from the different dietary treatment (480, 4040, 7600) they were  
19 marked on the head by different coloured pens. The chicks were kept in the  
20 experimental boxes for at least 30 min prior to beginning the trials. The test was based  
21 on the chick's natural behaviour to peck at small moving objects. The protocol  
22 originally described by Andrew (1991), was partially modified by introducing a  
23 pre-training treatment before the passive training, to increase retention and reduce  
24 variability (Burne and Rogers, 1997).  
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40 The test was composed of 4 different stages. In the pre-training stage chicks were  
41 trained to peck a white bead (2 mm diameter). In the training stage chicks were  
42 presented (one cm in front its beak) with a red bead and than a blue bead (both 4 mm  
43 diameter). In the testing stage the red bead bathed in a bitter liquid (methyl anthranilate  
44 - MA) was presented. Finally, in the post-testing stage the red bead and the blue bead  
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3 were presented again, as described for the training stage.  
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5 Two testers worked in pairs with the same testing group. The first tester operated  
6 as follows: he presented to each chick the bead fixed at the end of a wire 20 cm long (1  
7 mm cross section), announced the first peck and counted the number of pecks given by  
8 the chick. The second tester recorded the time elapsed (latency) between the bead  
9 presentation and the first peck given, announced the elapsed time, and recorded the  
10 number of pecks given (in 20 s in the pre-training stage and in 10 s in the training,  
11 testing and post-testing stages). Between each stage and single activity, pauses of  
12 different duration were observed. The whole procedure and pause durations are shown  
13 in Table 2.  
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22 In the post-testing stage the modification of the behaviour by learning is usually  
23 observed as much more marked avoidance in pecking the red bead than the blue bead.  
24 The intensity of this behavioural modification in each group is used as a neural  
25 development index.  
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### 30 Statistical analysis

31 All the observed times were converted into their reciprocal value because the original  
32 data were not normally distributed. Latencies longer than 20 s during the pre-training or  
33 longer than 10 s during training, testing or post-testing were considered infinite (equal  
34 to zero its reciprocal value). Latencies were analysed by ANOVA (by considering the  
35 effects of the n-3 content in the hen diet as a categorical variable). Number of pecks  
36 were analysed by the Wilcoxon/Kruskal-Wallis non parametric test (Rank Sums) in  
37 relationship to the categorical effect and by logistic fit (ordinal values) in relationship to  
38 the n-3 content. The differences between the post-testing stage and the training stage  
39 were analysed on post-testing crude values covariated per the training stage latencies.  
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3 Discrimination ratios (DR), calculated as blue colour latency (or pecks number) divided  
4 per red colour plus blue colour latencies (or pecks number) (Burne and Rogers, 1997),  
5 were first checked for the normality of their distribution and then analysed by ANOVA  
6  
7  
8  
9 (SAS Institute, 2002).

## 12 RESULT

### 14 **One-trial passive avoidance learning task**

16 The results of this task have been used to study temporal structure and memory  
17 formation in chicks (Andrew, 1991). In our study, 1-d-old partridge chicks were very  
18 active when they were just hatched and each chick pecked the bead at least once in two  
19 different stages of the entire trials (pre-training, training, testing or post-testing). Table 3  
20 reports the number of observations, latency time (mean values), number of pecks (mean  
21 values) and standard error of mean (pooled) and referred only to the training stage and  
22 post-testing stage. There were no treatment differences ( $P>0.05$ ) in the pre-training  
23 stage or testing stage.  
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32 A difference ( $P<0.05$ ) in latency times for pecking the red bead between the  
33 experimental group 7600 and the control group 480 (Table 3) was observed in the  
34 training stage. No difference ( $P>0.05$ ) was observed either for the blue bead or for the  
35 DR. In the post-testing stage (after experiencing MA) all the groups showed longer  
36 latency times. Group 4040 showed a longer latency to peck the red bead than groups  
37 480 and 7600 ( $P<0.05$ ). The ability to remember the unpleasant taste of the red bead  
38 and the consequently longer latency time showed by group 4040 chicks, was confirmed  
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3 The poor retention memory shown by group 480 was confirmed by the differences  
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5 between post and pre-testing stages for the red bead. In this case, the latency time  
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7 between group 4040 and group 480 was significant ( $P<0.05$ ). The number of pecks in  
8  
9 the training stage tended to increase in the chicks whose parents had been fed with  
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11 higher n-3 content diets (Table 3). In particular group 7600 pecked the blue bead more  
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13 than group 480 ( $P<0.05$ ).

14  
15 After the testing stage (after experiencing MA), all chicks markedly reduced their  
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17 number of pecks toward all beads (red and blue). Most of them pecked the red bead  
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19 only once and only pecked the blue bead slightly more (Table 3). Group 480 and group  
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21 7600 pecked the red bead more than group 4040 ( $P<0.05$ ). This evidence has been  
22  
23 partially confirmed by DR values in the post-testing stage and by different DR values  
24  
25 (post-testing – training stage), which were both significantly different, between the 4040  
26  
27 and 480 groups ( $P<0.05$ ).

#### 30 DISCUSSION

31  
32 The dietary n-3 content and/or the n-6/n-3 ratio can change the behaviour of animals  
33  
34 like mice and chicks (Bagliacca *et al.*, 2000; Raygada *et al.*, 1998). Our study suggests  
35  
36 that the behaviour of partridge chicks can be affected by the maternal partridge diet.  
37  
38 Diets with a low PUFA content and/or an incorrect ratio of n-3 and n-6 fatty acids  
39  
40 affected behaviour. A diet lower in PUFA and n-3 fatty acids concentration than the one  
41  
42 commonly eaten by game bird hens under natural conditions (Bagliacca *et al.*, 2000)  
43  
44 worsened the chicks' memory retention, when it was measured by means of the one-trial  
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46 passive avoidance task (Andrew, 1991). When maternal dietary PUFA and n-3 fatty  
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48 acid concentration was similar to that used in commercial diets the latency times,  
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3 number of pecks toward the previously-tested bitter bead and discrimination ability of  
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5 1-d-old chicks worsened compared to higher n-3 diet concentrations (4.04 g/kg). At the  
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7 same time, the lack of statistically significant difference between the intermediate and  
8  
9 the highest n-3 content diets, seems to demonstrate that n6/n3 ratio affected the  
10  
11 partridge chicks' behaviour more than n-3 diet content per se. As in mice (Wainwright,  
12  
13 2002), the superior behavioural score was reached at the n6/n3 ratio of 4, which is close  
14  
15 to that of our diet 4040 (3.1).

16  
17 In captive birds reared for release into the wild, the nutritional requirements  
18  
19 derived by studying Galliformes reared in captivity may be inadequate, so that using  
20  
21 diets, apparently equal in chemical composition but differing in their ingredients, may  
22  
23 impose an handicap when captive-reared birds are released. The n-3 content of the diet,  
24  
25 together with other unconvencient characteristics (Bagliacca *et al.*, 1998), must  
26  
27 always be evaluated so that birds are fully fit to survive after their release.

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30 Mr Pera and Volac Italia for having supplied the saponified palm oil, VETAGRO for  
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35  
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**Table 1.** *Ingredients and chemical composition of the diets.*

Ingredient (g/kg "as is basis")				
Soft wheat		220		
Soybean meal solv extr		190		
Barley		170		
Sunflower seed meal solv extr		150		
Maize		100		
Sorghum		50		
Fat supplement*		30		
Calcium Carbonate		46		
Lignosulphonate		10		
Vitamin and mineral premix**		4		
Sodium bicarbonate		3		
Sodium Chloride		3		
Dicalcium phosphate		14.85		
L-lysine HCl		1.65		
DL-methionine		7.50		
TOTAL		1000.00		
Calculated analysis	Unit	Diet 480	Diet 4,040	Diet 7,600
Metabolisable Energy	MJ/kg	11.1	11.2	11.3
Crude Protein	g/kg	210	209	209
Fat	g/kg	50.3	50.4	50.6
NSP (Non Starch Polysacc.)	g/kg	259	260	262
LA C18:2	g/kg	13.7	13.9	14.1
LNA C18:3 ω3	g/kg	0.48	1.43	2.80
EPA C20:5 ω3	g/kg	-	0.68	1.37
DPA C22:5 ω3	g/kg	-	0.33	0.67
DHA C22:6 ω3	g/kg	-	1.75	3.50
Total ω3	g/kg	0.48	4.04	7.60
Total ω6	g/kg	13.72	12.44	11.16
ω6/ω3		28.60	3.10	1.50

\* diet 480 30 g/kg saponified palm oil; diet 4,040 15 g/kg saponified palm oil and 15 g/kg microencapsulated oil mixture; diet 7,600 30 g/kg microencapsulated oil mixture.

\*\*Supplied (mg/Kg diet): retinol 4.5, cholecalciferol 0.075, dl-α-tocopherol 30, menadione 3, thiamin 2, riboflavin 8, pyridoxin 5, cyanocobalamin 0,03, d-biotin 0.1, nicotinic acid 40, pantothenic acid 15, folic acid 1.25, choline chloride 600, Mn 150, Zn 60, Fe 35, Co 0.5, Cu 10, J 0.5, Se 0.1, ethoxyquin 2.5.

**Table 2.** *Outline of the One-trial Passive Avoidance Task.*

Stage	Activity	Stimulus	Interval between trials (min)
1	Three chicks placed in each box	Rest	> 30
2	Pre-training treatment 1	Chrome bead (2 mm Ø) + water	20
	Pre-training treatment 2	Chrome bead (2 mm Ø) + water	30
3	Training treatment 1	Red bead (4 mm Ø) + water	6
	Training treatment 2	Blue bead (4 mm Ø) + water	30
4	Testing treatment	Red bead (4 mm Ø) + MA	30
5	Post testing - responding 1	Dry red bead (4 mm Ø)	6
	Post testing - responding 2	Dry blue bead (4 mm Ø)	

**Table 3.** Influence of n-3 content of the hen diet on the latency and number of pecks of the partridge chicks.

Group	Training stage			Post-testing stage			Variation			
	Red bead	Blue bead	DR*	Red bead	Blue bead	DR*	Diff red	Diff blue	Diff DR*	
Latency time										
480	n	149	149	149	149	149	149	149	149	
	Mean-s <sup>2</sup>	6.72 b	6.74	0.50	14.00 a	13.00	0.48 b	7.24 b	6.31	-0.02
4040	n	140	140	140	140	140	140	140	140	
	Mean-s <sup>2</sup>	5.23 ab	5.09	0.50	16.40 b	13.30	0.43 a	11.20 a	8.23	-0.07
7600	n	143	143	143	143	143	143	143	143	
	Mean-s <sup>2</sup>	4.28 a	4.87	0.51	14.00 a	12.00	0.46 ab	9.68 ab	7.17	-0.05
Pooled SEM		0.33	0.33	0.01	0.38	0.4	0.01	0.47	0.48	0.02
Number of pecks										
480	n	149	149	133	149	149	84	149	149	78
	Mean	3.33	3.10 B	0.49	1.08 a	0.90	0.51 b	-2.25	-2.20	0.01 b
4040	n	140	140	134	140	140	70	140	140	68
	Mean	3.41	3.50 AB	0.49	0.53 b	1.19	0.71 a	-2.89	-2.31	0.26 a
7600	n	143	143	138	143	143	88	143	143	86
	Mean	3.78	4.13 A	0.50	1.08 a	1.31	0.60 ab	-2.71	-2.83	0.12 ab
Pooled SEM**		0.15	0.16	0.02	0.09	0.09	0.03	0.16	0.17	0.03

Note:

Means with different letters differ ( $P < 0.05$ ).

\* DR = Blue bead value / (red bead value + blue bead value)

\*\* Calculated by analysis of variance.

## SHORT COMMUNICATION

**LEARNING ABILITY OF 1-D-OLD PARTRIDGES (*Alectoris rufa*) FROM  
EGGS LAID BY HENS FED WITH DIFFERENT n-3 FATTY ACID  
CONCENTRATIONS**

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**Running head : n-3 DIET AND LEARNING ABILITY**

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3 **Abstract.** 1. The diets of commercial strains of laying partridge are usually lower in  
4 PUFA and n-3 fatty acids than the diets of wild partridges. The aim of this experiment  
5 was to examine the effects of three different PUFA and n-3 concentrations in partridge  
6 laying diets.  
7

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9  
10 2. Offspring learning ability (passive avoidance test of 1-d-old chicks) was used to  
11 assess the effect of three different maternal diets (144 chicks were tested for each diet).  
12 A negative experience, allowing the bird to peck a bead bathed in a bitter liquid (methyl  
13 anthranilate - MA), was used for this purpose. The adults had been fed one of three  
14 different diets with n-3 contents of 0.48, 4.04 or 7.60 g/kg.  
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16  
17 3. There was better memory retention in the offspring of adults fed the intermediate n-3  
18 content compared to those fed the lower content. Discrimination ratio (DR) of the  
19 latency time toward the wrong (red) bead was less for the lower n-3 content (0.48) than  
20 for the middle n-3 PUFA content (0.43). DR of the number of pecks toward the wrong  
21 beads was greater for the lower n-3 content (0.51) than for the middle n-3 PUFA  
22 content (0.71).  
23

24  
25 4. The partridges fed the diet containing the lowest concentration of n-3 and PUFA  
26 were unable to express the expected behavioural score (neural embryo development  
27 index) given the genetic characteristics of the animals.  
28  
29

#### 30 INTRODUCTION

31  
32 Essential fatty acids contribute to the structure of all tissues and are involved in cell  
33 membrane synthesis. Long-chain polyunsaturated fatty acids (l.c. PUFA) are  
34 particularly represented in the brain, retina and other neural tissues and they seem to be  
35 involved in the functional activity of the brain. Lipids are about 50-60% of human brain  
36 dry matter of which approximately 35% are l.c. PUFA. Arachidonic acid (AA) and  
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3 docosahexaenoic acid (DHA) are the main constituents of the l.c. PUFA in the brain.  
4  
5 These fatty acids have two different main origins: a) through biosynthesis from their  
6  
7 precursors, primarily linoleic acid and  $\alpha$ -linolenic acid (LNA); b) directly from dietary  
8  
9 sources (Wainwright *et al.*, 1999).

10  
11 DHA, a 22-carbon fatty acid with 6 double bonds, the last of which in the n-3  
12  
13 position, is considered essential for neuronal plasticity and development (Itokazu *et al.*,  
14  
15 2000). Docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and LNA are n-3  
16  
17 fatty acids involved in the same processes and they can also be used as DHA precursors.  
18  
19 An inadequate PUFA content in the diet, particularly of n-3 and n-6 fatty acids, may  
20  
21 cause imperfect development of the tissues and their functionality might be impaired or  
22  
23 reduced. Some kinds of algae and fish industry by-products (mainly oil) are foods  
24  
25 particularly rich in PUFA. The algae are the primary producers and fish that eat the  
26  
27 algae store these fatty acids in the liver. These two different sources have a different  
28  
29 PUFA concentration and bio-availability in the digestive tract of different animal  
30  
31 species. Other conventional foods such as soybean and other oil seeds, some species of  
32  
33 fresh forages such as alfalfa, and unconventional food such as snails, worms and  
34  
35 insects, also contain high concentrations of n-3 PUFA (Bagliacca *et al.*, 2000).

36  
37 In bird species, the only source of PUFA for the embryo is the yolk. For this  
38  
39 reason, the yolk fatty acid composition is essential for the optimal development of the  
40  
41 neural embryo-tissues. Many studies have demonstrated the possibility of increasing the  
42  
43 n-3 yolk content by dietary alteration (Cherian and Sim, 1991). Learning ability and  
44  
45 memory performance have been studied on several mammalian species, such as  
46  
47 humans, pigs, mice and rats (Helland *et al.*, 2003) but few studies have been carried out  
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49 in avian species (Bagliacca *et al.*, 2000). The ingredients of commercial laying diets  
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3 greatly differ from the diet ingredients selected by partridge in their natural environment  
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5 (Bagliacca *et al.*, 2000).

6  
7 The aim of the present study was to examine the effect of three different n-3  
8  
9 PUFA diet contents supplied to red partridge laying hens (*Alectoris rufa rufa*), on CNS  
10  
11 functionality by means of the learning ability of their offspring (1-d-old chicks).  
12

## 13 14 15 METHODS

### 16 17 **Animals and diets**

18  
19 Eggs laid by three different parent groups (called 480, 4040 and 7600) were used for the  
20  
21 experiment. Each group was composed of 48 red partridge pairs, two and three years  
22  
23 old. Pairs were distributed at random in the groups and in the cages. The cages (45 x 80  
24  
25 x 35 cm: w x d x h, with a mesh floor of 1 x 1 cm, wire cross section 2.0 mm), each held  
26  
27 one pair of birds, were equipped with one feeder (20x7x15 cm, with a capacity of 1.2 kg  
28  
29 approximately) and were kept outdoors in a fenced area. An artificially-extended  
30  
31 photoperiod was used (natural + artificial 16L:8D; 35 lux light intensity), eggs were  
32  
33 collected daily, stored (14°C, RH 70%) for one week and incubated each week.  
34  
35 Each parent group was fed with a different diet starting from 30 d before the laying  
36  
37 period began. The only treatment difference was the diet of the breeding pairs.

38  
39 The diets, formulated for partridge laying hens, contained two different  
40  
41 commercial fat sources: diet 480, 30 g/kg palm oil (as fatty acid calcium salts); diet  
42  
43 4040, 15 g/kg palm oil (as fatty acid calcium salts), 15 g/kg oil-mixture (linseed oil 600  
44  
45 g/kg and fish oil 400 g/kg, micro-encapsulated); diet 7600 30 g/kg oil-mixture  
46  
47 (composed as described above). Diet ingredients and their fatty acid compositions, are  
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49 shown in Table 1.  
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3 The third, 7<sup>th</sup> and 11<sup>th</sup> hatches were used to test learning ability and memory  
4 retention and a total of 144 chicks (48 chicks for each group) were chosen at random  
5 from each hatch for this purpose.  
6  
7

### 8 **Passive avoidance test**

9  
10 A passive avoidance task (PAL) was used to assess brain development through the  
11 learning ability and memory retention of the 1-d-old chicks (Andrew, 1991). Sociable  
12 birds cannot be tested alone because they show signs of distress if placed alone in a  
13 strange environment and the presence of more than one bird in the same box seems not  
14 to affect the test results (Andrew, 1991). For this reason, three partridge chicks (just  
15 hatched) coming from three different parent groups were placed together in one shaving  
16 floor cardboard holding box (26 x 21 cm). The boxes were continuously warmed by 100  
17 W red bulbs (30 cm high) and the room was kept at a constant temperature of 27°C. To  
18 recognise chicks from the different dietary treatment (480, 4040, 7600) they were  
19 marked on the head by different coloured pens. The chicks were kept in the  
20 experimental boxes for at least 30 min prior to beginning the trials. The test was based  
21 on the chick's natural behaviour to peck at small moving objects. The protocol  
22 originally described by Andrew (1991), was partially modified by introducing a  
23 pre-training treatment before the passive training, to increase retention and reduce  
24 variability (Burne and Rogers, 1997).  
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40 The test was composed of 4 different stages. In the pre-training stage chicks were  
41 trained to peck a white bead (2 mm diameter). In the training stage chicks were  
42 presented (one cm in front its beak) with a red bead and than a blue bead (both 4 mm  
43 diameter). In the testing stage the red bead bathed in a bitter liquid (methyl anthranilate  
44 - MA) was presented. Finally, in the post-testing stage the red bead and the blue bead  
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3 were presented again, as described for the training stage.

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5 Two testers worked in pairs with the same testing group. The first tester operated  
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7 as follows: he presented to each chick the bead fixed at the end of a wire 20 cm long (1  
8  
9 mm cross section), announced the first peck and counted the number of pecks given by  
10  
11 the chick. The second tester recorded the time elapsed (latency) between the bead  
12  
13 presentation and the first peck given, announced the elapsed time, and recorded the  
14  
15 number of pecks given (in 20 s in the pre-training stage and in 10 s in the training,  
16  
17 testing and post-testing stages). Between each stage and single activity, pauses of  
18  
19 different duration were observed. The whole procedure and pause durations are shown  
20  
21 in Table 2.

22 In the post-testing stage the modification of the behaviour by learning is usually  
23  
24 observed as much more marked avoidance in pecking the red bead than the blue bead.  
25  
26 The intensity of this behavioural modification in each group is used as a neural  
27  
28 development index.

### 29 30 **Statistical analysis**

31  
32 All the observed times were converted into their reciprocal value because the original  
33  
34 data were not normally distributed. Latencies longer than 20 s during the pre-training or  
35  
36 longer than 10 s during training, testing or post-testing were considered infinite (equal  
37  
38 to zero its reciprocal value). Latencies were analysed by ANOVA (by considering the  
39  
40 effects of the n-3 content in the hen diet as a categorical variable). Number of pecks  
41  
42 were analysed by the Wilcoxon/Kruskal-Wallis non parametric test (Rank Sums) in  
43  
44 relationship to the categorical effect and by logistic fit (ordinal values) in relationship to  
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46 the n-3 content. The differences between the post-testing stage and the training stage  
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48 were analysed on post-testing crude values covariated per the training stage latencies.  
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3 Discrimination ratios (DR), calculated as blue colour latency (or pecks number) divided  
4 per red colour plus blue colour latencies (or pecks number) (Burne and Rogers, 1997),  
5 were first checked for the normality of their distribution and then analysed by ANOVA  
6  
7  
8 (SAS Institute, 2002).  
9

## 10 11 12 RESULT

### 13 14 **One-trial passive avoidance learning task**

15  
16 The results of this task have been used to study temporal structure and memory  
17 formation in chicks (Andrew, 1991). In our study, 1-d-old partridge chicks were very  
18 active when they were just hatched and each chick pecked the bead at least once in two  
19 different stages of the entire trials (pre-training, training, testing or post-testing). Table 3  
20 reports the number of observations, latency time (mean values), number of pecks (mean  
21 values) and standard error of mean (pooled) and referred only to the training stage and  
22 post-testing stage. There were no treatment differences ( $P>0.05$ ) in the pre-training  
23 stage or testing stage.  
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26  
27 A difference ( $P<0.05$ ) in latency times for pecking the red bead between the  
28 experimental group 7600 and the control group 480 (Table 3) was observed in the  
29 training stage. No difference ( $P>0.05$ ) was observed either for the blue bead or for the  
30 DR. In the post-testing stage (after experiencing MA) all the groups showed longer  
31 latency times. Group 4040 showed a longer latency to peck the red bead than groups  
32 480 and 7600 ( $P<0.05$ ). The ability to remember the unpleasant taste of the red bead  
33 and the consequently longer latency time showed by group 4040 chicks, was confirmed  
34 by the DR values, which were different ( $P<0.05$ ) between group 4040 and group 480.  
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3 The poor retention memory shown by group 480 was confirmed by the differences  
4 between post and pre-testing stages for the red bead. In this case, the latency time  
5 between group 4040 and group 480 was significant ( $P<0.05$ ). The number of pecks in  
6 the training stage tended to increase in the chicks whose parents had been fed with  
7 higher n-3 content diets (Table 3). In particular group 7600 pecked the blue bead more  
8 than group 480 ( $P<0.05$ ).  
9

10  
11 After the testing stage (after experiencing MA), all chicks markedly reduced their  
12 number of pecks toward all beads (red and blue). Most of them pecked the red bead  
13 only once and only pecked the blue bead slightly more (Table 3). Group 480 and group  
14 7600 pecked the red bead more than group 4040 ( $P<0.05$ ). This evidence has been  
15 partially confirmed by DR values in the post-testing stage and by different DR values  
16 (post-testing – training stage), which were both significantly different, between the 4040  
17 and 480 groups ( $P<0.05$ ).  
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#### 30 DISCUSSION

31  
32 The dietary n-3 content and/or the n-6/n-3 ratio can change the behaviour of animals  
33 like mice and chicks (Bagliacca *et al.*, 2000; Raygada *et al.*, 1998). Our study suggests  
34 that the behaviour of partridge chicks can be affected by the maternal partridge diet.  
35 Diets with a low PUFA content and/or an incorrect ratio of n-3 and n-6 fatty acids  
36 affected behaviour. A diet lower in PUFA and n-3 fatty acids concentration than the one  
37 commonly eaten by game bird hens under natural conditions (Bagliacca *et al.*, 2000)  
38 worsened the chicks' memory retention, when it was measured by means of the one-trial  
39 passive avoidance task (Andrew, 1991). When maternal dietary PUFA and n-3 fatty  
40 acid concentration was similar to that used in commercial diets the latency times,  
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3 number of pecks toward the previously-tested bitter bead and discrimination ability of  
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5 1-d-old chicks worsened compared to higher n-3 diet concentrations (4.04 g/kg). At the  
6  
7 same time, the lack of statistically significant difference between the intermediate and  
8  
9 the highest n-3 content diets, seems to demonstrate that n6/n3 ratio affected the  
10  
11 partridge chicks' behaviour more than n-3 diet content per se. As in mice (Wainwright,  
12  
13 2002), the superior behavioural score was reached at the n6/n3 ratio of 4, which is close  
14  
15 to that of our diet 4040 (3.1).

16  
17 In captive birds reared for release into the wild, the nutritional requirements  
18  
19 derived by studying Galliformes reared in captivity may be inadequate, so that using  
20  
21 diets, apparently equal in chemical composition but differing in their ingredients, may  
22  
23 impose an handicap when captive-reared birds are released. The n-3 content of the diet,  
24  
25 together with other unconv nutrient characteristics (Bagliacca *et al.*, 1998), must  
26  
27 always be evaluated so that birds are fully fit to survive after their release.

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35  
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