SEASON OF HATCH, n-3 LEVEL OF PARENTS DIET AND LEARNING ABILITY OF DAY-OLD RED-LEGGED PARTRIDGES (*ALECTORIS RUFA RUFA*)

PERIODO DI NASCITA, LIVELLO DI n-3 DELLA DIETA DEI GENITORI E CAPACITÀ DI APPRENDIMENTO DEI PULCINI DI PERNICE ROSSA (*ALECTORIS RUFA RUFA*)

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SUMMARY

The fatty acids, content of the egg can vary in relationship to the *PUFA* content of the diet and during the season of lay. For this reason, we wanted to detect the effect of the season of hatch (starting middling and ending of lay) in red-legged partridge chicks, offspring of parents fed three different PUFA and n-3 laying diets.

The offspring learning ability have been tested in 48 chicks for each week and each parents diet (432 total). The negative experience consisted in the pecking of a bead bathed in a bitter liquid (Methyl Anthranilate), differently coloured from the previously pecked beads bathed in a sweet solution (Passive Avoidance Learning task – PAL – of day old chicks). The eggs were collected during the 3rd, the 7th and the 11th week of lay. The n-3 content of the three used diets were (mg/kg) 480, 4,040 and 7,600.

Discrimination Ratio difference (DRd) between post and pre-test (MeA experiencing) has shown a lack memory retention with the increasing of the number of eggs laid. The DRd between latencies were statistically significant between the 3rd and the 11th laying week; the DR observed in the 7th laying week did not differ either from the 3rd or the 11th laying week. The same trend was confirmed by the DR between number of pecks: differences were statistically significant between the 3rd and the 7th laying week *vs.* the 11th laying week.

Our study showed, in addition to the possibility to change the behavioural task of the birds by means of their parents diet, a possible effect of the week of lay probably linked with the worsening of the egg quality.

Key words: Partridge, behaviour, egg laying, n-3 fatty acids, PAL task.

RIASSUNTO

Gli acidi grassi contenuti nelle uova possono variare sia in relazione al contenuto in PUFA della dieta, sia al periodo di deposizione. Per questa ragione abbiamo voluto studiare l'effetto

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del periodo di deposizione su pulcini schiusi da uova deposte da genitori alimentati con diete a differente tenore in n-3 e PUFA.

La capacità di apprendimento è stata testata in 48 pulcini di un giorno di età per ciascuna tesi alimentare e per ciascun periodo di schiusa (totale 432 pulcini). L'esperienza negativa consisteva nel beccare una pallina imbevuta in una sostanza amara, metilantranilato, di colore diverso da quelle precedentemente beccate imbevute in una soluzione zuccherina (Passive Avoidance Learning task – PAL). I pulcini erano nati dalle uova deposte durante la 3°, la 7° e 11° settimana di deposizione e le 3 diete dei riproduttori presentavano il seguente tenore in n-3: 480, 4040 e 7600 mg/kg.

La differenza tra la discriminazione fra le palline colorate (DRd) osservata dopo e prima il contatto con la pallina amara, ha mostrato una diminuzione della ritenzione della memoria con il procedere della deposizione. La DRd relativa al tempo di latenza è risultata significativa solamente tra la 3° e l'11° settimana di deposizione. La diminuzione della ritenzione della memoria con il procedere della deposizione è stata confermata anche dal DRd relativo al numero di beccate per il quale differenze significative sono state rilevate tra la 3° e la 7° settimana di deposizione rispetto alla 11°.

Il nostro studio ha mostrato, oltre alla possibilità di modificare la capacità di apprendimento dei pulcini tramite la formulazione delle diete fornite ai genitori, una differenziazione della capacità di apprendimento in funzione del periodo di deposizione, probabilmente legato al peggioramento della qualità dell'uovo.

Parole chiave: pernici, comportamento, deposizione, acidi grassi n-3, PAL task.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) and particularly n-3 fatty acids are essential for neural plasticity, development and tissues functionality (Fernstrom, 1999; Itokazu et al., 2000). Lipids are about 50-60% of human brain dry matter and approximately 35% of them are l.c. PUFA. In particular, Arachidonic acid (AA) and Docosahexaenoic acid (DHA) are the main constituent of the l.c. PUFA brain (Innis, 2000; Lauritzen et al., 2001; Uauy et al., 2001; Wainwright, 2002). Many studies have shown that it is possible to modify the n-3 content of eggs by mean of nutrition (Barber et al., 1998; Cheriam & Sim, 1991; Meluzzi et al., 1998). In natural condition avian find these lipids in some unconventional food as snails, worms and insects, commonly containing high level of n-3 and others PUFA (Bagliacca et al., 2000). Artificial laying partridge diets are usually lower in PUFA and n-3 fatty acids than the natural diets. An inadequate PUFA diet's content, particularly of n-3 and n-6 fatty acids, cause a not perfect development of important tissues such as neuronal tissues and, consequently, the functionality of these tissues might be impaired or reduced (Wainwright et al., 1999; Watkins, 1995). In fact partridge parents fed with three different n-3 (and PUFA) level had a different learning ability and memory retention score (Fronte et al., 2007). Since yolk composition may change in relationship to the laying season (Romboli et al., 1984) with the present research we wanted to detect the influence of the laying week on offspring learning ability and memory retention.

MATERIALS AND METHODS

Animals and Diets - Three hatching coming from three groups called 480, 4040 and 7600 respectively, each one composed of 48 red partridge pairs were used for the experiment. The cages with the paired parents were randomly chosen and fed with three different diets, starting from 30 days before the laying start. Egg laying was obtained under an artificially extended photoperiod (natural + artificial 16L:8D); eggs were daily collected and weekly incubated. The 3rd, 7th and 11th hatches were used to tests learning ability and memory retention. 48 chicks for each group, 144 chicks total, were randomly chosen from each hatch. The parents diets contained two different fat sources: -Diet 480, 3.0 kg/t palm oil (palm oil 100% whole as fatty acid calcium salts); -Diet 4040, 1.5 kg/t palm oil and 1.5 kg/t oil-mixture (linseed oil 60% and fish oil 40%, micro-encapsulated); -Diet 7600, 3.0 kg/t oil-mixture (Fronte et al., 2007).

Test - Passive Avoidance Task (PAL) (Andrew, 1991) was used to check the brain development through the learning ability and memory retention of the day old chicks. The negative experience consisted in the pecking of a bead bathed in a bitter liquid (Methyl Anthranilate, MeA), differently coloured from the previously pecked beads bathed in a sweet solution (Fronte et al., 2007). Three one day-old partridge chicks, each one randomly selected from a different experimental group, were placed together in one shaving floor cardboard holding boxes (cm 26x21). Sociable birds cannot be tested alone since they show signs of distress if placed alone in a strange environment. The presence of more than one bird in the same box seem do not affect the test results (Andrew, 1991; De Vaus et al., 1980). The boxes were continuously warmed by 100 Watt red bulbs (30 cm high) and the room was kept at a constant temperature of 27°C. The chicks were kept in the experimental boxes at least 30' prior to begin the trials. The original test, based on the natural chick's behaviour that readily peck at a small moving objects, was partially modified; a pre-training treatment was added since pre-training before the passive training increases retention levels and reduces variability (Andrew 1991; Marples & Roper, 1997). The whole procedure and pauses duration between different stages are shown in Tab. I.

Tab. I. Outline of the One-trial Passive Avoidance Task

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

Statistical analysis - Before the analysis every observed times were converted into their reciprocal value. Latencies longer than 20" during the pre-training or longer than 10" during training, testing or post-testing, were considered infinite. Discrimination Ratio (DR), calculated as blue colour latency (or pecks number) divided per red colour plus blue colour latencies (or pecks number) (Burne & Rogers, 1997), were previously checked for distribution and then analysed by ANOVA. The minimum significant differences were calculated by the Tuckey post test method (SAS Institute, 2002).

RESULTS AND DISCUSSION

The results of the task have been used to establish the detailed temporal structure and the memory formation in the chicks (Andrew, 1991). In the present study, day-old partridge chicks showed a remarkable vitality just hatched and every chicks have pecked the bead at least in two different stages of the entire trials (pre-training, traning, testing or post-testing).

The DR difference between latencies observed during post and pre-testing stages (MeA experiencing), have shown a negative trend of the performances between the 3rd, 7th and 11th week, respectively for each group (Tab. II). Between the 3rd and the 11th laying week differences were statistically significant. The DR differences observed in the 7th laying week did not differ either from the 3rd or the 11th laying week. The same trend was confirmed by the DR differences between number of pecks observed during post and pre-testing stages. Between the 3rd and the 7th laying week *vs.* the 11th laying week differences were statistically significant. Regarding the interaction with the n-3 content of the diet the medium level (4040 mg/kg) seem the best solution to preserve memory retention during the laying period in fact, during the last hatching week (11th

Tab. II. Discrimination Ratio differences between post and pre-testing stages.

	3rd laying week		**7th laying week**		**11th laying week**		**Main effect diet**	
Latency time								
Diet 480	-0.089	abc	0.016	ab	0.021	ab	-0.017	u
Diet 4040	-0.155	c	-0.020	ab	-0.045	abc	-0.073	u
Diet 7600	-0.072	ab	-0.010	bc	0.032	a	-0.048	u
Main effect season	-0.105	n	-0.036	mn	0.003	m	**MSE = 0.327**	
Pecks number								
Diet 480	0.137	abc	0.020	bc	0.052	c	0.027	v
Diet 4040	0.304	a	0.215	abc	0.257	a	0.258	u
Diet 7600	0.206	abc	0.213	ab	0.001	bc	0.140	uv
Main effect season	0.216	a	0.136	n	0.069	m	**MSE = 0.474**	

Note: Means with different letters differ per P<0.05.

week) the chicks hatched by parents fed with this diet show the best mean values either for latency time or for pecks number. In the latter case, the difference reached the minimum significant difference.

The DR difference is the best parameter to evaluate the behavioural score of day-old chicks because it is able to better show the behavioural changing of day-old chicks after the negative experience (MeA experiencing). In addition to the possibility to change the behavioural task of animal by means of their parents diet (Fronte et al., 2007), this study showed an effect of the week of lay. The memory retention level of the chicks, measured by mean of the PAL, seem to worsen with the advancing of the laying season, probably in relationship to the worsen hens ability in storing n-3 and PUFA into the egg yolk.

CONCLUSION

In captive birds reared for release into wildlife, nutritional requirement data might be considered incomplete. Furthermore, even if diet are equal in the main chemical parameters but different in the ingredients, birds physiological and behavioural development can reduce or impair toward the end of the laying season before of the worsening of the laying and the hatching rates. The moment of hatch in relationship to the laying season and in n-3 diet content and other unconventional nutrient characteristics (Bagliacca et al., 1998; Wainwright et al., 1999), must always be evaluated to obtain birds more fit to survive in the wild after release.

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