BEHAVIOURAL REACTIONS, SEMEN QUALITY AND TESTOSTERONE LEVELS IN COCKS: GENETIC IMPLICATIONS

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Abstract *Animal Welfare 2001,* ***10:*** 269-279

*Intense selection for productivity may have indirectly affected some behavioural traits in poultry. Intensive husbandry systems change rapidly, and the animals may have difficulties in coping with their environment and management. The aims of this study are to examine the fear reactions of two strains of chicken (*Gallus gallus domesticus*) and to test the relationship between these fear reactivity levels and the chickens' semen characteristics. Semen characteristics may indicate the effect of genetic selection both on the productivity and fitness of the animals. Forty cocks of two genetics strains (an egg-type strain and a meat-type strain), housed in single cages, were used in the study. During the breeding period, semen was collected twice a week from each animal. Each cock was submitted to an open-field test and a tonic immobility test. The results show that strong genetic selection, carried out over a long period on domestic chickens in order to improve egg and meat production, seems to affect some aspects of behaviour. The reactions to the fear tests show many differences between the two strains: in the open-field test, the egg-type cocks show higher levels of exploratory behaviour and lower general fearfulness (eg lower frequency of vocalisations and head movements). On the other hand, the meat-type cocks show a significantly lower duration of tonic immobility, indicating a lower level of fear specifically towards humans. Moreover, a key nearest neighbours analysis carried out using the behavioural data allows us to discriminate between the two strains with an error rate of 0%. These results suggest the potential for genetic selection aimed at reducing fear reactions, both towards novel environments and towards human beings, which may significantly improve the welfare of cocks.*

Keywords: *animal welfare, egg-type cocks, fear reaction, meat-type cocks, semen quality*

Introduction

Domestic animals have been genetically selected for high levels of productivity. In particular, chickens have been selected for reproductive efficiency, egg production, and rapid growth (Ottinger 1983). This genetic selection for productivity may also have affected, at least indirectly, some behavioural traits, and these behavioural traits may differ between chickens even more widely than do their productivity levels.

Domestication has certainly determined the increase of some productivity traits and, possibly, the adaptability to intensive housing and husbandry. However, in spite of a higher level of adaptability in comparison with animals not genetically selected, intensive husbandry systems change so rapidly that animals may have great difficulties in coping with their environment and management. They may be frightened by the presence of many stimuli, including human beings. Freedom from fear is considered to be very important for farm animals' welfare, as suggested by the Farm Animal Welfare Council (UK) in 1979 (Jones 1987a).

In order to measure fearfulness in chickens, behavioural tests such as the open-field test and the tonic immobility (TI) test may be useful (Jones 1987b). Fear reactions in the open field, which is "a novel enclosure that is bigger than the home cage" (Gallup & Suarez 1980), are widely used in behavioural research (Faure 1975; Gallup *et al* 1976; Jones 1982; Jones *et al* 1995; Murphy & Wood-Gush 1978; Webster & Hurnik 1989, 1990). According to Gallup and Suarez (1980), the behaviour of chickens in the open-field test could be related to predator defence, which includes reactions to the presence of human beings. According to Ginsburg *et al* (1974), ambulation in the open field could be facilitated by the habituation of the animals to the human presence.

According to Ratner (1967), TI may be considered, from an evolutionary point of view, to be an anti-predator response. It is found in many species, and the longer the duration of TI, the higher the propensity to be easily frightened. TI may be affected by several factors such as previous handling, management and taming, genetics, social factors and housing systems (Jones 1986). TI may be a measure of a specific fear of human beings, rather than a general underlying fear response (Hansen *et al* 1993). Moreover, TI has been shown to correlate very closely with other fear tests (Jones *et al* 1991).

It is important to examine the relationships between behavioural and reproductive traits in order to verify the possible roles both of the environment and of genetic selection on the adaptability of poultry reared in intensive husbandry systems. The aim of this research is to study behavioural reactions and some reproductive traits in two different genetic strains of chickens (*Gallus gallus domesticus*) that have been genetically selected for productive efficiency. The results should allow us to verify whether genetic selection for productivity traits (ie semen characteristics) also affect behavioural reactions. In order to evaluate the semen characteristics, we used volume, concentration, motility, and forward progressive motility (FPM), which are considered the common values for expressing semen quality (Etches 1996).

Methods

Animals

In this study, forty parent cocks (20 ISA Warren egg-type breeders and 20 Cobb meat-type breeders) were used. All the birds were housed in single cages 42 cm wide x 43 cm long x 60 cm high in a controlled environment (14 h light : 10 h dark; temperature = 18–20 °C) for the whole reproductive period (from 20 to 54 weeks of age) and fed with a standard male breeder diet (12.5% crude protein, 11.5 MJ metabolisable energy kg^{-1}). The egg-type breeders were fed *ad libitum* and the meat-type breeders were fed a restricted diet, according to the conventional management of the two strains of cocks. All the animals were weighed before the beginning of the trial.

Behavioural tests

At 41 weeks of age, each cock was individually submitted to an open-field test and then, three days later, to a TI test. No repetition was carried out in order to avoid the risk of habituation of the animals to the test apparatus.

Open-field test

The open-field test was individually carried out in an apparatus comprising an area 4 m^2 with a rubber floor and wooden walls. The floor was divided into 16 squares. Each session of the test lasted 10 minutes and was video-recorded. The behaviours observed were: time in seconds before making the first movement (latency [s]); freezing time (s); standing still (s); movement in the centre of the area (frequency); movement at the sides of the area (frequency); attempts to escape from the area (frequency); vocalisation (frequency); feather ruffling (frequency); flapping the wings (frequency); pecking the floor (frequency); head movement (frequency); and self grooming (frequency).

Tonic Immobility (TI) test

TI was induced by placing each bird on its back on a planar surface and restraining it for 15 s according to the method used by Jones and Faure (1982). The latency in seconds from induction until the bird righted itself was measured. The maximum duration of the test was two minutes.

Blood sampling

Blood samples were collected in heparinized tubes (Vacutainer® Becton Dixon) from the wing vein in each cock two days before the open-field test, at the same time in the morning (1000 h), in order to evaluate testosterone levels. The heparinized samples were immediately frozen until the analysis. The testosterone radioimmunoassay determination was performed according to the method of Gaiani *et al* (1984).

Semen collection and evaluation

During the whole reproductive period, twice weekly, semen was collected from each cock in order to evaluate the following sperm quality parameters: volume (ml); concentration (spermatozoa [spz] x 10^9 ml^{-1}); motility; and forward progressive motility (FPM), which is an evaluation of the linearity of the spermatozoa's movement. Semen was collected using the abdominal massage method (Lake & Stewart 1978).

Semen volume was evaluated using graduated tubes. Sperm concentration was calculated using a calibrated spectrophotometer (λ = 535 nm; Spectronic® Genesys™ 5 Milton Roy). Motility (%) and FPM (0–4) were measured using CASA (Computer Assisted Semen Analysis; CellSoft Cryo Resources Ltd., New York, USA).

Statistical analysis

The means and standard errors for all the variables were calculated. Productivity traits, semen characteristics, TI duration (s), testosterone levels and reactivity levels in the open-field test were analysed using a non-parametric analysis of variance (Kruskal-Wallis test) using the strain as the factor (Statistical Analysis System Institute Inc. 1987). The key nearest neighbours (Knn; Frank & Friedman 1989) classification analysis was used in order to verify the correct classification of the two strains according to the variables above. Principal component analysis (PCA) was used in order to evaluate the variables' distribution (Jackson 1991).

Results

As displayed in Table 1, the meat-type breeders showed a lower volume of ejaculate, higher spermatozoa concentration and higher spermatozoa motility compared to the egg-type breeders.

Table 1 **Means ± Standard Error for productive traits, semen quality and testosterone levels.** spz, spermatozoa.

	Productivity traits		
	Egg-type	**Meat-type**	***P***
Weight (g)	2731 ± 35.22	4745.55 ± 82.87	< 0.001
Semen quality	**Egg-type**	**Meat-type**	***P***
Volume V (ml)	0.42 ± 0.06	0.28 ± 0.03	< 0.01
Concentration C (spz x 10^9 ml^{-1})	2.8 ± 0.24	4.24 ± 0.27	< 0.001
Total number of spz (V x C)	1.39 ± 0.20	1.3 ± 0.13	0.79
Motility (%)	38.23 ± 4.14	56.29 ± 3.59	< 0.01
FPM (0–4)	2.11 ± 0.18	2.05 ± 0.12	0.4
Testosterone levels (ng ml^{-1})	**Egg-type**	**Meat-type**	***P***
	1.81 ± 0.36	1.56 ± 0.35	0.83

The results of the behavioural tests are shown in Table 2. The duration of TI was significantly higher in the egg-type cocks. In the open-field test, meat-type cocks showed a movement latency six times longer than that seen in the egg-type strain. Egg-type cocks showed higher frequencies of escape attempts, vocalisations, wing flapping, head movements and floor pecking. The meat-type cocks showed neither escape attempts nor head moving. The time spent standing still was four times longer in the egg-type cocks. It should be noted that neither egg-type cocks nor meat-type cocks showed freezing behaviour.

Table 2 **Means ± Standard Error for TI and open-field tests.** f, frequency.

	Egg-type	**Meat-type**	***P***
TI TEST (s)	91.73 ± 9.12	61.35 ± 9.90	< 0.05
OPEN-FIELD TEST	**Egg-type**	**Meat-type**	***P***
Latency (s)	50.05 ± 11.03	303.60 ± 45.62	< 0.001
Movement in centre (f)	2.4 ± 0.84	2.55 ± 0.54	0.36
Movement at side (f)	3.20 ± 0.75	2.60 ± 0.60	0.53
Escape attempts (f)	0.75 ± 0.10	0.00	< 0.001
Vocalizations (f)	16.25 ± 2.07	3.45 ± 1.19	< 0.001
Standing still (s)	400.1 ± 15.44	102.75 ± 30.59	< 0.001
Ruffling feathers (f)	1.35 ± 0.47	2.25 ± 0.52	0.16
Flapping wings (f)	10.05 ± 1.86	3.65 ± 1.04	< 0.01
Head movements (f)	7.40 ± 1.62	0.00	< 0.001
Floor pecking (f)	3.50 ± 0.76	0.35 ± 0.25	< 0.001
Self-grooming (f)	0.9 ± 0.19	0.55 ± 0.23	0.07

Figures 1a, 1b and 1c show the Knn analysis results. Figure 1a shows the classification of the cocks when considering all the variables together (ie semen characteristics, testosterone levels, open-field reactions and TI times). The cross-validated misclassification is 5.0 per cent. Figure 1b shows that when only the semen characteristics and testosterone levels are considered, the cross-validated misclassification increases to 35 per cent. Figure 1c shows that when only the behavioural reactions in the open-field and the TI tests are considered, the cross-validated misclassification is zero.

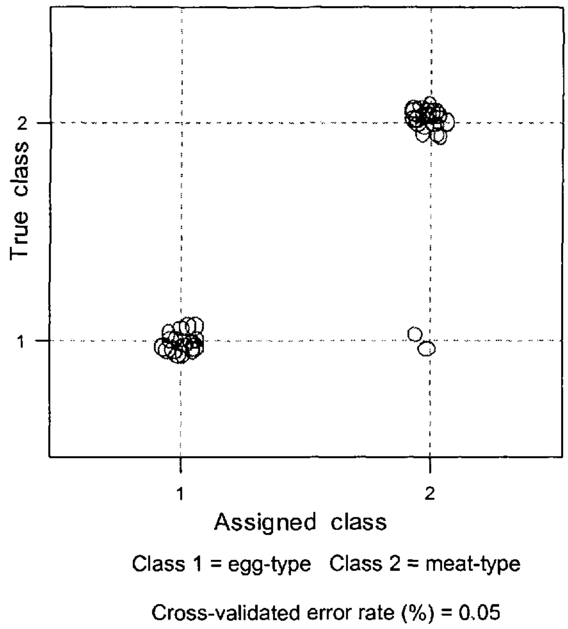

Figure 1a **Knn cross-validated class assignment considering all the variables**

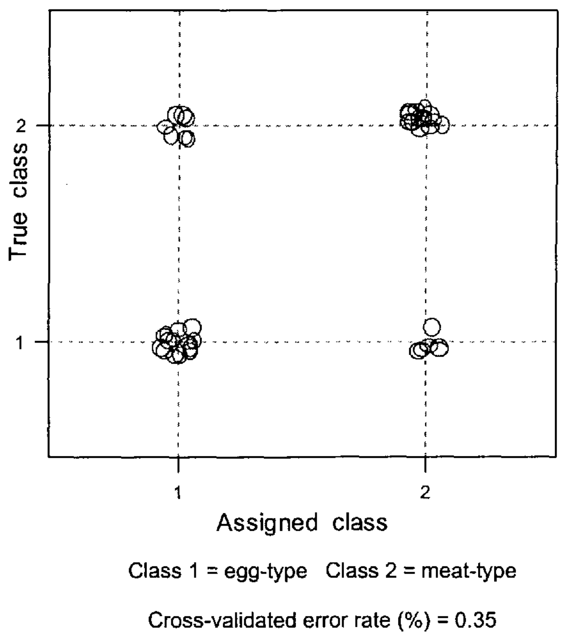

Figure 1b **Knn cross-validated class assignment considering semen characteristics and testosterone levels**

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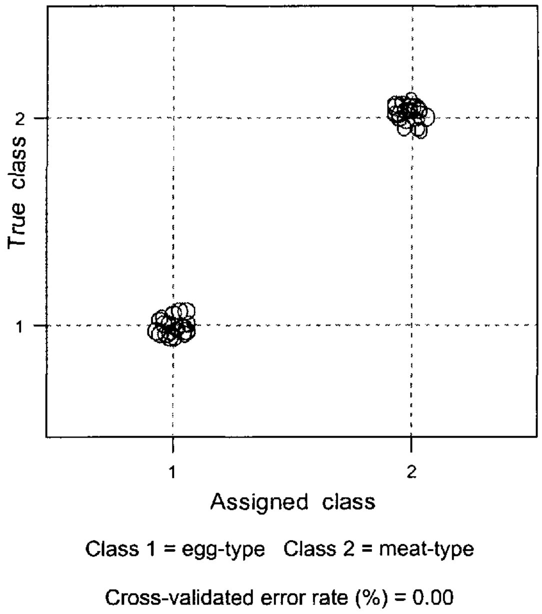

Figure 1c **Knn cross-validated class assignment considering the variables of the behavioural tests**

The PCA analysis (see Table 3 and Figure 2) reveals that the first five components explain 71.1 per cent of the total variance. The five components can be identified as follows:

(i) The first component, explaining 28.9 per cent of the total variance, represents movement latency (negatively correlated with escape, vocalisations, head movements, wing flapping and floor pecking).

(ii) The second component, explaining 15.6 per cent of the total variance, represents locomotion in the centre and along the side walls of the open field and feather ruffling, negatively correlated with standing still.

(iii) The third component, which explains 10.4 per cent of the total variance, represents the total number of spermatozoa.

(iv) The fourth component, which explains 8.6 per cent of the total variance, represents the FPM, negatively correlated with self-grooming and tonic immobility duration.

(v) The last component, which explains 7.6 per cent of the total variance, represents the testosterone levels and the motility of the spermatozoa.

Discussion

These results show that the total number of spermatozoa in the ejaculate is equivalent in the two genetic strains of chickens, although it is known that egg-type chickens generally have a higher reproductive performance than meat-type chickens (North & Bell 1990). There is no significant difference in testosterone levels between the two genetic strains. In fact, a relationship exists between androgen levels and copulative behaviour in chicks (Andrew 1978), as well as in many other species of birds and mammals, and the levels of testosterone measured in this study are comparable to those found by other authors (Freeman 1984).

Table 3 Principal component analysis undertaken on all the variables under consideration

	Eigenvalue	Explained variance (%)	Cumulative explained variance (%)
PC1	4.62	28.9	28.9
PC2	2.49	15.6	44.5
PC3	1.66	10.4	54.9
PC4	1.38	8.6	63.5
PC5	1.22	7.6	71.1

ID	Variables	PC1	PC2	PC3	PC4	PC5
1	latency	**–0.378**[1]	0.118	–0.276	0.001	0.076
2	movement in centre	0.227	**0.457**	0.138	–0.057	0.093
3	movement at sides	0.261	**0.426**	0.19	0.075	0.184
4	escape attempts	**0.321**	–0.239	0.028	0.078	0.132
5	vocalisations	**0.32**	–0.137	–0.082	–0.211	–0.103
6	standing still	0.292	**–0.402**	0.068	0.08	–0.166
7	ruffling feathers	0.128	**0.43**	0.006	–0.262	0.191
8	flapping wings	**0.265**	–0.026	0.22	0.25	–0.21
9	head movements	**0.345**	0.038	–0.264	–0.133	0.048
10	floor pecking	**0.322**	–0.056	–0.153	–0.22	0.248
11	self-grooming	0.145	–0.083	0.176	**–0.475**	–0.396
12	tonic immobility	–0.301	–0.072	0.31	**–0.329**	0.076
13	testosterone	–0.103	–0.315	0.31	–0.012	**0.447**
14	motility	0.111	–0.203	–0.269	0.203	**0.553**
15	forward progression	0.057	0.103	0.33	**0.577**	–0.092
16	volume x concentration	0.014	–0.07	**0.562**	–0.118	0.274

[1] The most significant variables for each component are in bold.

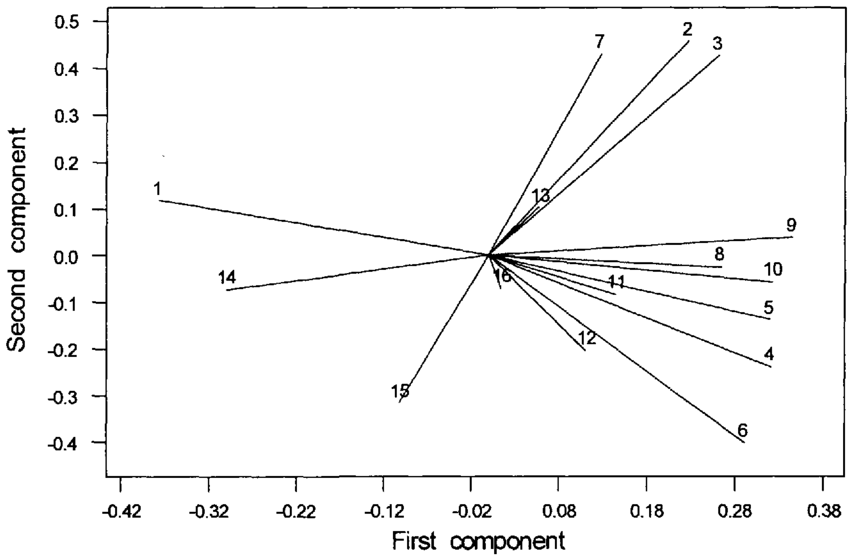

Figure 2 Principal component loading plot for all the considered variables

The behavioural test results show significant differences between the two types of cocks. Meat-type breeders show a lower TI duration than egg-type breeders. Many factors such as

https://doi.org/10.1017/S0962728600024040 Published online by Cambridge University Press

the husbandry system, age and genetic selection may affect TI duration in chickens. As far as genetic selection is concerned, Kujiyat *et al* (1984) failed to find differences in the duration of TI among controls and stocks successfully selected for egg production when kept in multiple-hen cages.

In the present study, the significant differences in TI times between the two groups may be related to a genetic underlying effect, as all the other environmental and management variables were the same for all the birds. This trait seems to be open to genetic manipulation. Craig and Muir (1989) were able to estimate the heritability of TI, indicating that this trait is subject to directional natural selection relating to innate fear of predators. Campo and Alvarez (1991), studying two pure poultry breeds and their cross, found that the duration of TI was significantly shorter in the cross breed, indicating heterosis for this trait. Gallup (1974) found a high value for the heritability of TI. On the other hand, some authors have reported low to medium values of heritability for TI (Benoff & Siegel 1976; Gerken & Petersen 1992).

The results of the open-field test are also statistically different between the two types of breeders. The meat-type breeders show higher latencies before movement compared to the egg-type breeders. Kerr-Kerr *et al* (1996) stated that it is difficult to compare stocks with differences in their inherent level of locomotory activity using activity-based measures as fear indicators but, in the present study, the differences between the cocks' reactivity in the open-field test involve not only their movement. In fact, the egg-type cocks show longer durations of standing still. This behaviour may be considered to be an attempt to maintain control over the environment, a proposal that is reinforced by the fact that standing still is displayed together with a series of other reactions, such as escape attempts, vocalisations, wing flapping, head movements and floor pecking — all of which behaviours are statistically higher in egg-type than in meat-type cocks.

Considering in further detail the open-field behaviour and its possible relationship with adaptability and welfare, according to Gallup and Suarez (1980) it may reflect, in domestic chickens, a combination of opposite reactions to the threats of predation and of social isolation. As stated by Ginsburg *et al* (1974), shorter ambulation latencies are shown in chicks that have been extensively handled prior to testing. In the present study, because of the fact that the only difference between the two samples is genetic stock, the significant differences in TI and open-field behaviour must be related to genetic selection, which may have indirectly affected the reactivity.

The two strains studied here differ substantially in behavioural reactions, as shown also by the Knn classification (cross-validated error rate = 0%). On the other hand, semen traits and testosterone do not discriminate between the strains (Knn cross-validated error rate = 35%). When all the variables are considered together, the classification results are rather intermediate: in fact, the Knn cross-validated error rate is 5 per cent.

Moreover, the PCA results stress the meaning of the grouped variables: in fact, the first five components explain 71.1 per cent of the total variance. However, the majority of the total variance (44.5%) is explained by the first two components, whose directions are determined mainly by the behaviour reactions in the open-field test. This means that, beside a very accurate distinction among the groups of variables, behaviour reactions show a higher variability than semen quality and testosterone levels in the whole studied population.

No significant relationships have been found between semen quality, testosterone levels and behavioural reactions. Archer (1973a) did not find any consistent difference in escape behaviour in an open-field test, for example jumping at the chamber wall, between birds treated with testosterone and controls. However, latency times were reduced in testosterone-treated birds (Archer 1973a,b), which also showed a reduction in freezing times.

Moreover, extreme or intense novelty evokes fear reactions, whereas a moderate amount of novelty leads to exploration (Grigor *et al* 1995). If this is the case, our results may indicate lower levels of general fearfulness in egg-type breeders, as they show higher levels of exploration in the new environment compared to meat-type breeders.

In conclusion, egg-type breeders have lower levels of general fearfulness in a new environment, whereas meat-type breeders show lower levels of specific fear towards human beings. TI may indeed be a better measure of specific fear of human beings than of general underlying fear responses (Hansen *et al* 1993).

Conclusions

The results of the present study suggest that the strong genetic selection carried out over many generations on these strains of domestic chickens has allowed the development of homogeneous traits as far as productivity is concerned. On the other hand, the chickens' reactions on specific behavioural tests show some differences between strains. The two strains of cocks that have been studied here, which have been genetically selected for specific products (eggs and meat), show significantly different reactions in the open-field test and in the TI test, whereas their semen quality and testosterone levels do not appear to be significantly different.

Considering the high levels of heritability found by some authors for behavioural reactions, mainly in TI duration (Campo & Alvarez 1991; Craig & Muir 1989; Gallup 1974), it is reasonable to propose that there is a high possibility of affecting the basic behavioural reactions of chickens in intensive husbandry systems through genetic selection. Such selection could be used effectively in order to reduce fear reactions, both towards new environments and, more specifically, towards human beings.

Animal welfare implications

Animal welfare in intensive husbandry systems is strongly affected by the presence of environmental stressors and by the coping ability of the animals. In order to improve animal welfare, the reduction of fear levels, which are an important behavioural indicator of welfare, may result in an improvement of the quality of the animals' lives. This reduction may be effected by the improvement both of housing systems and management, and of the adaptive capabilities of the animals.

In the present study, the differences found between the behaviour of the two genetic stocks, relating to general fearfulness in a new environment and to specific fear of human beings, suggest that a high genetic variability exists as far as these traits are concerned. For this reason, further research is needed in order to better understand the relationships between the behaviour of the animals and their other characteristics, and to increase the possibility of improving their coping capabilities and their welfare. In conclusion, the present results may improve the opportunities for the evaluation of fear reactions in genetically selected animals in order to optimise their welfare level.

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