

Immunocompetence and feather pecking:  
Effects of rearing condition and differences  
between commercial selection lines in the White Leghorn.



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## Preface

In this paper I present my first doctoral study, carried out at the Biological Centre in Haren (University of Groningen), at the department of Ethology. I studied immunity in the White Leghorn, in relation to feather pecking, from October 1999 to April 2000. Two commercial selection lines were used, one with a high propensity to feather peck (HP) and the other with a low propensity to feather peck (LP); the birds were raised in two different housing conditions, where groupsize (density), presence of a mother and egg type were factors that differed.

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## Abstract

A study was performed in White Leghorn chicks to assess immunocompetence in relation to feather pecking. Effects of housing condition, selection line, and age were investigated.

Two commercial selection lines were used, that differ in their propensity to feather peck: One line shows a high propensity to feather peck (HP) and the other line shows a low propensity to feather peck (LP). In addition to this behavioural dissimilarity, the lines differ in their physiological reaction to stress.

Two housing conditions were created, one with a large group size (commercial) and one with a small group size (semi-natural), for investigating whether group size influences feather pecking and immunocompetence; furthermore, presence of a (foster) mother in the commercial group, and egg type were additionally varying factors. There were eight commercial groups and eight semi-natural groups. Half of the groups in each condition consisted of LP birds, and half of the groups consisted of HP-birds.

For measuring immunocompetence the PHA wing-web response (dermal immune response) was used for measuring cell mediated, and injection of sheep red blood cells (SRBC) was used for measuring humoral immunity.

An effect of age was found in the dermal immune response. During the experiment the dermal immune response increased some 3-fold. Significant effects of sex in the model of line and housing condition used were not found.

Birds in the *commercial* condition showed a slightly higher (but not significant) response to PHA than the birds in the semi-natural condition, at the age of 6 and 12 weeks, but not at 18 weeks. Birds in the *semi-natural* housing condition of 18 weeks old showed a significantly higher IgG response at day(6) post-immunisation, than birds in the *commercial* condition.

HP birds showed a slightly higher (but not significant) PHA response at the age of 12 weeks. The IgG response in birds of 12 weeks is significantly higher in HP birds (day(6) post-immunisation); however in 18-week-old birds it is higher in LP birds (day(6) and day(10) post-immunization).

## INTRODUCTION

### STRESS

The publication of a one-page article in *Nature* in 1936 by Selye and the subsequent description of details of the *General Adaptation Syndrome* (GAS) (Selye, 1937) defined the biological concept of stress and has become the basis for a vast number of studies of stress in animals. In Selyean terminology, stress describes an animal's defence mechanisms. A stress stimulus (stressor) is any circumstance that elicits the defence responses (Selye, 1963).

Considering the type of response, a defence mechanism can be categorised as *specific* or *non-specific*. Specific defence mechanisms are those in which particular conditions elicit specific responses related to these conditions. For example, when the ambient environment causes a rise in body temperature of a bird, surface blood vessels dilate to allow rapid heat dissipation, while feathers are rearranged to reduce insulative value. Behaviourally, limbs are held away from the body and the bird seeks cool surfaces for conductive heat loss. Non-specific defence mechanisms take place when, regardless of the stressor, the bird responds in a generalised manner, going into a state of general stress: For example the production of corticosteroids due to a rise of ambient temperature. Production of corticosteroids is observed also when the animals are caught by the researcher. These defence mechanisms are not mutually exclusive, but may occur simultaneously.

Stress has important consequences for the animal, especially stressors that affect energy metabolism and interactions with the immune system. With regard to energy metabolism, neurogenic amines, such as adrenaline and noradrenaline, potentially activate the breakdown of glycogen to glucose in the liver of birds (Assenmacher, 1973). Combined with an accelerated delivery system, such as rises in blood pressure and pulse rate, free living birds are rapidly provided with resources to deal with injury or to escape predation.

The most fundamental effects attributed to corticosteroids (in birds primarily corticosterone: Baylé, 1967) are those on metabolic processes. Corticosteroids increase plasma glucose and enhance glycogenolysis in several species of birds (Riddle, 1937; Stamler *et al.*, 1954; Snedecor *et al.*, 1963). An increase in uric acid excretion (Adams, 1968; Siegel and van Kampen, 1984) gives rise to an increased urine flow. This is necessary to clear the additional uric acid excretion and thus water consumption rapidly increases (Siegel and van Kampen, 1984).

### GLUCOCORTICOIDS AND THE IMMUNE SYSTEM

Glucocorticoids interfere with the immune system in many ways. In man, cortisol in high doses reduces total serum IgG concentrations (Butler and Rossen, 1973). Glucocorticoids can also affect monocyte and macrophage populations as well as their functions. High levels of cortisone markedly reduce the phagocytic abilities of mouse liver phagocytes (Gotjamos, 1970). High levels of cortisol have general anti-inflammatory effects (Claman, 1975). However, lower levels (0.1–1.0  $\mu\text{M}$ ) are necessary for the induction of the immune response (Ambrose, 1964, 1970; Cooper *et al.*, 1979). In summary, glucocorticoids at large doses (a) reduce antibody formation and suppress cell-mediated immunity; (b) interfere with phagocytosis. At low doses, these corticosteroids may enhance the inductive phase of lymphocyte reactions.

## COPING & FEATHER PECKING

The environment can be seen as a composite of interacting stressors that, in the broadest sense, includes all the conditions in which the bird lives – external (temperature, light, social or behavioural environments), as well as internal (disease organisms, toxins). The success of the bird in coping with its environment therefore depends on the severity of the stressors and its physiological ability to respond properly. Significant changes in the environment stimulate regulatory processes that attempt to maintain or re-establish the equilibrium or homeostatic state; Koolhaas *et al.* (1997) defined coping as the behavioural and physiological processes necessary to reach this homeostasis.

In primate and rodent populations individuals with an extreme difference in behavioural coping and stress reaction co-exist. These extremes display either an active behavioural response (fight/flight) or a passive behavioural response (conservation/withdrawal) (Engel & Schmale, 1972) in response to a threat. The active coping style is associated with high neurosympathetic activity but low corticosteroid level, whereas during the passive behavioural coping style high cardiac parasympathetic activity and high corticosteroid level occur (Bohus *et al.*, 1987).

Recently it has been suggested that also in the domestic fowl these two coping strategies exist. Two commercial lines of White leghorn laying hens have been shown to differ consistently in the propensity to feather peck. One line (High feather Pecking line/HP line) feather pecks significantly more than the Low feather Pecking line (LP line). In addition to this behavioural dissimilarity, the lines differ in their physiological response to a stressor. The high peckers react with a noradrenaline response, while the low peckers react with a corticosterone response to manual restraint (Korte *et al.*, 1997). This difference in neuroendocrine response patterns together with the behavioural differences lead Korte *et al.* to suggest that the HP and LP lines may represent two distinct coping styles.

## FEATHER PECKING

Feather pecking is considered to be an aberrant behaviour and is a problem in commercial poultry farming (Vestergaard *et al.*, 1996). It varies from gentle pecking at the plumage, to pulling out feathers (severe feather pecking) from cagemates; the removal of feathers results in the formation of wounds. The blood is likely to attract more birds, and cannibalism can occur. In laying hens egg production reduces and because of the loss of feathers the animals suffer extra heat loss. Consequently the birds have to consume more food.

At this moment beak trimming is carried out, to reduce the damage inflicted by feather pecking. However from animal welfare point of view this method is questionable, and fortunately will be prohibited in the near future. Moreover, this method does not change the actual problem; feather pecking does not stop, but the effects are less serious; it is better to investigate what are the causes of such behaviour.

In commercial housing systems laying hens are kept in extremely large groups, which is stressful. Awadalla (1998) found that overstocking of chickens lead to elevation of corticosterone levels. Birds have a high chance to be disturbed in their behaviour by other birds, and social interactions are affected. Unfavourable social or behavioural environments effectively initiate non-specific stress responses in birds (Gross & Siegel, 1973). Acute stress may increase the

potential for short-term survival, but chronic stress, reflected in elevated corticosterone levels, may be deleterious: In young birds growth and skeletal development may be depressed (Siegel & Latimer, 1970), and stress is implicated in feather pecking and coping (Korte *et al.*, 1997).

In mammals much research has been done on the relationship between crowding (as a stressor) and the immune system. Crowding in mice has reduced granuloma formation (an inflammatory response) to subcutaneously implanted cotton pellets (Christian and Williamson, 1958). Overcrowding of adult rats was found to reduce both the primary and secondary antibody responses to the salmonella flagellin whereas low-voltage shock proved to be ineffective (Solomon, 1969).

This might be the case too in the domestic fowl. Overstocking of the birds may lead to chronic stress, due to the abnormal large group size. As a consequence, the birds in this crowded situation have elevated corticosterone levels, which suppresses the immune system (Munck *et al.*, 1984).

Birds from the LP-line may be more sensitive to stress than birds from the HP-line, considering the intensity of the corticosterone response to a stressor; it might be especially in a crowded situation that these animals perform worse when challenged with an antigenic substance.

To investigate these hypotheses two housing conditions were created, one with a small group size (semi-natural), and one with a large group (commercial). Additional varying factors were the presence of a foster mother in the semi-natural housing condition (in the semi-commercial housing condition no adult (mother) chicken was present), and background of the eggs (see methods). Chicks were reared under these two conditions; in addition to observations of feather pecking, the performance of the immune system was assessed.

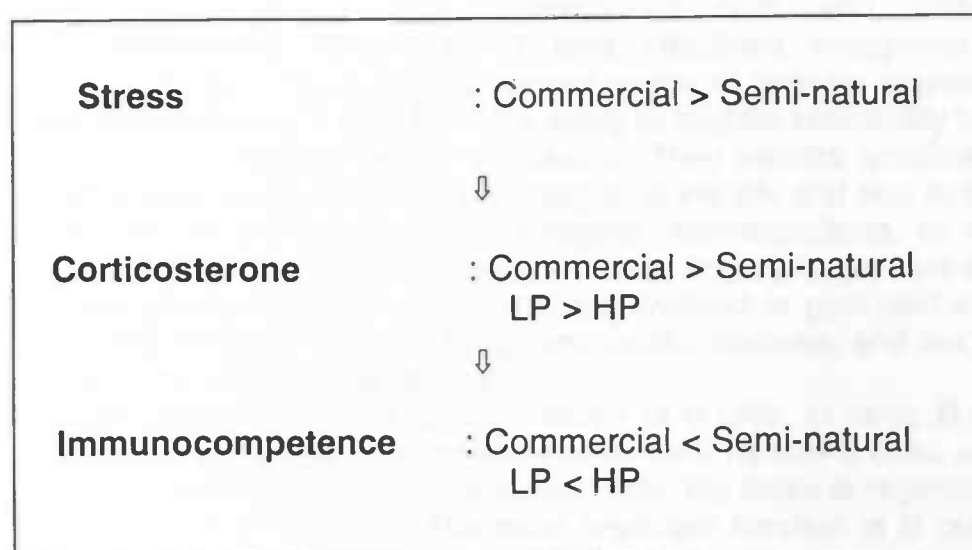


Figure 1. Hypotheses

# The immune system

In 1798 Jenner's observations gave rise to the science of immunology: By inoculating a boy with pus from a cowpox lesion of a dairy maid. Some weeks later, when the boy was reinoculated with infectious pus from a patient suffering from smallpox, the disease failed to occur; this led to his classical paper that vaccination leads to immunity against smallpox.

The lymphocytes are the cells of the immune system and have the ability to recognize and to respond to foreign materials or antigens. There are two major categories of lymphocytes: those that mature in the thymus (T-lymphocytes), and those that become immunocompetent in the bursa of Fabricius (birds) or in the bone marrow (mammals, B-lymphocytes). All lymphocytes arise from the multipotential stem cells, which reside in the bone marrow.

In both birds and mammals, hemopoietic cells first appear in the yolk sac. Later in embryonic development, they migrate through the blood stream to colonize the liver in mammals and the spleen in both birds and mammals, before they home to their permanent residence in the bone marrow. It was demonstrated (Wu et al., 1967&1968) that the morphologically and functionally very different cells of the erythrocyte, lymphocyte, monocyte-macrophage, and granulocyte series all originate from the same multipotential stem cells.

The thymus, bursa, and the bone marrow are known as the primary lymphoid organs. Mature T and B lymphocytes from these organs home to the secondary lymphoid organs, namely the spleen, lymph nodes, and mucosal lymphoid tissues, where some of them will undergo further antigen-driven differentiation, as required.

At least four functional classes of T lymphocytes may be distinguished: helper, suppressor, delayed-type hypersensitive and cytotoxic T cells. Helper T cells are needed for the antigen-driven differentiation of all effector lymphocytes (delayed-type hypersensitive-, killer-, suppressor-T cells, and antibody-secreting B lymphocytes). Suppressor T cells effectively antagonize the initiation of new effector cells during the normal course of immune responses. Delayed-type hypersensitive T cells have the ability to migrate specifically to the sites of minor antigen deposits within the tissues. They secrete lymphokines that attract other mononuclear cells (macrophages) to the site and also activate the recruited cells for the elimination of antigenic microorganisms, or other antigenic material. Killer (cytotoxic) T cells are able to destroy target cells in an immunologically specific fashion in vitro and are involved in graft and tumor rejection, in defense against viruses, fungi, and certain bacteria, and are also responsible for some autoimmune reactions.

The other major category of lymphocytes consists of B cells. In birds, B cells mature in the Bursa of Fabricius that contains stem cells, mature B cells, and a small number of T cells. In comparison with mammals, the bursa is regarded as a bone marrow equivalent in birds. The most important function of B cells is antibody formation; upon stimulation by an antigen, they transform from the small lymphocytic stage into plasma cells, which secrete large amounts of immunoglobulin. Virgin precursor cells have antigen receptors of the IgM class, regardless of the class of antibody eventually secreted. Exposure to antigen induces a shift in receptor class from IgM to IgG.



## METHODS

### ANIMALS & HOUSING

In this experiment two commercial lines of the White Leghorn were used. These two lines originate from different breeding lines and are the result of a commercial selection programme, which included criteria such as number of eggs, eggshell quality, and mortality. The lines differ in their propensity to feather peck: one line shows a high propensity to feather peck (HP line), and the other line shows a low propensity to feather peck (LP line).

Two housing conditions were created: In the *commercial housing condition* 45 chicks were placed in a cage of 3.5 square meters. For the first three weeks they were restricted to an area of one square meter; the fourth week the cage was enlarged to two square meters, and from the fifth week until the end of the experiment the cage was maximized to 3.5 square meters. In the *semi-natural housing condition* 6 chicks were placed in an identical cage of 3.5 square meters, for the duration of the experiment.

Besides groupsize, background of the eggs and presence of a foster mother in the natural housing condition (in the commercial housing condition no mother was present) were additional varying factors. In the commercial housing condition the eggs were obtained from a commercial poultry farm; in the semi-natural housing condition the eggs were produced by hens living in groups of 4 (including a rooster). These hens had hatched from commercially produced eggs, but were kept in large outdoor cages with an indoor part.

The duration of the experiment was 20 weeks.

### VACCINATION & FOOD

In the first week of life the chicks were vaccinated for Marek's disease and Newcastle disease, by intramuscular injection and eyedrops, respectively. Food was given *ad libitum*: 0-8 weeks, Crumms 1; 8-16 weeks, Crumms 2; subsequently standard pellets for laying hens (*dfk legkorrel blauw*).

### IMMUNOLOGY

#### *Cell-mediated response*

Cell-mediated immunity (CMI) was measured using the dermal Phytohaemagglutinin (PHA= PHA-P, Sigma-Aldrich) reaction in the wing web. The skin test provides a measure of the proliferative response of circulating T-lymphocytes to an injected mitogen; PHA has long been recognized for its mitogenic and blastogenic properties (Hungerford *et al.* 1959), and injection of PHA for a localised *in vivo* inflammatory response in birds is used since a long time to measure cell-mediated immunity (Stadecker *et al.* 1977; Lamont & Smith, 1984).

Birds were injected intradermally in the right wing web at the age of 6, 12 and 18 weeks (fig. 2, next page) with 100  $\mu$ l PHA (1.0  $\mu$ g/ $\mu$ l) in phosphate buffered saline (PBS). Half of the 6-week-old birds were treated with PHA, prior to SRBC (see humoral immunity) treatment. The degree of swelling in the wing web 24 h after injection was measured by an analogue micrometer. Correlation analyses

were made for the response to PHA with bodymass and the mean wing web thickness before injection. The thickness of the wing web before injection possibly influences the response to PHA. Thicker patagia could swell more than smaller ones; receiving the same amount of PHA, they may be able to receive more incoming cells.

	6 weeks	12 weeks	18 weeks
Group1:	PHA/SRBC	PHA/SRBC	PHA/PRBC
Group2:	-----	PHA/SRBC	PHA/SRBC

Figure 2. Time schedule of the experiment. Half of the focals were challenged at 6 weeks, with PHA and SRBC. Additionally group 1 was tested with pig red blood cells (PRBC) at the age of 18 weeks (appendix X)

#### *Antibody response (humoral immunity)*

At the age of 6, 12 and 18 weeks birds were injected intra-peritoneally with 0.5 ml 2% sheep red blood cells (SRBC) (6 weeks) or 1.0 ml 2% SRBC (12 and 18 weeks), suspended in PBS. Treatment with SRBC was always five days later than PHA-treatment, avoiding possible interference of the immune responses. Blood samples were taken six and ten days post-immunization, from the *Vena jugularis*, centrifuged at 11.000 rpm for 10 min; serum was stored at -20 °C until analysis. Antibodies were measured in duplo using haemagglutination (Hudson & Hay, 1989). Titres were scored as the highest dilution at which antibodies were detectable, and are based on a <sup>2</sup>log scale.

Before analysis sera were heat-inactivated (56 °C, 30 min). Primary response samples were diluted in 20 µl PBS (dilutions ranged from 2<sup>1</sup> to 2<sup>12</sup>), and an equal amount of sheep erythrocyte solution was added. After incubation of 1 hour at 37 °C titres were scored; this was done a second time after at least 12 hours of incubation at room temperature, and the mean of both scores was taken. Besides analysing the total amount of immunoglobulines (see primary response), secondary response samples were analysed using mercapto-ethanol, which is known to break down the IgM immunoglobulines. As with the primary response, samples were first heat-inactivated, but were diluted in 20 µl of mercapto-ethanol suspended in PBS (0.2 M) and incubated 1 hour at room temperature. Then further dilutions were made in PBS alone.

#### FEATHER PECKING

Feather pecking was recorded twice a week, starting one week post-hatching. Five weeks post-hatching observations were continued once a week, until the end of the experiment. The frequency of feather pecking was recorded, as well as the frequency of being feather pecked.

During 15 minutes the chicks were observed and all the feather-related pecks were recorded (pecks of the focal bird to another birds' feathers and pecks received by the focal bird directed towards the feathers). Observations were carried out between 09:00 and 14:00, always one day after the commercial

cages were cleaned (the semi-natural cages did not need to be cleaned every week due to the smaller group size). See table 1 for the definitions used. Correlations were made with the dermal immune response and the antibody response.

Table 1: Ethogram

Feather pecks	All the pecks directed to the feathers of a cagemate of the observed focal animal
Being feather pecked	All the received pecks directed to the feathers of the observed focal animal

#### CORTICOSTERONE SAMPLING

Blood samples used for corticosterone analyses were always taken within 2 minutes after entering the cage for catching. Samples were centrifuged by 11.000 rpm for 10 min. and stored for further analysis. Unfortunately these samples could not be analysed within the time span of this project.

#### STATISTICS

SPSS-win v9.0 was used for all statistical analyses. PHA responses were analysed using univariate analyses of variance (Univ. ANOVA). Antibody responses were analysed using repeated measures analyses of variance (rep. mes. ANOVA). The difference in titre between day(0)/day(6) and day(0)/day(10) was compared using the *simple contrast* option of the program. With this function the program subtracts day(0) from day(6) and day(0) from day(10), thus comparing the antibody-titres after stimulation with the resting level antibody titre. The day(6) sample should give an indication of the amplitude of the immune response, the day(10) sample was taken to get an indication of the rate of decreasing of antibody titres. Correlation analysis was used to test for relationships between feather pecking and antibody responses.

## RESULTS

### (a) CELL-MEDIATED IMMUNITY (PHA)

At the age of 6 weeks (fig. 3) no clear difference in PHA-response occurs between the two commercial lines ( $p=0.322$ , table 3), but chicks in the semi-natural housing condition have slightly lower (but not significant) responses ( $p=0.094$ , table 3). Note that the mean increase at this age is  $\pm 0.40$  mm (0.4154 exactly). One focal animal died (semi-nat/LP), and two focals did not respond (semi-nat/HP and comm/LP).

The large variance in the semi-nat/HP group is due to one non-responder (0.09 mm) (whereas the other two values make 0.505 and 0.590 mm). There was no correlation of the response to PHA with bodymass ( $p=0.997$ , table 2). The mean wing web thickness before injection was significantly higher in HP birds ( $p=0.026$ ); there was no correlation with the response to PHA ( $p=0.314$ , table 2). With 7-week-old chicks an additional PHA-test was performed (Appendix IX), no difference in response was found.

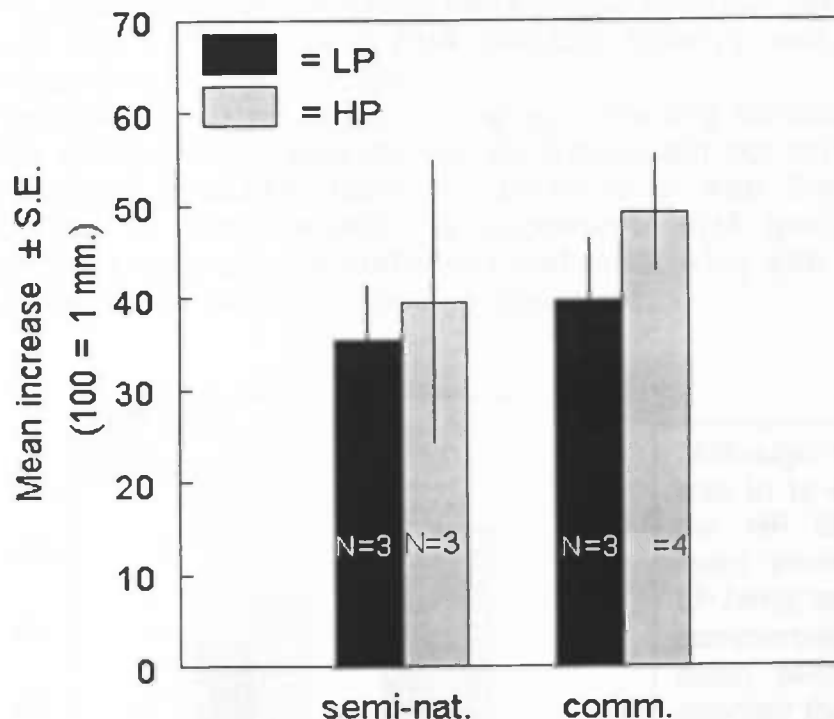


Figure 3. Response to PHA in the right wing web in 6-week-old chicks

\*No significant results; however, birds in the commercial rearing condition seem to respond stronger to PHA than birds in the semi-natural condition, and HP birds seem to react stronger than LP birds, according to the hypotheses.

Table 2. Correlation analysis for mean wing web thickness \*\* X response to PHA and for bodymass X response to PHA.

	AGE		
	6 weeks	12 weeks	18 weeks
Response X mean wing web thickness	0.314	0.121	0.203
Response X bodymass	0.997	0.038 *	0.412

\*\* the mean wing web thickness before injection was calculated as the sum of the right and the left wing web thickness, before injection, divided by 2.

In 12-week-old chicks (fig. 4) the mean response to PHA has increased to almost 0.90 mm (0.8945), that is more than a 2-fold increase, as compared with the mean response in 6-week-old chicks. HP birds tend to have a higher response than LP birds ( $p=0.068$ , table 3), and birds in the semi-natural housing condition tend to have a lower response to ( $p=0.072$ , table 3). No correlation was found between the response to PHA and mean wing web thickness ( $p=0.121$ , table 2), however HP birds had significantly bigger patagia. Bodymass correlated with the response to PHA ( $p=0.038$ , table 2), also HP birds were heavier than the LP birds ( $p=0.020$ ).

At 18 weeks (fig. 5) no effect of line and housing condition was found ( $p=0.321$  and  $p=0.952$  respectively). Bodymass did not correlate with the PHA-response ( $p=0.412$ , table 2). Mean wing web thickness before injection did not correlate with the response to PHA ( $p=0.203$ , table 2). No significant difference in bodymass and mean wing web thickness was found between the two lines/housing conditions.

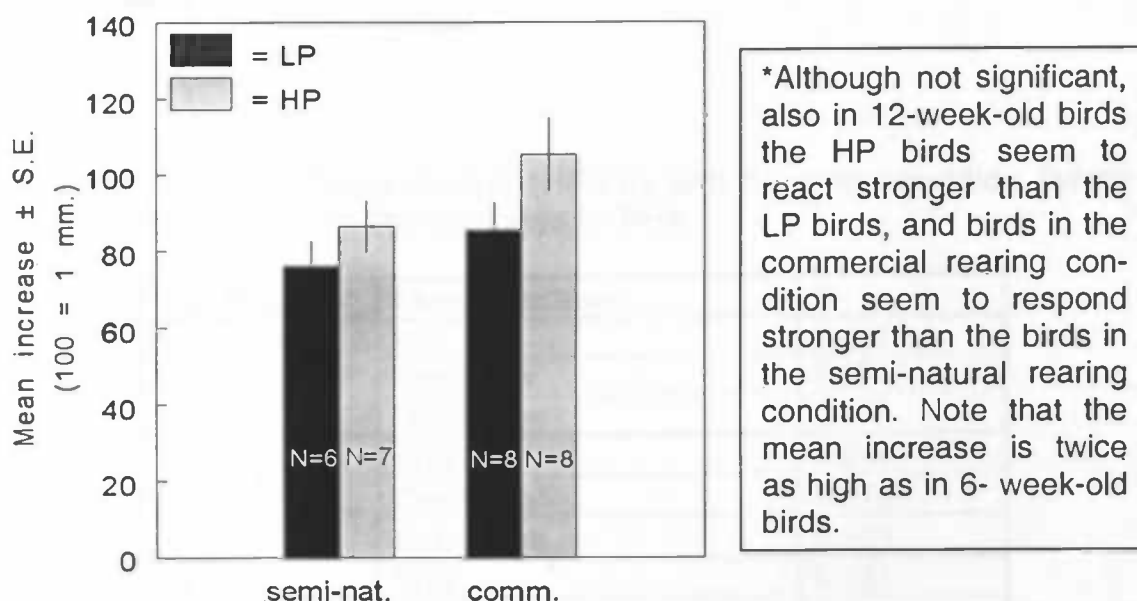


Figure 4. Response to PHA in the right wing web of 12-week-old chicks.

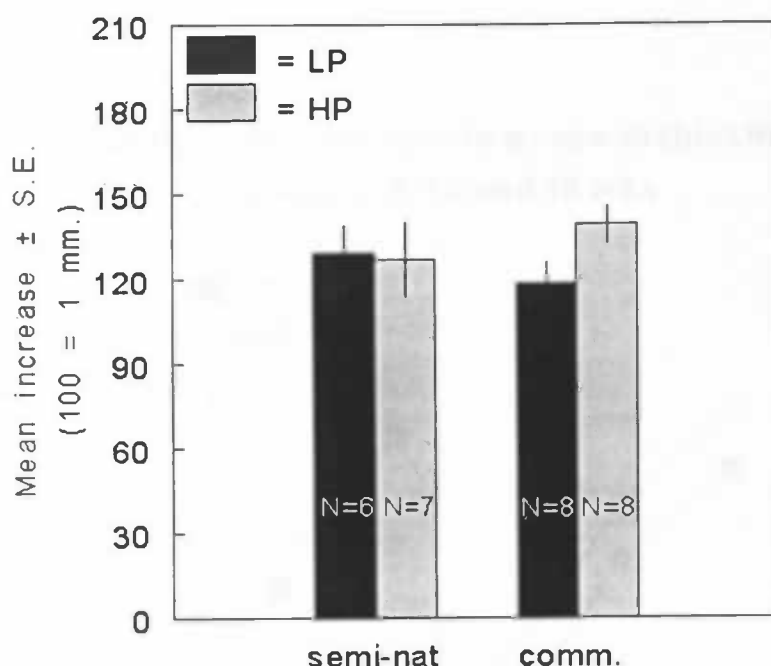
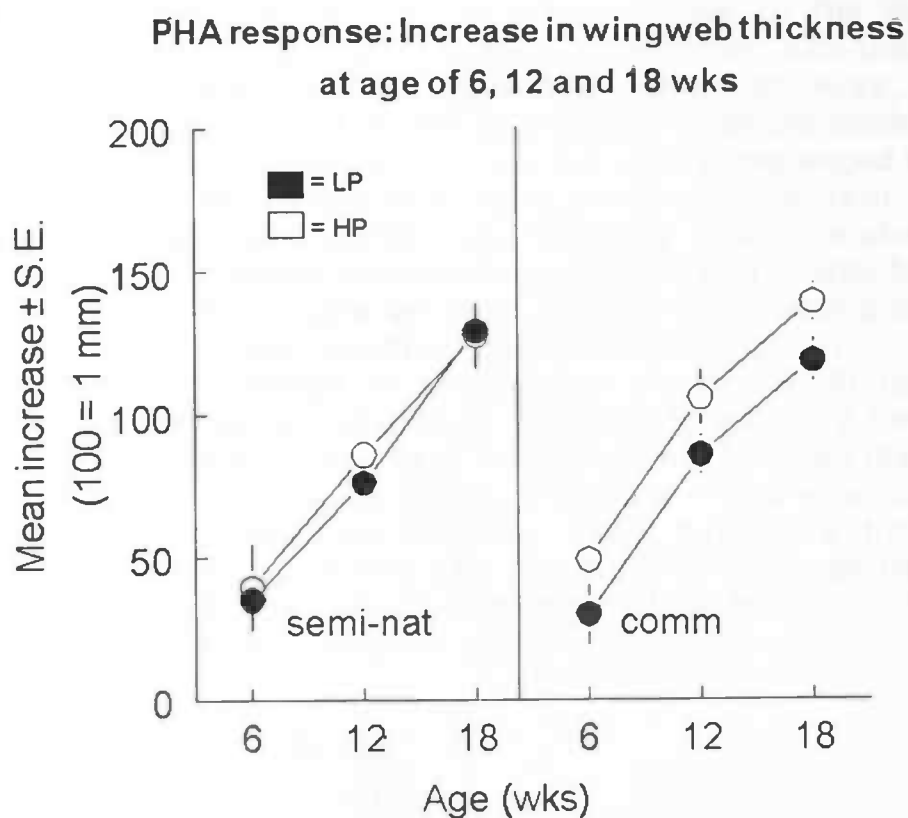


Figure 5. Response to PHA in the right wing-web in 18 wk old chicks

\*At this age, no significant differences are found between the lines and rearing conditions. But note that in 18-week-old birds, the mean increase is about three times as high as in 6-week-old birds.

Table 3: Effects of line (HP/LP) and housing condition (semi-nat/comm) on the response to PHA.

Age (weeks)	Line/Condition	P
6	Line	0.322
	Condition	0.094
	Interaction	0.163
12	Line	0.068
	Condition	0.072
	Interaction	0.523
18	Line	0.321
	Condition	0.952
	Interaction	0.244



*Figure 6.* The increase in right wing web-thickness (100=1mm) after treatment with PHA at the age of 6, 12 and 18 weeks.

In fig. 6 the response to PHA is depicted with increasing age. The response increases significantly some 3-fold in twelve weeks; however no difference in increase was found between the lines or housing conditions.

#### CELL MEDIATED IMMUNITY

- Contrary to the hypothesis, birds in the semi-natural condition seem to respond less than birds in the commercial condition
- As was expected, LP birds respond less (nearly significant) than HP birds
- At 18 weeks no differences are found
- There appears to be an effect of age; the response increases some 3-fold in 12 weeks (from week 6 – week 18)

(b) HUMORAL IMMUNITY

In 6-week-old birds (fig. 7) the effect of line on the increase in titre between day(0) and day(10) is almost significant, with  $p=0.060$  (table 3): HP birds almost have a significantly larger increase; no significant differences were found on this age. With 7-week-old chicks an extra test was carried out (Appendix VIII); the birds were challenged with SRBC, but no significant differences were found ( $p$ -values not shown).

The first challenge in 12-week-old birds (fig. 8) did not show a significant difference in response between the two lines, and neither between the two housing conditions. Line tends to interact with housing condition in the increase in titre from day(0) to day(6) ( $p=0.091$  table 3).

The second challenge in 12-week-old chicks (fig. 9) didn't show any significant difference looking at the total Ig antibody titre for line and housing condition. There were no interactions between line and condition (table 3). However, a significant difference in line was found in the IgG response (of 12-week-old birds)(fig. 10) at day(6) (i.e. the increase from day(0) to day(6)),  $p=0.023$ . The difference in response between the two lines at day(10) is nearly significant ( $p=0.058$ , table 3): HP birds show a higher increase than LP birds.

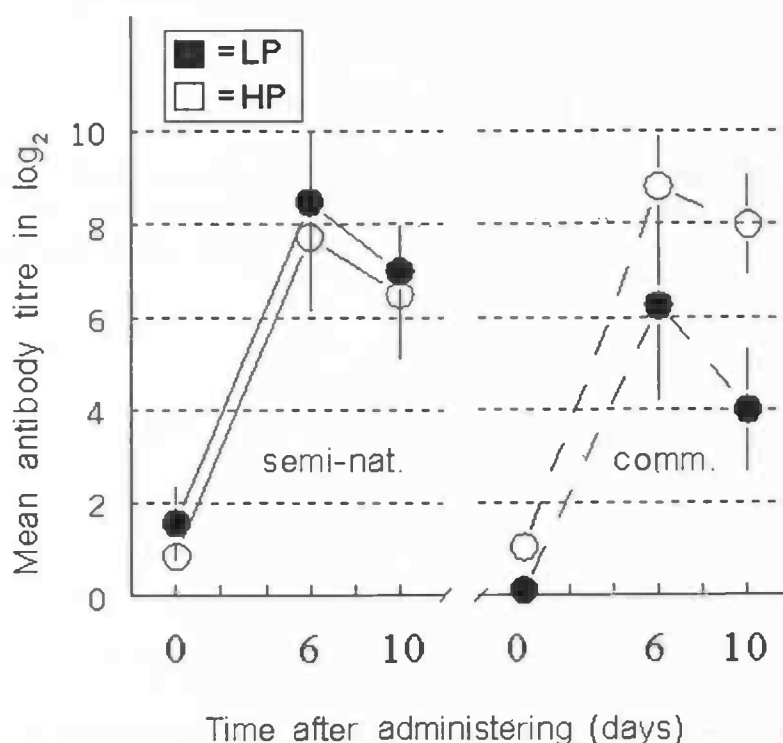


Figure 7. The antibody response (first challenge, IgM+IgG) to 0.5 ml 2% Sheep Red Blood Cells, in 6-week-old birds (N=3 in the semi-natural housing condition/N=4 in the commercial housing condition).

\* The increase in titre day(0)/day(10) is nearly significant between HP and LP birds.



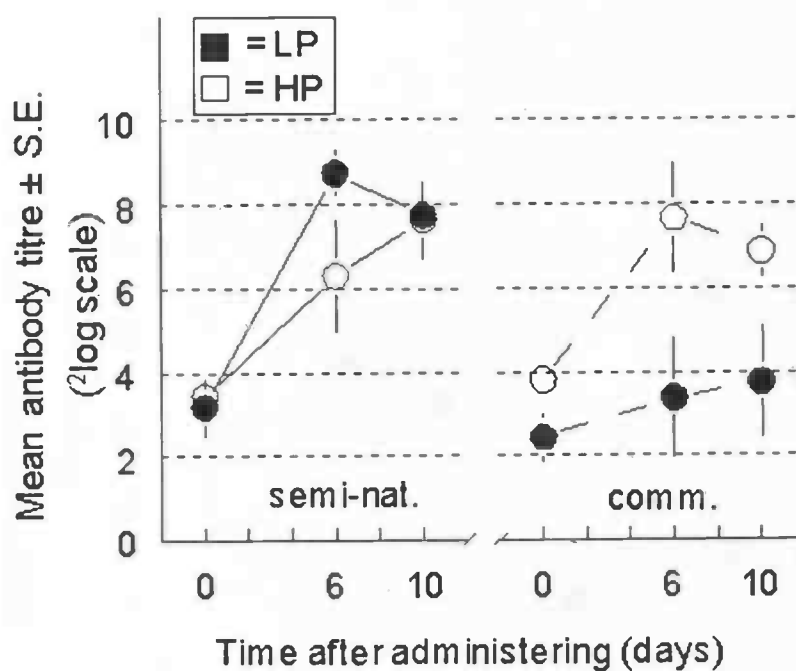


Figure 8. First challenge antibody titres (IgM+IgG) for SRBC (1.0 ml 2%), in 12-week-old chicks (N=3 for LP/semi-nat; N=4 for the remaining three groups).

\*No significant differences were found at this age.

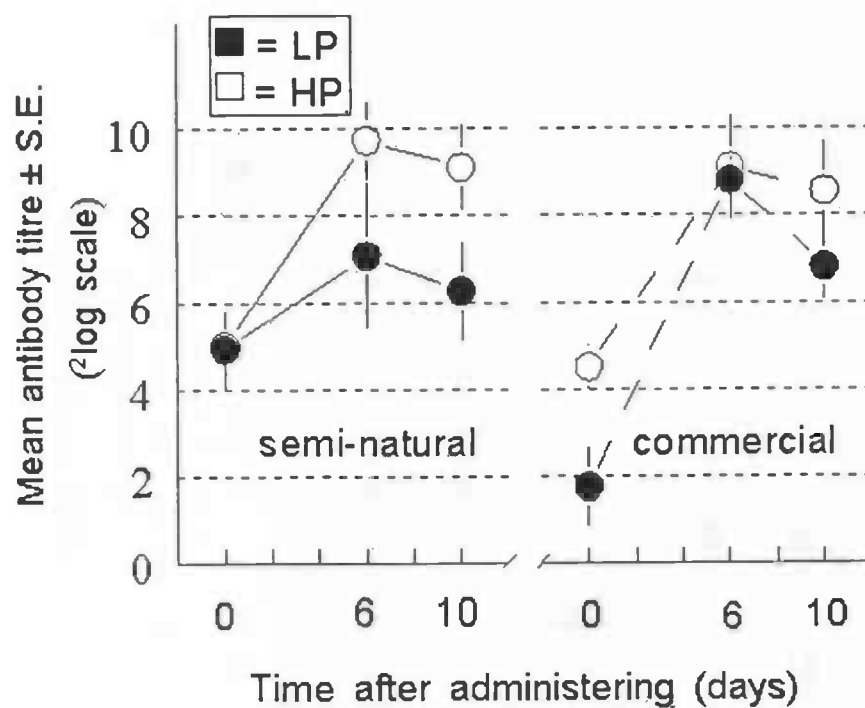
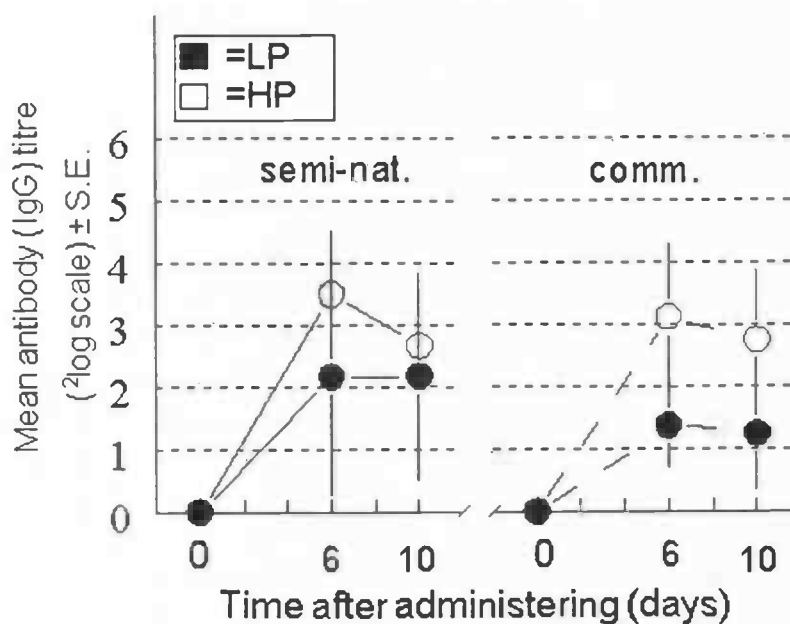


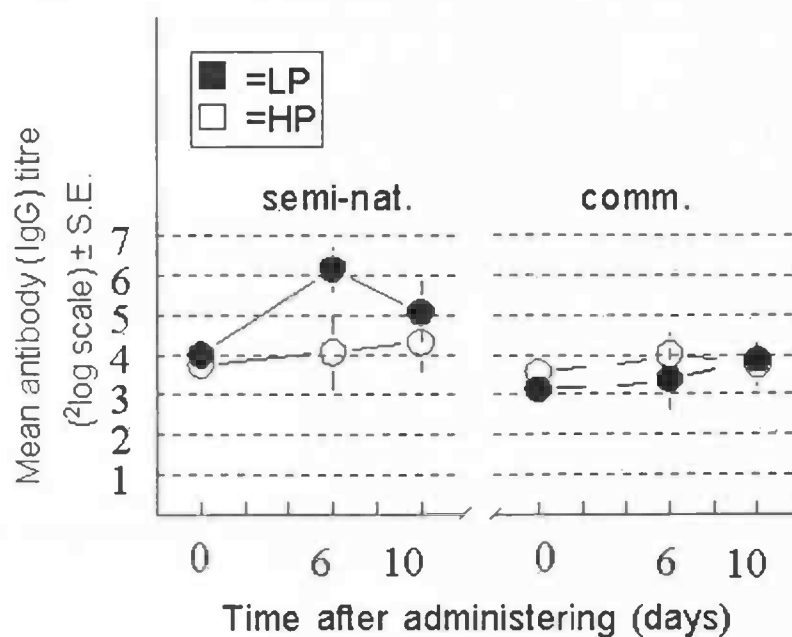
Figure 9. **Second** challenge antibody titres (IgM+IgG) for SRBC (1.0 ml 2%), in 12-week-old chicks (N=3 for the semi-natural housing condition/N=4 for the commercial housing condition).

\*No significant differences were found.



*Figure 10. IgG-antibody response (for SRBC) at the age of 12 weeks, of two lines in two different conditions (mercapto-ethanol treated samples).*

\*HP birds have a higher increase in antibody titre at day(6) than LP birds. This is nearly significant at day(10).



**Figure 11. IgG-antibody response (for SRBC) at the age of 18 weeks, two lines in two different conditions.**

\*Both at day(6) and day(10), LP birds have a significantly higher increase in antibody titre than HP birds (in contrast with 12-weeks-old birds: HP birds having a higher increase than LP birds in IgG antibody titre).

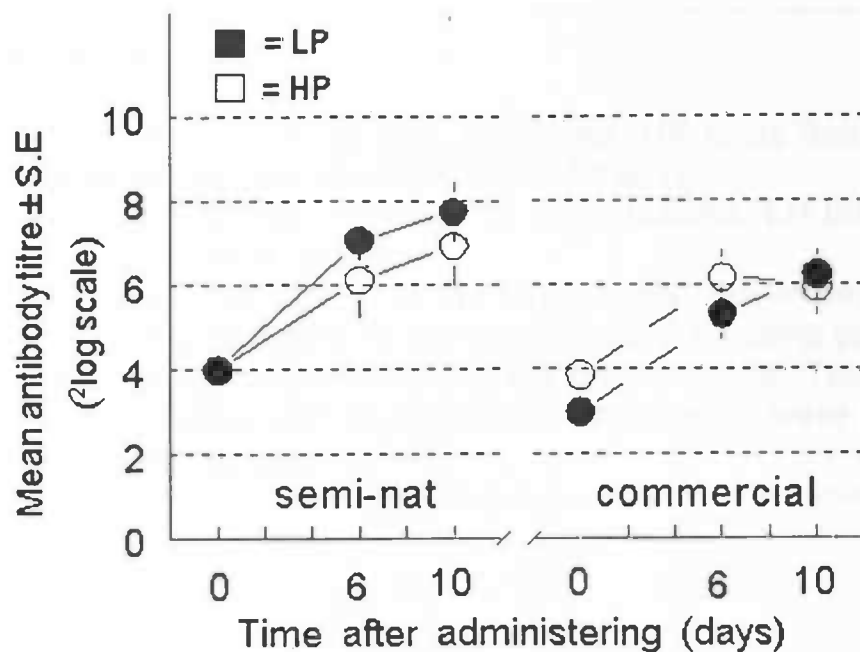


Figure 12. **Second** challenge (IgM+IgG), response to SRBC (1.0 ml 2%) in 18-week-old chicks (N=3 for LP/semi-natural; N=4 for the rest).

\*No significant differences were found.

At the age of 18 weeks, the response to SRBC (second challenge, fig. 12) does not differ between the lines and housing conditions. However, looking at the IgG response at 18 weeks (fig.11), a significant difference in line both in the increase from day(0) to day(6) and from day(0) to day(10) is found,  $p < 0.010$  and  $p = 0.008$  respectively. Contrary to expectations, LP birds have a larger increase in antibody titre. In addition there is a difference in titre between the housing conditions with respect to the increase from day(0) to day(6),  $p = 0.019$  (see table 3); as was expected, the increase in the commercial housing condition is smaller. An interaction between line and housing condition occurs, with respect to the increase in titre from day(0) to day(6). No significant correlations with bodymass were found.

#### HUMORAL IMMUNITY

- At 12 weeks of age, as was expected, HP birds have a higher increase in IgG titre than LP birds; however,
- At 18 weeks of age, contrary to expectations, LP birds have a higher increase in IgG titre.
- At 18 weeks, according to the hypothesis, the increase in IgG antibody titre is higher in the semi-natural housing condition,.
- There does not seem to be a clear effect of age: The response did not increase with age. Second challenges were not higher than first challenges.

*Table 3.* Repeated measures ANOVA with simple contrast for antibody responses to SRBC. INC6 is the increase in antibody titre from day(0) to day(6), INC10 is the increase in antibody titre from day(0) to day(10).

Age	First/Second Challenge	Line/ Condition	P	P
			INC6	INC10
6 (Ig-total)	First challenge	Line	0.246	0.060
		Condition	0.411	0.368
		Interaction	0.376	0.106
12 (Ig-total)	First challenge	Line	0.741	0.719
		Condition	0.282	0.203
		Interaction	0.091	0.362
12 (Ig-total)	Second challenge	Line	0.796	0.345
		Condition	0.158	0.163
		Interaction	0.069	0.086
12 (IgG)	Second challenge	Line	0.023*	0.058
		Condition	0.792	0.733
		Interaction	0.560	0.812
18 (Ig-total)	Second challenge	Line	0.607	0.290
		Condition	0.753	0.461
		Interaction	0.607	0.839
18 (IgG)	Second challenge	Line	<0.01*	0.008*
		Condition	0.019*	0.481
		Interaction	0.002*	0.379

### (c) FEATHER PECKING

Feather pecking was recorded during the experiment. To correlate feather pecking with the immune responses, I calculated the total cumulative amount of feather pecks observed for each bird, at the moment the birds were challenged, and made a quotient by dividing it through the total cumulative time (in seconds) observed (appendix I).

In 6-week-old chicks no correlation (Pearson, all animals together) was found for the immune response and feather pecking. Surprisingly, in 12-week-old birds the response to PHA and being feather pecked shows a positive correlation: The more feather-pecks a bird receives, the stronger the skin response to PHA. In 18-week-old birds, according to the hypothesis, the humoral IgG response from day(0) to day(6) is negatively correlated with being feather pecked: Being feather pecked causes stress, which is associated with elevated levels of corticosterone, and apparently reduces the antibody IgG-response.

Table 4: Pearson correlation analysis of the immune response with feather pecking and being feather pecked, for all the birds. Not separated for sexe, line or housing condition.

Age	Type of response	Feather pecks P:	Being feather p. P:
6 weeks	PHA-response	0.713	0.899
	Resp_6 (SRBC1)	0.258	0.646
	Resp_10 (SRBC1)	0.271	0.314
12 weeks	PHA-response	0.676	0.006 (pos.) **
	Resp_6 (SRBC1)	0.379	0.228
	Resp_10 (SRBC1)	0.604	0.611
	Resp_6 (SRBC2)	0.509	0.396
	Resp_10 (SRBC2)	0.307	0.311
	Resp_6 (IgG)	0.472	0.143
	Resp_10 (IgG)	0.596	0.116
18 weeks	PHA-response	0.534	0.909
	Resp_6 (SRBC2)	0.600	0.265
	Resp_10 (SRBC2)	0.214	0.080
	Resp_6 (IgG)	0.673	0.042 (neg.) *
	Resp_10 (IgG)	0.322	0.113



## DISCUSSION & CONCLUSIONS

### CELL-MEDIATED IMMUNITY

In the time course of this experiment, it was shown that the dermal immune response in 6- and 12-week-old White Leghorn chicks is not fully developed; in 12 weeks (week 6-week 18), the skin response for PHA increased some 3-fold. The idea arose that the increase in response could be due to a memory response, as with humoral immunity. However, half of the 12-week-old focals were injected for the first time with PHA, and half of the focal birds were injected for a second time (the first challenge was received when 6 weeks old; see figure 2, page 10). It turned out that the mean response of the first group ( $0.92 \text{ mm} \pm 0.065$ ) was not different in magnitude from the mean response of the second group ( $0.87 \text{ mm} \pm 0.057$ ) (appendix VI), which eliminated this hypothesis.

No significant differences were found between the lines/housing conditions for the wing web response to PHA (table 3). The pattern of responses found in 12-week-old birds closely resembles the pattern of responses found in 6-week-old birds, as if more 'accentuated'; but in 18-week-old chicks this was no longer clear.

Although not significant, birds in the commercial condition in general show a higher skin response to PHA than in the semi-natural housing condition, but this is not the case in birds of 18-weeks-old. At first sight this is in contradiction with the hypothesis, that, with increased (social) stress the immune response is smaller in the commercial condition. However, it may be because of the higher frequency of feather pecking in the commercial cages, that the skin becomes stimulated by all the pecks and pulling of the feathers: The pecks to the skin and the pulling of the feathers may cause some slight stimulation of the skin. When the skin is challenged with a mitogenic substance, it then may result in a stronger response. A larger sample size may yield significant results.

The LP birds respond almost significantly less than HP birds, in both housing conditions; however, this is only found in 12-week-old birds. This is according to the hypothesis that the immune response is expected to be smaller in the LP birds, due to higher levels of corticosterone. Again, repeating the experiment with a larger sample size may yield significant results.

### HUMORAL IMMUNITY

Six-week-old birds show no significant difference in response, dependent on line/housing condition; however HP birds have an almost significantly higher increase in antibody titre at day(10). There were exceptionally few non-responders: Only one (see Apanius (1998): Ontogeny of immune function).

At the age of 12 weeks, the birds were challenged for a second time and, surprisingly, the response did not appear to be stronger. The apparently identical intensity of the responses suggest that the secondary responses have peaked already before day(6) (higher than the first challenge), and therefore is decreasing already at day(6). In this way the two responses would be of equal intensity. This theory also explains why in 12-week-old birds, the secondary response is not much stronger than the response of the first challenge in 12-week-old birds. However, this hypothesis was ruled out by a pilot study, carried out in 19-week-old birds, challenged for a second time (appendix XIII). Although performed under different conditions (only HP birds, living in a group of 8

individuals, sex ratio 50% males:50% females), the peaks in antibody titre were found to be between day(6) and day(10) post-immunization.

With respect to the IgG response, a significant difference in line was found at day(6) post-immunization; as was expected, the HP birds have a higher increase. However, at 18 weeks this seems to be inverted: LP birds have a higher increase in antibody IgG titre, at both day(6) and day(10) post-immunization. In 18-week-old HP birds bodymass was positively correlated with the IgG response (at both day(6) and day(10)), as this was not the case in 12-week-old birds. Because of that I would give more importance to the statistical result of the difference in IgG response in 12-week-old birds.

In 18-week-old chicks the secondary response for sheep red blood cells is even smaller than the response found at the age of 6 and 12 weeks. The LP birds seem to respond less in the *commercial* housing condition, whereas for the HP birds the humoral immune responses in the two housing conditions are more or less of equal intensity. This finding may lead to the conclusion that LP birds are more sensitive to (social) stress than HP birds.

No correlations were found for the cell mediated immune response with the humoral immune response, a high dermal immune response apparently does not implicate also a high antibody response or *vice versa*.

#### FEATHER PECKING AND IMMUNITY

No relationship was found between feather pecking and being feather pecked. It does not seem to be the case that birds who peck a lot are not or very little being feather pecked, or birds who are being feather pecked a lot, perform less feather pecking themselves: Peckers are also receivers.

In 6-week-old chicks no correlations were found; however in 12-week-old chicks a positive correlation was found for being-feather-pecked with the response to PHA (table 4): The more the birds are being feather pecked, the higher turns out to be their dermal immune response. This is in concordance with the result discussed in the previous section: Birds in the *commercial* housing condition (more feather pecking) seem to have a slightly stronger dermal response than in the natural condition. Although in contradiction with the hypothesis that feather pecking causes stress and thus suppresses the immune system, birds in the *commercial* rearing condition have a higher response. A very plausible explanation is that the increased feather pecking leads to priming of the skin, which results in a stronger dermal immune response. This hypothesis is supported by the positive correlation that was found between the dermal response to PHA, and being-feather-pecked; the cell-mediated immunity may be less sensitive to corticosterone levels.

In 18-week old chicks a negative correlation is found between being feather pecked and the increase in IgG level from day(0) to day(6). The negative correlation is exactly what is expected. The more a bird is being feather pecked, the more it is stressed and this is associated with elevated levels of corticosterone, which suppresses the immune system. This finding supports the hypothesis that high group sizes (commercial rearing condition) -that may be associated with an increased frequency of feather pecking- have a negative impact on the immune system of the animals.

## SUMMARY

The PHA wing-web response did not bring any significant results. A higher sample size however may yield significant results, especially in 6- and 12-week-old birds. However, the dermal skin reaction appeared to be age-dependent, almost increasing 3-fold in 12 weeks. In 6-week-old birds, no significant results were found. In 12-week-old birds, HP birds had significantly higher IgG titres than LP birds. This was according to the hypothesis that due to lower resting corticosterone levels these birds would have a more competent immune system. In 18-week-old birds, LP birds were found to have significantly higher IgG titres, but due to a correlation with bodymass, this statistical result is questionable. The LP birds seem to have a smaller increase in antibody titre in the commercial condition, whereas the HP birds didn't show this difference. This may lead to the conclusion that LP birds are more sensitive for (social) stress.

At the age of 12 weeks a positive correlation was found between being-feather-pecked and the response to PHA. This lead to the conclusion that the pecking may cause priming of the skin that therefore becomes stimulated; the immunological reaction of the skin may be enhanced. At the age of 18 weeks a negative correlation was found between being-feather-pecked and the day(6) IgG response. This supports the hypothesis that feather pecking (associated with high groupsize) negatively influences the humoral immune response.

## FURTHER RESEARCH

Repeating the experiment with larger sample sizes may yield significant results, especially for the PHA wing-web response.

The corticosterone samples that were taken during the experiment should be analysed, for understanding the relationships between stress, corticosterone and immunity.

To obtain a more detailed understanding of the effect of groupsize, several groups of increasing groupsize can be created, to assess immunity on different moments in life. Other varying factors should be excluded.

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## APPENDICES

### Appendix I

Rates (pecks per second) of feather pecking and being feather pecked. The cumulative amount of feather pecks was divided by the cumulative time observed; **Sex:** 0=female, 1=male; **Condition:** 0=semi-natural, 1=commercial; **Line:** 0=LP, 1=HP

Tag	Sex	Cond	Line	Feather pecking 6 weeks	Feather pecking 12 weeks	Feather pecking 18 weeks	Is Feather Pecked 6 weeks	Is Feather pecked 12 weeks	Is Feather pecked 18 weeks
102	0	0	0	0.000808	0.001159	0.00101	0.000202	0.000386	0.000269
176	0	0	0	0.002	0.00114	0.001069	0	0.001667	0.001384
196	0	0	0	0.000333	0.001842	0.001509	0	0	0.000126
197	0	0	0	0.001975	0.001164	0.001075	0	0.000635	0.000645
163	1	0	0	0.000202	0.000483	0.000337	0.00101	0.000483	0.000337
179	1	0	0	0.006889	0.003535	0.003125	0.000667	0.000505	0.000417
223	0	0	1	0.010575	0.005683	0.003956	0.00069	0.000656	0.00044
235	0	0	1	0.003908	0.002623	0.001905	0.025057	0.012131	0.009011
240	0	0	1	0.004646	0.002222	0.00202	0.015354	0.007343	0.005253
255	0	0	1	0.001556	0.001212	0.00125	0.002444	0.001212	0.001042
281	0	0	1	0.005556	0.002727	0.002222	0.000222	0.000404	0.000278
209	1	0	1	0.002222	0.00152	0.00152	0.001212	0.001404	0.001404
217	1	0	1	0.003434	0.002126	0.002559	0.001616	0.00087	0.000741
268	1	0	1	0.008687	0.006184	0.004377	0.002424	0.001159	0.001347
144	0	1	0	0.002222	0.002512	0.002761	0.001818	0.001159	0.000875
159	0	1	0	0.001556	0.00202	0.001597	0.001778	0.002525	0.002569
167	0	1	0	0.003232	0.001643	0.002492	0.00101	0.001643	0.001414
178	0	1	0	0.001778	0.001616	0.003403	0.003556	0.002222	0.002153
103	1	1	0	0.001616	0.001643	0.001212	0.00101	0.002319	0.001751
120	1	1	0	0.000444	0.000404	0.000625	0.002	0.001616	0.002639
136	1	1	0	0.001111	0.001414	0.0025	0	0.001414	0.001736
181	1	1	0	0.002716	0.003175	0.002724	0.000741	0.008889	0.012115
207	0	1	1	0.006222	0.006566	0.008958	0.027111	0.016566	0.011667
285	0	1	1	0.00642	0.018413	0.01319	0.004198	0.003598	0.002652
288	0	1	1	0.003232	0.002126	0.001886	0.005859	0.028986	0.020673
291	0	1	1	0.004	0.003131	0.003125	0.000222	0.000404	0.002778
201	1	1	1	0.001333	0.002626	0.002083	0.010444	0.004747	0.003264
221	1	1	1	0.002222	0.006667	0.005069	0.005111	0.005152	0.004792
274	1	1	1	0.000889	0.001212	0.004931	0.000222	0.002323	0.002778
280	1	1	1	0.001778	0.010202	0.010556	0.001778	0.004545	0.003125

## Appendix II

Bodymasses (grams) of the focal birds; **Sex:** 0=female, 1=male;  
**Condition:** 0=semi-natural, 1=commercial; **Line:** 0=LP, 1=HP

Tag	Sex	Cond	Line	0 weeks	3 weeks	5 weeks	7 weeks	9 weeks	13 weeks	20 weeks
102	0	0	0	40.07	131.6	266.4	463.3	699.9	944	1467
176	0	0	0	36.18	101.4	215.3	380.6	557.6	937	1320
196	0	0	0	39.79	123.7	250.4	432.4	622.5	991.3	1328
197	0	0	0	37.35	113.8	212.8	392	591.6	958	1257
163	1	0	0	36.93	134.3	268.1	506.1	773.4	1295	1675
179	1	0	0	38.27	152.8	299	525	824.3	1343	1753
223	0	0	1	35.1	145.2	264.5	487.9	759.6	1090	1461
235	0	0	1	41.16	156.4	306.1	546.6	826.9	1143	1544
240	0	0	1	36.61	112.7	242.7	433	637.3	963.4	1242
255	0	0	1	36.88	143.7	262.4	459.1	711.8		1343
281	0	0	1	36.6	171	340.3	567	848.6	1192	1685
209	1	0	1	38.76	181.9	375.1	631.2	885.4	1055	dead
217	1	0	1	43.09	158.6	359.2	636.6	994.6	1646	2000
268	1	0	1	38.18	168.4	347.6	604.9	900.8	1366	1770
144	0	1	0	40.12	169.8	284.5	479	688.4	1006	1340
159	0	1	0	49.79	167.9	310.5	505	742.3	1089	1505
167	0	1	0	42.18	170.3	268.7	457	670.9	979	1328
178	0	1	0	43.2	169.9	309	539.3	778.8	1159	1530
103	1	1	0	41.4	151.6	275.7	514	739.4	1290	1865
120	1	1	0	43.73	190.6	314.1	594.5	932.6	1433	1930
136	1	1	0	41.44	149.1	276.5	427	718.8	1226	1810
181	1	1	0	40.09	196.6	341	596.9	920.6	1470	1935
207	0	1	1	39.39	179	309.3	492	701.9	976	1326
285	0	1	1	41.93	171.9	266.1	541.9	783.4	1125	1508
288	0	1	1	39.99	199.8	354	563	813.6	1108	1460
291	0	1	1	42.21	177.8	319.9	536.7	760.5	1065	1282
201	1	1	1	39.28	194.4	359	620.5	937.8	1483	1750
221	1	1	1	41.57	229.6	421.7	731.4	1113	1760	2146
274	1	1	1	42.42	204.1	367.7	620	871.8	1325	1572
280	1	1	1	43.24	210	387.5	548.5	966.1	1429	1690



# Appendix III

Antibody (IgM+IgG) titres to SRBC for the focal animals. The second series of values are secondary response values, the third series are antibody titres for Pig Red Blood Cells. **Condition:** 0=semi-natural, 1=commercial. Note: all tag numbers ranging from 100-199 are LP birds, and all tag numbers ranging from 200-299 are HP birds.

	Tag	Cond	Total IgG											
			SRBC 1				SRBC 2				PRBC			
			Age (wks)	Day 0	Day 6	Day 10	Age	Day 0	Day 6	Day 10	Age	Day0	Day6	Day10
Group 1	223	0	6	0.5	4	4	12	5.25	12	12	18	9.5	9	10
	163	0	6	0	6.5	6.5	12	3.25	5.75	4.75	18	7	8.25	8
	281	0	6	0	7	4.5	12	6	8.5	7.5	18	8.5	8	8.5
	197	0	6	2.75	11.5	9	12	5	10.5	8.5	18	8.5	9	7.5
	196	0	6	1	12	10	12	6.5	5	5.5	18	5.75	12	10
	268	0	6	2	7.5	5.5	12	3.75	10.5	9	18	8.25	10.5	9.75
	282	0	6	2	8	7.5	12	5	8	8	18	7.75	9	9.25
	167	1	6	0	9	5.25	12	2.5	9.25	7.75	18	7.75	8.5	8
	207	1	6	1	8.75	8.25	12	4	9.5	8.5	18	8.5	7.5	8
	103	1	6	0	8	4.75	12	0.5	10	8	18	7	7.5	8
	274	1	6	0.5	7.5	5	12	3.5	6	5.75	18	8	9	8.5
	178	1	6	0.5	8	6	12	4	7.5	7	18	7.5	9.5	9
	288	1	6	0.5	12	10.25	12	6	12	11.5	18	9	9	9
	181	1	6	0	0	0	12	0	8.5	4.5	18	5.5	8	6.75
	280	1	6	2.25	7	8.5	12	4.5	9	8.5	18	9.25	9.5	9
Group 2	235	0	12	3	5	6	18	4	3.75	4				
	102	0	12	4.5	8.75		18	4	6.5	7				
	217	0	12	3.75	9.5	9	18	4	6.5	8.25				
	179	0	12	3	7.75	7.5	18	3.5	7.25	9.25				
	176	0	12	2	9.75	8	18	4.5	7.5	7				
	240	0	12	3	3.25	9.5	18	3.25	6	7.5				
	255	0	12	4	7.5	6	18	4.75	8.25	8				
	120	1	12	3			18	3	4.5	4.5				
	221	1	12	4	8.5	8	18	3.75	7.25	6.5				
	144	1	12	3.75	4	3.5	18	4	4	6.5				
	285	1	12	3.25	9.5	8	18	3.5	5	4				
	136	1	12	1	7.25	6.25	18	3	6.75	7.5				
	201	1	12	3	5	5.5	18	3	7.5	7				
	159	1	12	2	1.25	1.5	18	2	6	6.5				
	291	1	12	5		6	18	5.25	5	6.25				



	Cond	Line	Age	Day 0	Day 6
	0	0	12	0	0
	0	0	12	0	6
	0	0	12	0	0.5
	0	1	12	0	5.5
	0	1	12	0	2
	0	1	12	0	3
	0	1	12	0	1
	1	0	12	0	3
	1	0	12	0	2
	1	0	12	0	0.5
	1	0	12	0	0
	1	1	12	0	4
	1	1	12	0	0.5
	1	1	12	0	6
	1	1	12	0	2
	0	0	18	3.5	5
	0	0	18	4	7
	0	0	18	4.5	6.5
	0	1	18	3.5	2
	0	1	18	4	5.5
	0	1	18	4.5	2.75
	0	1	18	3	6
	1	0	18	3.5	4.5
	1	0	18	4	3
	1	0	18	3	4.75
	1	0	18	2	1.25
	1	1	18	3	5.5
	1	1	18	4.25	4
	1	1	18	3	3.75

## Appendix V

Measures of the wing web at the age of 6 weeks (group1), with the calculated mean thickness and the bodymass (BM, 5 wks); **Sex**: 0=female, 1=male. **Line**: 0=LP, 1=HP. **Cond**: 0=semi-natural, 1=commercial. **MeanL/R**= mean of two measures of the left respectively the right wing web. **MeanLaft/MeanRaft**= mean of two measures of the left respectively the right wing web after challenging with PHA. The **Response** is calculated as the difference in wing web thickness before and after challenging with PHA. **Meanth**= mean of the left and right wing web thickness, prior to injection.

Tag	Sex	Line	Cond	MeanL	MeanR	MeanLaft	MeanRaft	Response	BM	Meanth
163	1	0	0	35.5	36.0	43.5	67.5	31.5	268.1	35.75
196	0	0	0	38.5	30.5	53.5	57.5	27.0	250.4	34.50
197	0	0	0	33.0	28.5	37.0	76.0	47.5	212.8	30.75
223	0	1	0	54.5	32.0	64.0	82.5	50.5	264.5	43.25
268	1	1	0	78.5	89.5	70.5	80.5	-9.0	347.6	84.00
281	0	1	0	55.5	30.5	74.0	89.5	59.0	340.3	43.00
209	1	1	0	60.0	74.5	92.0	83.5	9.0	375.1	67.25
167	0	0	1	56.0	65.0	57.0	107.5	42.5	268.7	60.50
178	0	0	1	73.0	42.5	39.0	92.5	50.0	309.0	57.75
181	1	0	1	45.5	41.0	44.0	67.5	26.5	341.0	43.25
207	0	1	1	51.0	81.5	43.5	124.0	42.5	309.3	66.25
274	1	1	1	55.5	40.5	45.0	98.0	57.5	367.7	48.00
280	1	1	1	57.5	61.5	62.5	123.5	62.0	387.5	59.50
288	0	1	1	73.0	62.0	87.0	96.5	34.5	354.0	67.50
103	1	0	1	45.5	29.5	73.5	30.5	1.0	275.7	37.50

## Appendix VI

Measures of the wing web at the age of 12 weeks (group 1+group 2), with the calculated mean thickness and the bodymass (BM, 13 wks); **Sex:** 0=female, 1=male. **Line:** 0=LP, 1=HP. **Cond:** 0=semi-natural, 1=commercial. **MeanL/R=** mean of two measures of the left respectively the right wing web. **MeanLaft/MeanRaft=** mean of two measures of the left respectively the right wing web after challenging with PHA. The **Response** is calculated as the difference in wing web thickness before and after challenging with PHA. **Meanth=** mean of the left and right wing web thickness prior to injection, not meas.= not measured.

	Tag	Sex	Line	Cond.	MeanL	MeanR	MeanLaft	MeanRaft	Resp.	BM	Meanth
Group 1	103	1	0	1	40.00	38.00	42.00	124.00	86.00	1,290.00	39.00
	163	1	0	0	47.00	49.50	58.50	130.50	81.00	1,295.00	48.25
	167	0	0	1	45.50	45.50	52.50	119.00	73.50	979	45.50
	178	0	0	1	39.50	38.00	45.00	112.50	74.50	1,159.00	38.75
	181	1	0	1	42.50	40.00	45.50	137.50	97.50	1,470.00	41.25
	196	0	0	0	48.50	54.00	51.00	124.00	70.00	991.3	51.25
	197	0	0	0	43.00	38.00	43.00	114.00	76.00	958	40.50
	207	0	1	1	46.00	47.50	55.50	159.50	112.00	976	46.75
	223	0	1	0	50.50	37.50	53.50	107.50	70.00	1,090.00	44.00
	268	1	1	0	47.50	53.00	76.50	177.00	124.00	1,366.00	50.25
	274	1	1	1	53.00	50.00	59.50	127.50	77.50	1,325.00	51.50
	280	1	1	1	50.50	51.50	63.50	178.50	127.00	1,429.00	51.00
	281	0	1	0	47.00	60.00	60.50	142.00	82.00	1,192.00	53.50
	282	0	1	0	37.50	35.50	56.50	117.50	82.00	not meas.	36.50
	288	0	1	1	60.00	51.00	71.00	194.50	143.50	1,108.00	55.50
Group 2	102	0	0	0	43.50	35.50	41.50	139.50	104.00	944	39.50
	120	1	0	1	39.00	34.50	47.00	150.00	115.50	1,433.00	36.75
	136	1	0	1	36.00	38.00	37.50	90.50	52.50	1,226.00	37.00
	144	0	0	1	31.00	33.50	41.50	113.00	79.50	1,006.00	32.25
	159	0	0	1	45.00	36.00	54.50	143.00	107.00	1,089.00	40.50
	176	0	0	0	56.50	54.00	61.00	109.00	55.00	937	55.25
	179	1	0	0	44.50	44.50	45.00	116.00	71.50	1,343.00	44.50
	201	1	1	1	54.50	54.50	68.50	142.00	87.50	1,483.00	54.50
	217	1	1	0	41.50	40.00	62.50	109.00	69.00	1,646.00	40.75
	221	1	1	1	47.50	46.00	74.50	155.50	109.50	1,760.00	46.75
	235	0	1	0	49.50	47.00	62.50	130.50	83.50	1,143.00	48.25
	240	0	1	0	38.50	45.00	54.00	130.50	85.50	963.4	41.75
	255	0	1	0	48.00	45.50	53.50	137.50	92.00	not meas.	46.75
	285	0	1	1	35.50	45.00	42.00	107.50	62.50	1,125.00	40.25
	291	0	1	1	59.00	51.50	55.50	176.50	125.00	1,065.00	55.25

## Appendix VII

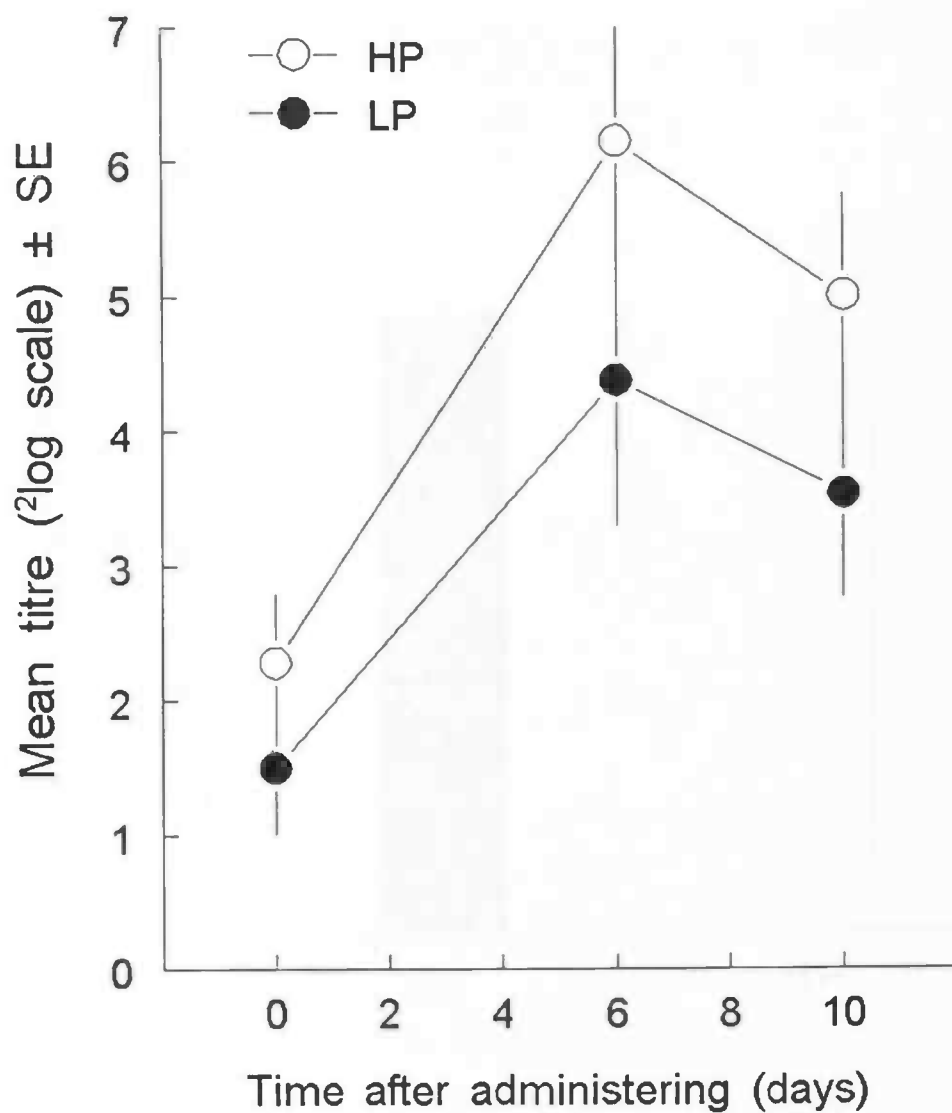
Measures of the wing web at the age of 18 weeks (group 1 + group 2), with the calculated mean thickness and the bodymass (BM, 19 wks); **Sex:** 0=female, 1=male. **Line:** 0=LP, 1=HP. **Cond:** 0=semi-natural, 1=commercial. **MeanL/R=** mean of two measures of the left respectively the right wing web. **MeanLaft/MeanRaft=** mean of two measures of the left respectively the right wing web after challenging with PHA. The **Response** is calculated as the difference in wing web thickness before and after challenging with PHA. **Meanth=** mean of the left and right wing web thickness prior to injection.

	Tag	Sex	Line	Cond	MeanL	MeanR	MeanLaft	MeanRaft	Resp	Meanth	BM
Group 1	103	1	0	1	73.5	54.5	51.50	150.00	95.50	64.00	1865
	163	1	0	0	55.0	67.5	72.50	170.00	102.50	61.25	1675
	167	0	0	1	60.0	62.5	54.50	168.00	105.50	61.25	1328
	178	0	0	1	66.0	52.5	73.00	181.00	128.50	59.25	1530
	181	1	0	1	54.5	61.5	61.50	187.50	126.00	58.00	1935
	196	0	0	0	41.5	45.5	39.00	177.00	131.50	43.50	1328
	197	0	0	0	46.5	40.0	45.50	167.00	127.00	43.25	1257
	207	0	1	1	43.5	51.5	60.50	178.00	126.50	47.50	1326
	223	0	1	0	49.0	49.5	51.00	180.00	130.50	49.25	1461
	268	1	1	0	73.5	93.5	79.00	276.50	183.00	83.50	1770
	274	1	1	1	66.0	52.5	73.00	208.00	155.50	59.25	1572
	280	1	1	1	81.0	64.5	81.50	180.00	115.50	72.75	1690
	281	0	1	0	69.0	60.5	65.50	182.50	122.00	64.75	1685
	282	0	1	0	66.5	58.5	67.00	188.00	129.50	62.50	dead
	288	0	1	1	57.0	59.0	80.00	184.50	125.50	58.00	1460
Group 2	102	0	0	0	45.0	45.0	63.50	154.00	109.00	45.00	1467
	120	1	0	1	81.0	89.5	75.50	212.00	122.50	85.25	1930
	136	1	0	1	49.0	55.5	56.50	140.00	84.50	52.25	1810
	144	0	0	1	55.0	45.0	51.00	178.50	133.50	50.00	1340
	159	0	0	1	57.5	51.0	60.50	202.50	151.50	54.25	1505
	176	0	0	0	42.5	59.5	49.00	231.50	172.00	51.00	1320
	179	1	0	0	47.0	62.5	57.00	194.50	132.00	54.75	1753
	201	1	1	1	60.5	81.0	69.00	219.00	138.00	70.75	1750
	217	1	1	0	68.5	72.5	76.50	221.00	148.50	70.50	2000
	221	1	1	1	140.5	65.5	96.50	211.50	146.00	103.00	2146
	235	0	1	0	57.0	62.5	71.50	198.50	136.00	59.75	1544
	240	0	1	0	44.5	60.5	45.50	131.00	70.50	52.50	1242
	255	0	1	0	47.5	73.0	59.00	171.50	98.50	60.25	1343
	285	0	1	1	54.5	53.5	53.50	182.50	129.00	54.00	1508
	291	0	1	1	68.5	53.0	76.50	229.00	176.00	60.75	1282

## Appendix VIII

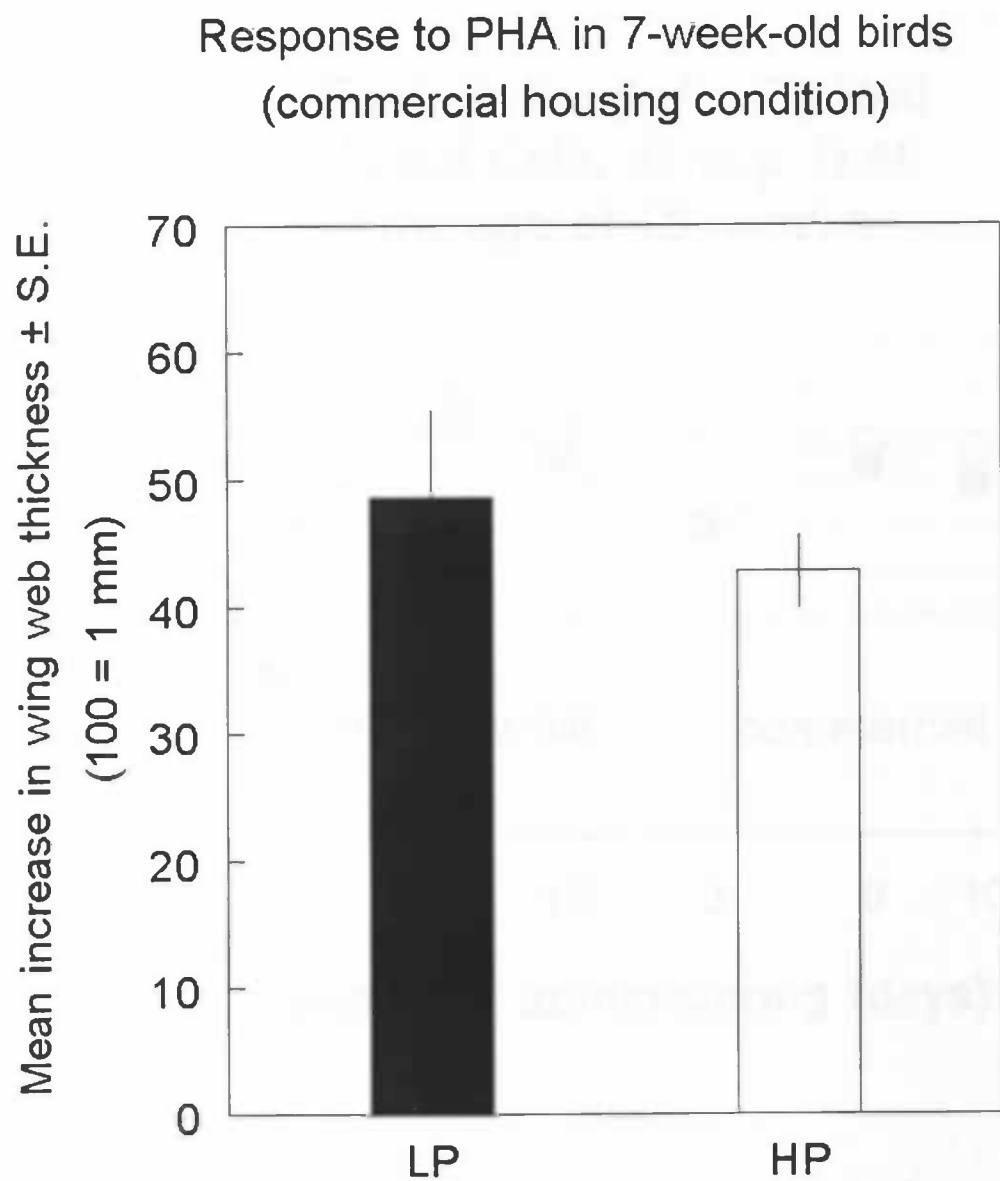
7-week-old chicks in the commercial housing condition were challenged with SRBC.

### Antibody titres to SRBC (0.5 ml 2%) in 7-week-old birds



## Appendix IX

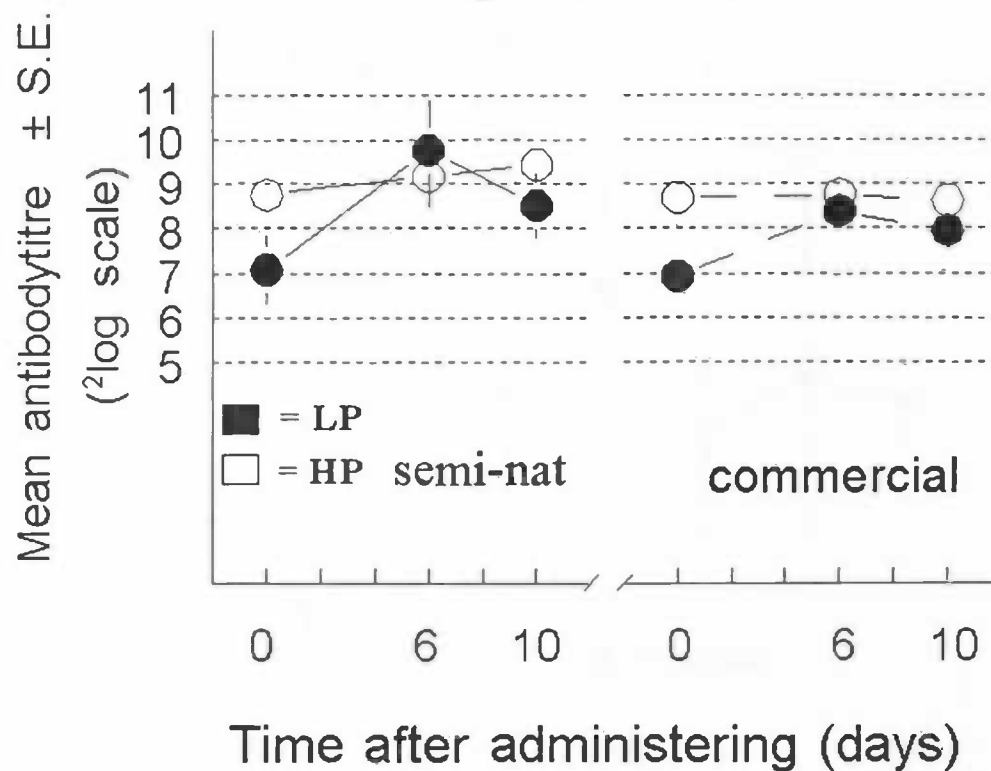
Response to PHA in 7-week-old chicks in the commercial housing condition



## Appendix X

18 wk. old chicks challenged with Pig Red Blood Cells.

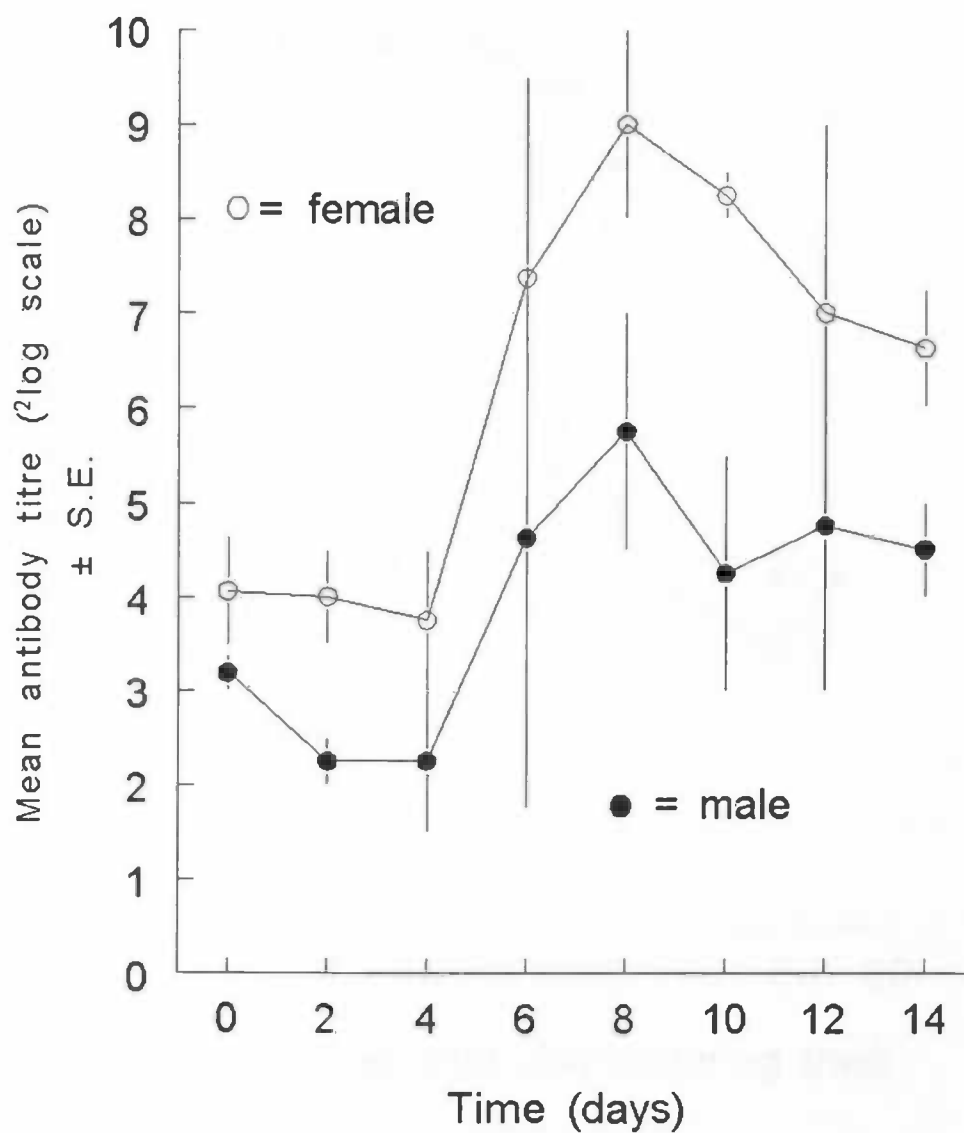
### First challenge to Pig Red Blood Cells (Group 1) at the age of 18 weeks



## Appendix XI

A pilot study carried out to estimate the immune response to SRBC in chicks of 13 weeks.

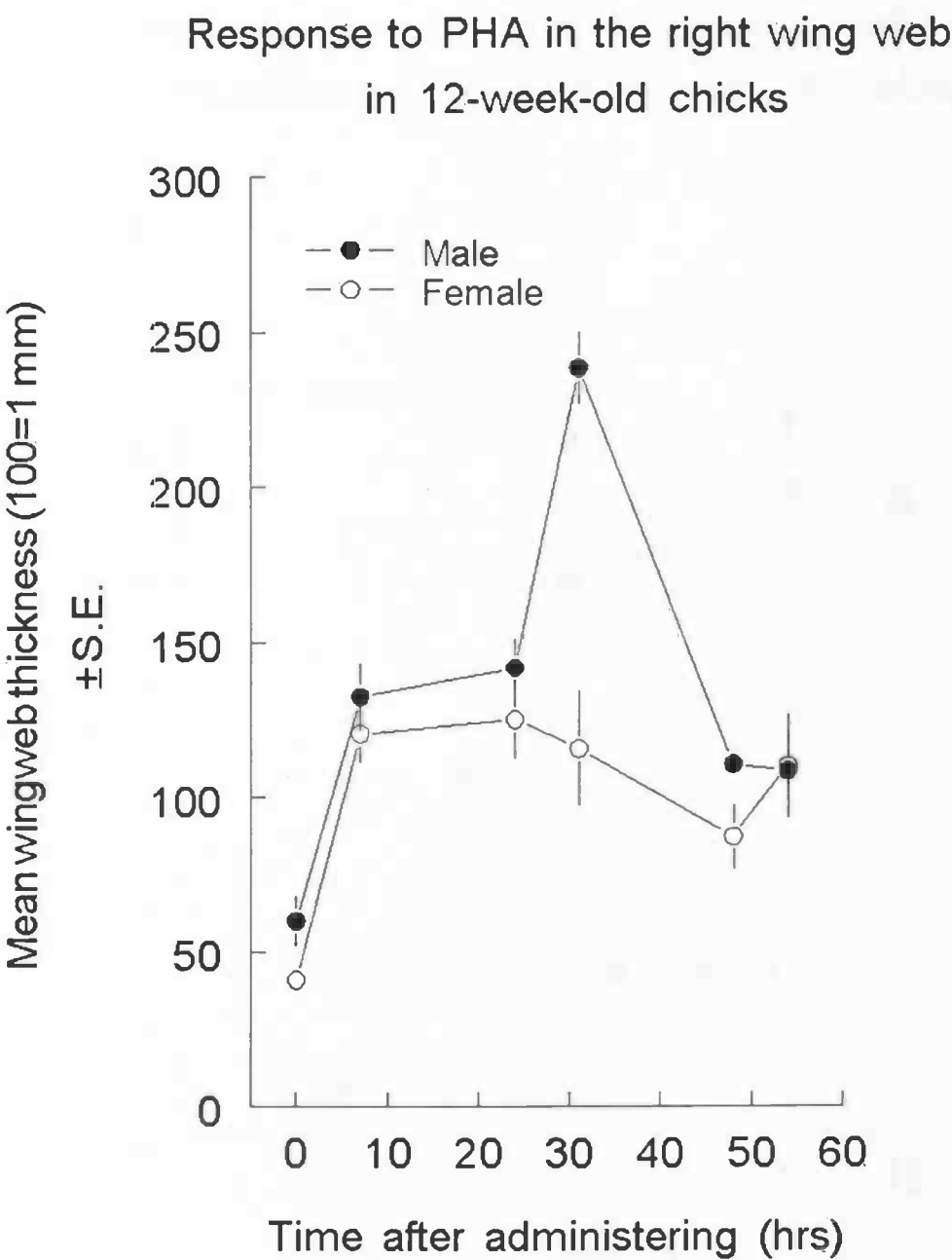
### Antibody titres for Sheep Red Blood Cells in 13-week-old chicks (4 females, 4 males)





Appendix XII

A pilot study carried out to assess the skin immune response to PHA, in chicks of 12 weeks.



### Appendix XIII

A pilot study carried out to estimate the immune response to SRBC in chicks of 19 weeks.

#### Antibody titres for Sheep Red Blood Cells in 19-week-old chicks (4 females, 4 males)

