Thermal influences on antibody production and metabolism in chicken lines divergently selected for immune responsiveness.

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Voor *mijn ouders*

Alwine

Marqué

Er is zelden iets dat ik zeg of denk
of ook het tegenovergestelde gaat door mijn gedachten.

[Godfried Bomans]

Stellingen

1. Selektie op antilichaamproduktie bij pluimvee leidt niet tot een verhoogde stressgevoeligheid.
   [Dit proefschrift, Hst. I, II, III]

2. Verschillen tussen de "hoge" en "lage" selektielijn in humorale immuunrespons worden deels verklaard door verschillen in het aantal antilichaamproducerende cellen.
   [Dit proefschrift, Hst. IV]

3. Het al dan niet optreden van immuunsuppressie is onbruikbaar als parameter voor de mate van "stress" waaraan een dier is blootgesteld.
   [Kelley, 1985. Animal stress p.193-223; Siegel, 1985. WPSA-journal 41: 36-44; Dit proefschrift]

4. Selektie op een hoge humorale immuunrespons kan uitgevoerd worden zonder negatieve gevolgen voor andere immunologische kenmerken en produktie-eigenschappen.

5. De potentiële waarde van selektie op weerstandskenmerken is voldoende onderbouwd om nader onderzoek in challenge-proeven en beproeving in commerciële fokprogramma's te rechtvaardigen.

6. Analyse van DNA-sequenties biedt goede perspektieven voor het opsporen van ziekte-risikofaktoren en resistentie-genen in het genoom van zowel mens als dier.

7. Bij voortgaande toepassing van moderne reproduktietechnieken bij landbouwhuisdieren dient meer aandacht geschonken te worden aan het voorkomen van genetische erosie.

8. Gebruik maken van "DNA-fingerprinting" als selektiekriterium bij het akseptatiebeleid voor verzekeringen of in sollicitatieprocedures is in essentie niet verschillend van diskriminatie op grond van ras of sekse, en is derhalve in strijd met de grondwet.

9. Indien aanzuren van mest geaksepteerd wordt als grootschalige oplossing om de ammoniakuitstoot te reduceren, zal het plateau van Margraten alsnog afgegraven moeten worden.

10. Het verhogen van de resistentie van PC's tegen virusinfekties vereist regelmatige financiële injekties voor de aankoop van up-to-date kiemvrije software.

11. Doordat het huidige sociale en fiscale beleid eenzijdig gericht blijft op stimulering van de traditionele "hoeksteen van de samenleving", wordt het ouderschap in tweeverdiener-relaties nog steeds ontmoedigd.

12. Sinds melkquota verhandelbaar zijn, komt (wit-)goudkoorts vaker voor dan melkerskoorts.

13. Uit het feit dat ze zichzelf kunnen voeden, leiden sommige mensen onterecht af dat zij voedingskundigen zijn.

14. Wanneer de groene lobby bereid is haar witte motor aan een zorgvuldige revisie te onderwerpen en regelmatig onderhoud te plegen, kan die ook aan toekomstige strenge milieunormen voldoen.

Proefschrift R.A. Donker.
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Wageningen, 19 mei 1989.

Voorwoord

De wijze waarop een skriptie tot stand komt is ooit eens vergeleken met het volbrengen van een dracht. De totstandkoming van een proefschrift als dit kan naar mijn idee echter beter vergeleken worden met een immuunrespons.

Na gedurende mijn propaedeuse en kandidaats studie met een breed scala van antigenen (studievakken) in aanraking te zijn gekomen, heb ik in mijn doktoraalstudie enkele duidelijke immunisaties gehad met fokkerij en immunologie. De resultaten van die immunisaties waren enkele skripties, en een ir. titel. Na een korte tussenperiode heb ik, in de vorm van dit promotie-assistentschap een forse reimmunisatie ontvangen met immunologie. De respons bij een reimmunisatie vertoont doorgaans een heftiger verloop, een hogere piek en houdt langer aan. Het resultaat van deze respons ligt voor u. Zoals u kunt konstateren is de hoeveelheid antilichaam in ieder geval voldoende voor een flinke respons.

Zoals een imuunrespons tot stand komt door een groot aantal samenwerkende cellen (o.a. macrofagen, plasma cellen, B-cellen, T-helper, T-suppressor en nul cellen), zo komt een proefschrift slechts tot stand door een groot aantal samenwerkende personen. Een aantal wil ik op deze plaats met name noemen.

Mijn promotor, Prof. dr. Arie Hoogerbrugge, wil ik bedanken voor het vertrouwen dat hij in mij gehad heeft en de geboden begeleiding. Ik heb het zeer gewaardeerd dat u ook na uw onverwacht vervroegde emiritaat altijd bij het onderzoek betrokken hebt willen blijven.

Mijn co-promotor, dr.ir. Akke van der Zijpp, bedank ik voor de geboden kans dit onderzoek te doen en de vrijheid die je me daarbij gelaten hebt. Door de jaren heen is jouw stimulerende invloed van essentieel belang gebleken, niet alleen voor dit onderzoek, maar voor het hele immunologisch onderzoek bij de vakgroep.

Dr. Gerard Beuving dank ik voor zijn inbreng vanuit het COVP 't Spelderholt in het onderzoek, en in de diskussies in de begeleidingskommissie. Waardevolle bijdragen in de begeleiding zijn eveneens geleverd door ir. Marijke Kreukniet en dr.ir. Andre Henken.

Mike Nieuwland neemt uiteraard een unieke plaats in in dit rijtje mensen. Zonder zijn denk-, hand- en lachwerk zou nog niet de helft van dit proefschrift tot stand gekomen zijn. Peter Vos heeft, met name in de latere respiratiecel-proeven, een belangrijk deel van het (vele) monnikenwerk voor zijn rekening genomen.

De bedrijfsleider van de pluimvee-akkomodatie, Roel Terluin, heeft, met zijn medewerkers Arie den Dool en Aad Rodenburg, op inventieve wijze telkens oplossingen gevonden voor de problemen welke ik wist te creëren. Op vergelijkbare wijze heeft Peter van den Berg, met de verzorgers Victor Schemkes en Wouter Hiskemuller, op uitstekende wijze de proef in de toen

splinternieuwe stal op het Spelderholt verzorgd; na van de eerste schok bekomen te zijn. De belangeloze terbeschikkingstelling van de faciliteiten op het Spelderholt door de direktie van het COVP is zeer gewaardeerd.

De proeven in de respiratiecellen zouden absoluut onmogelijk geweest zijn zonder de 24-uur-per-dag 7-dagen-per-week toewijding van ing. Prins van der Hel, ir. Henk Brandsma, Koos van der Linden en ing. Marcel Heetkamp.

De hormoonassays uit de laatste hoofdstukken zijn in een prettige samenwerking uitgevoerd door Prof.dr. E. Decuypere van de K.U. Leuven.

De (toenmalige) studenten Hilde Kock, Gerard Scheepens, Carlo van Haren, Tom Schneijdenberg, Willem Oostenbrink, Carolien Makkink, Anja Swinkels, Saskia Beers, Rein van de Wal en Lia Jansen zullen meer of minder van hun werk terugvinden in dit proefschrift. Tijdens hun stage van de HAS hebben eveneens hun steentje bijgedragen Karin van Belzen en Trienke Hofstra.

De ernstigste engelse taalblunders zijn uit de manuskripten gehaald door Mw. Hèlen West, dr. Annemarie de Passillé en dr. Jeff Rushen.

De publikatie van dit proefschrift is mede mogelijk gemaakt door substantiële financiële bijdragen door het LEB-fonds en het Fonds voor Pluimveebelangen.

Alle mensen noemen die op één of andere wijze direkt of indirekt bij de proeven betrokken zijn geweest noemen, maakt dit proefschrift nog dikker dan het al is. Echter, niet vermeld is nog niet vergeten.

Gedurende drie jaar intensieve samenwerking is uiteraard niet alles altijd rozegeur en maneschijn geweest. Ikzelf ben echter blij dat altijd alles bespreekbaar is gebleken. Zodoende blik ik nu (al) terug op een prettige periode bij een gezellige vakgroep. Bedankt.

Tot slot wil ik nog drie namen noemen. Mijn paranimfen ir. Nicoline Soede en Ger de Vries Reilingh hebben in de afgelopen drukke periode reeds bewezen naast goede vriendinnen trouwe supporters te zijn. Dank, met jullie erbij kan mij niets gebeuren de 19e. Mijn kamergenoot van de afgelopen drie jaar (dr.)ir. Harm Ploeger bedank ik voor de prettige tijden. Jouw vaardigheid op de cursortoetsen zal ik nooit evenaren. Dat je me aan een koffie-verslaving hebt geholpen vergeef ik je, daar kan ik prima mee leven; maar dat je probeerde om mijn drop-hobby te ondergraven door telkens tijdens mijn afwezigheid de voorraad uit te putten...

Richard Donker.

Contents

Rubriekscode: 3	ISN:
3	Ill.; Tab.; refs.
3	Sum.: (da) , (de) , (en) , (es) , (fr) , (it) , (ja) , (nl) , (no) , (ru) , (sv)

Referaat:

Thermal influences on antibody production and metabolism in chicken lines divergently selected for immune responsiveness.

General Introduction.

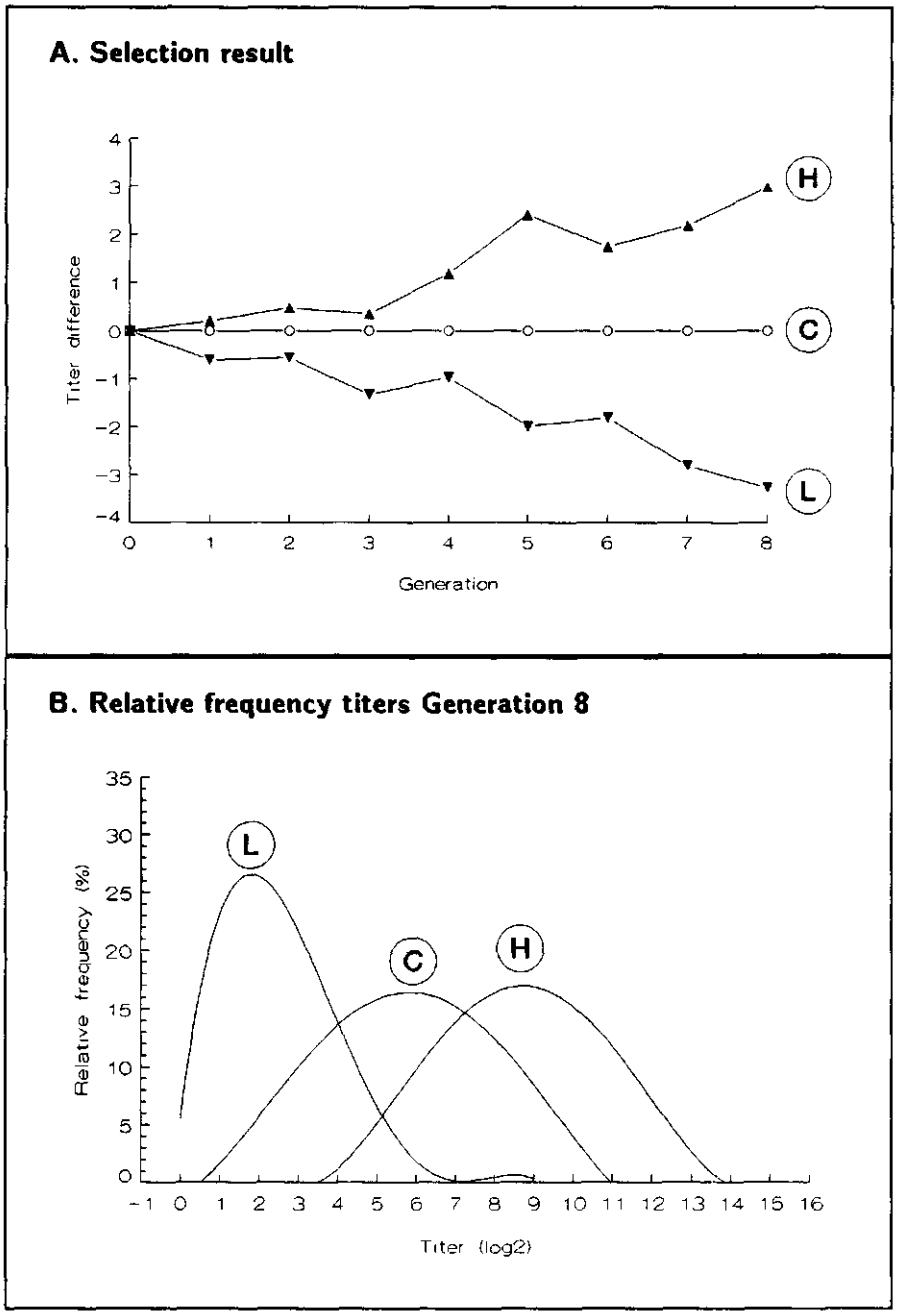
The developments in agriculture during recent decennia are characterized by a steady increase in specialization, mechanization and population density. Particularly in the poultry industry large, highly automated farms have evolved with very high concentrations of birds. With the increased population density, the infection pressure, and consequently the incidence of infectious diseases increased. Management precautions (isolation and strict hygiene), medication and extensive vaccination programs minimize the risk of disease outbreak. However, medication and vaccination programs are expensive, both in labor and drugs, and the still occurring disease outbreaks frequently cause enormous production loss, or may even require a stamp out of the whole flock.

Selection for immune responsiveness

An alternative (additional) method of minimizing health-risk is that of improving the genetic disease resistance of our flocks. A relatively simple approach to improve disease resistance is to include in the breeding program selection for responsiveness to a primary a-pathogenic antigen which stimulates the immune system (Siegel and Gross, 1980; Van der Zijpp, 1982). Using sheep red blood cells (SRBC) as antigen, three selected lines were obtained which diverged steadily during eight generations (Fig. 1A). Within each selection line (High response, randombred Control and Low response line) a normal distribution of antibody titers prevailed (Fig. 1B), however, values in the L line congregated around 1, the detection limit of the present assay. The steady variation within lines indicates the polygenic character of the selected trait. Comparable selection in mice (Biozzi *et al.*, 1979) involved approximately 10 independent genes.

The chicken lines derived have been found to show comparable differences in their response to other antigens which, like SRBC, stimulate the systemic response (Van der Zijpp *et al.*, 1986), but not in cellular immunity or phagocytic capacity. Moreover, the mortality after an experimental contact infection with Mareks' disease was found to be decreased in the H line compared to the L line, indicating lower susceptibility for this disease (Van der Zijpp *et al.*, 1988).

Figure 1. Total antibody titers to SRBC 5 days after immunization. H: High line; C: random bred Control line; L: low line.



Genotype x environment interactions

Related to any such selection, other traits may change accordingly. Either because of physiological relations with the selection criterium, or by genetic linkage. Monitoring possible consequences in primary production traits is therefore important for the poultry industry, and is subject to study. Altered genetic constitution also introduces the possibility of interactions with different environments, which could undo the obtained selective advantage.

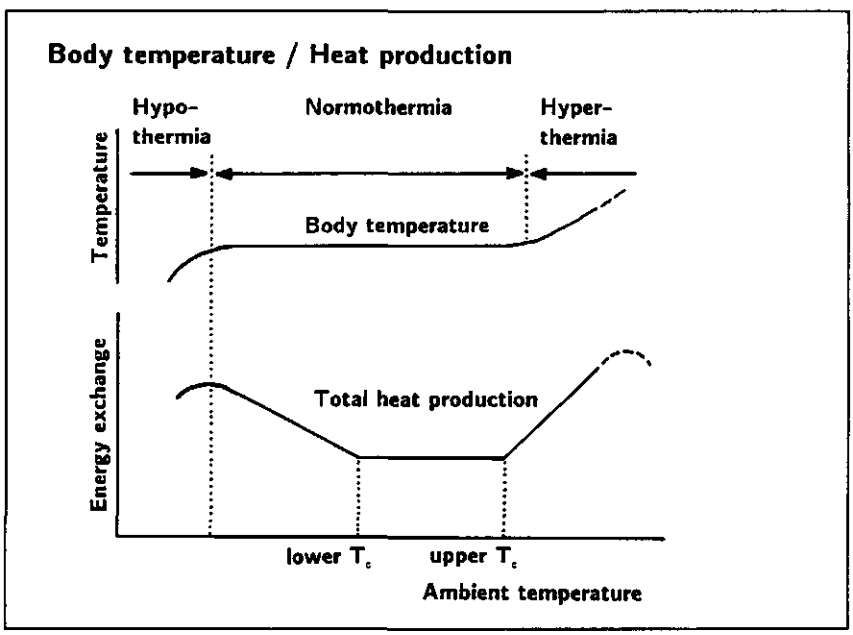
There are two major reasons for being concerned about possible genotype x environment interactions in these lines. One reason is based on the existence of a difference in the average bodyweight between the selection lines. The high selection line has a lower body weight than the low line. Differences in body weight could imply altered metabolic rate, energy and protein turnover.

The other reason is related to immunological self-regulation. Several factors involved in this regulation also play a role in the stress response. These two reasons will be discussed in more detail hereafter.

Environmental temperature

Homeotherms, including birds, maintain a more or less constant body temperature independently of environmental temperature. This is possible only within certain limits (indicated as "normothermia", Fig. 2). When the environmental temperatures is outside these limits hyper- or hypothermia occur, which eventually may cause death of the animal.

Figure 2. Schematic representation of relations between environmental temperature, heat production and body temperature in homeotherms. T_c: critical temperature (After Mount, 1979).

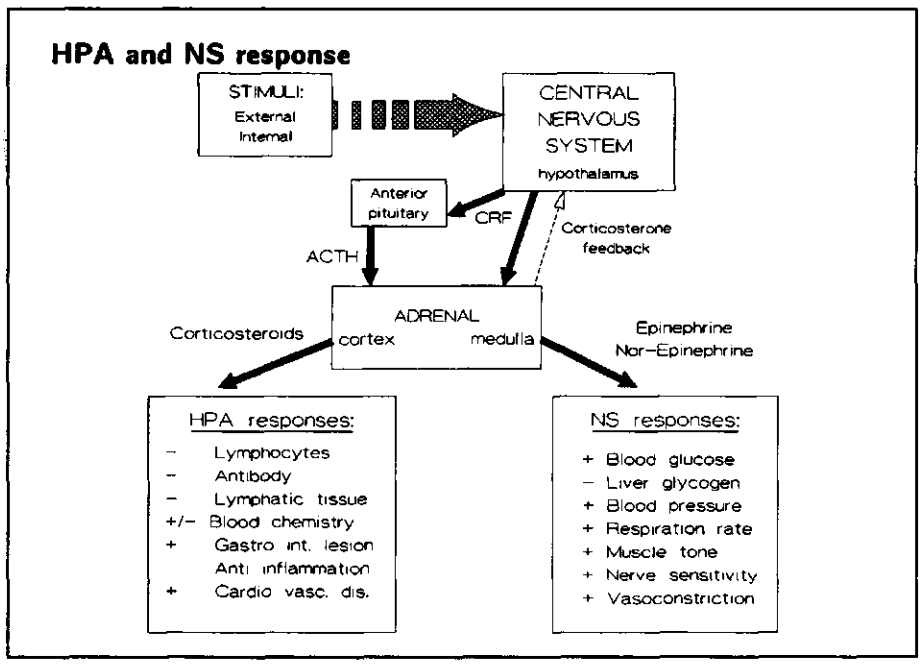


But also within this zone of normothermia temperature stress may be evident. Above the upper critical temperature thermoregulatory effort has to be undertaken to maintain body temperature constant: heat loss from the body has to be increased, and heat production decreased. Below the lower critical temperature heat loss from the body has to be restricted and heat production increased to prevent hypothermia. The regulatory mechanisms to maintain body temperature within these limits are neural, behavioral as well as endocrine (Hillman *et al.*, 1984).

Stress and adaptation

Abrupt changes in environmental temperature or other stressful stimuli initially provoke aspecific responses in behaviour, and in nervous and endocrine systems (Siegel, 1980; Fig. 3). Corticosteroids and catecholamines (epinephrine and nor-epinephrine) are some of the most potent hormones released. Corticosteroids and catecholamines cause important changes in cellular activity and energy mobilization, to prepare the body for acute action ("the fight or flight" response).

Figure 3. Schematic representation of reaction pattern to stressors. HPA: hypothylamus-pituitary-adrenal system; NS: neurogenic system; CRF: corticotripin releasing factor; ACTH: adrenocorticotropin. (After Siegel, 1980).



When the stressor prolongs, specific adaptive regulation will prevail, for example in behaviour or feed intake, to minimize the discomfort. Thyroid activity plays an important role in acclimation to changed environmental temperature. Thyroxine (T4) and the biologically active form triiodothyronine (T3) regulate feed intake, and thermoregulation (reviewed by Hillman *et al.*, 1984).

Several physiological changes induced by a stressor may be utilized to evaluate the severity of the stressor and to compare differences in impact between (e.g.) selection lines. Increased plasma corticosterone levels, changes in leukocyte numbers or morphology, lymphatic regression and changes in acute phase proteins might prove useful for this purpose. Also measurement of the metabolic rate and changes therein, caused by an altered environment can provide insight in the thermal demand of the environmental temperature on the animal.

Immunological regulation

In the vertebrates' immunology key functions are fulfilled by phagocytosis, cellular immunity and the systemic response. The latter, the evolutionary most refined, results in the production of highly specific and effective immunoglobulins and the founding of immunological memory. This requires the integrative action of various cell types. Communication between these cells and thus regulation of the response is realized by interleukins and through feedback on the hypothalamo-pituitary-adrenal axis (Besedovski *et al.*, 1983, 1986; Glick, 1984). Antigen stimulated lymphocytes produce compounds (lymphokines) that also stimulate CRF release in the hypothalamus (Besedovski *et al.*, 1983, 1986) and thus corticosteroid production (compare Fig. 3). Antigen stimulated lymphocytes may also excrete "ACTH-like" substances (Siegel, 1987), which may directly act on the adrenals. This corticosterone feedback is probably needed to suppress proliferation of lymphocytes with low affinity to the antigen, and thus prevents an over-reaction of the immune system which could cause autoimmunity (Glick, 1984; Munck *et al.*, 1984; Besedovski *et al.*, 1986; Trout *et al.*, 1988). This feedback is probably also essential in cessation of the immune response after the antigen has been cleared.

Temperature and stress effects on immunity

Because some endocrine factors, especially corticosterone, are related to both the stress response and immunological regulation, stressors can interfere with immune function. If an immunological stimulus is given during a stressful event suppression of the responses is found. In poultry, immuno suppressive activity on antibody production has been reported with acute heat stress (Subba Rao and Glick, 1970, 1977; Henken *et al.*, 1983; Thaxton and Siegel, 1970, 1972, 1973), with social stress (Gross and Siegel, 1965; Siegel and Gross, 1965; Gross and Colmano, 1969; Siegel and Latimer, 1975; Edens *et al.*, 1983; Gross, 1986) and with administration of adrenocorticotropin hormone (ACTH) or corticosteroids

(Thaxton and Siegel, 1973; Gross and Siegel, 1973; Gross *et al.*, 1980; Davison *et al.*, 1987, 1988; Powell and Davison, 1986; Davison and Misson, 1987).

Metabolic activity and immunity

After an initial aspecific stress response, acclimation to an altered situation (e.g. high temperature) will occur. After such an acclimation, stimulatory activity on the immune system may even be evident (Henken *et al.*, 1983; Anderson and Kühn, 1988). This stimulatory effect was postulated to be associated with thyroid activity, and alterations in energy and protein metabolism (Henken *et al.*, 1983). Thyroid involvement in regulation of the immune response is evident (Gause and Marsh, 1985; Kai *et al.*, 1987, 1988; Marsh *et al.*, 1984a, 1984b; Mashaly *et al.*, 1983; Scott *et al.*, 1985; Yam *et al.*, 1981), but the route of action is still unclear.

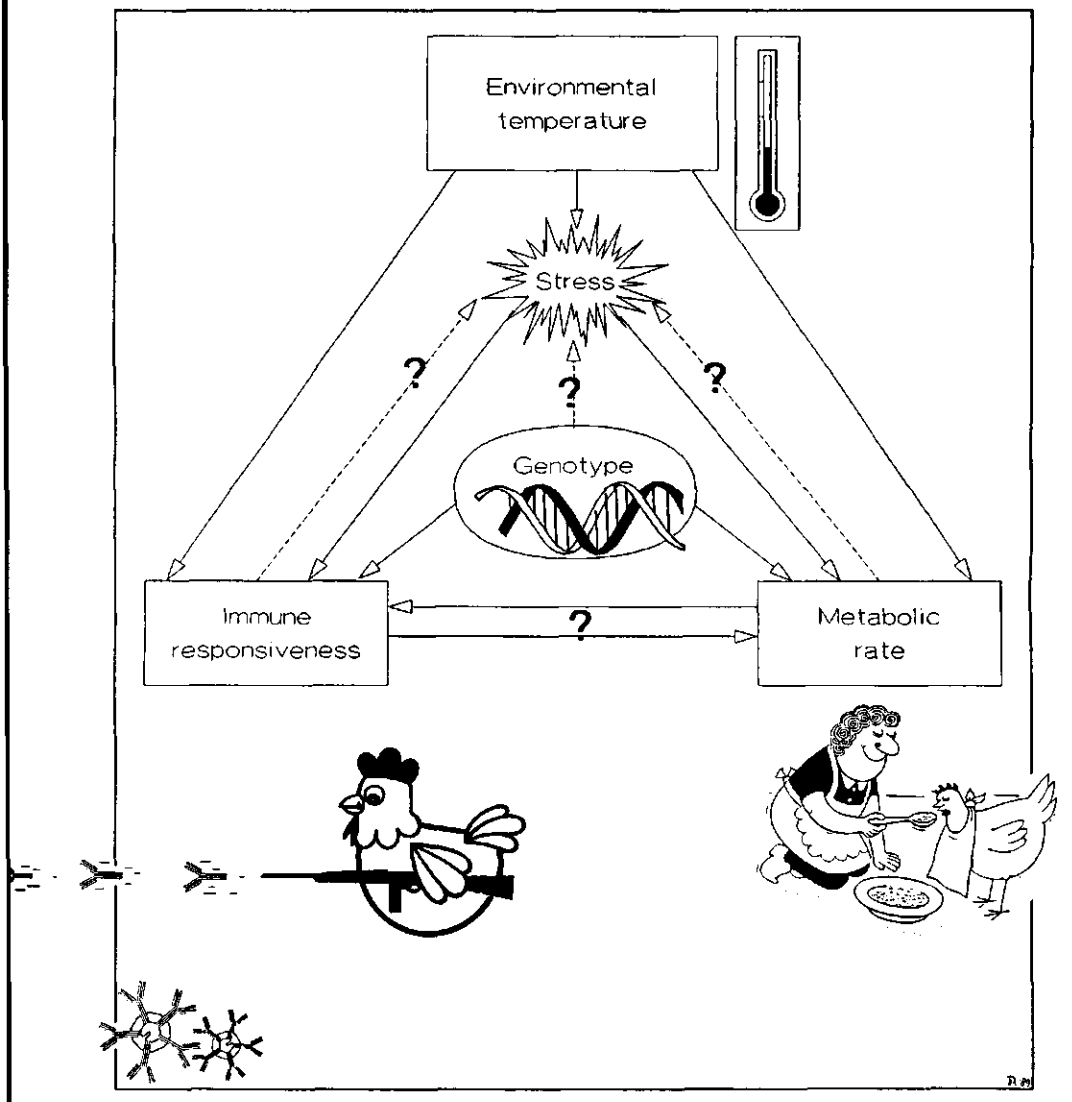
The immune response itself affects the animal's metabolism. During an immune response a reduced heat production was found, and fat deposition was favored over protein retention (Henken and Brandsma, 1982; Henken *et al.*, 1982; Siegel *et al.*, 1982).

Synthesis

Thus a complex network of interference between immunity, environmental temperature and metabolic rate is unfolding (Fig. 4). Within these, interactions with the genotype might take place on several levels. Based on the described observations the following possible interactions are hypothesized:

- Selection for high antibody production is based on a low level of endocrinological feedback after immunization (see "*Immunological regulation*"). In the high line lymphocytes can proliferate which excrete antibodies with a relatively low affinity for the antigen. External stressors could have a more profound impact on the immune response in high than in low line chickens, since the adrenal corticosteroids, released after a stressor, would easily suppress the proliferation of these lymphocytes that excrete the "low-affinity" antibodies.
- On the other hand, if the above would prove untrue, the number of lymphocytes excreting antibodies with high affinity to the antigen should be increased in the high line birds. Alternatively, the proliferative ability of these lymphocytes may be increased. These populations of lymphocytes would not be differently sensitive to external stressors, resulting in equal responses to stress or environmental temperature in either line. The selective advantage in antibody production would not be lost after stressors that cause immunosuppression.

Figure 4. Schematic representation of interactions between environment, immune response and metabolic traits.



- Selection for high antibody production may be associated to differences in endocrine and metabolic activity between the lines. The high antibody producing line birds are easily stimulated by antigen, or any environmental stimulus. These environmental stimuli would cause relatively high base levels of corticosteroids. Lymphocytes adapt to these high base levels by blocking their corticosterone-receptors, or reducing the numbers of these on their surface. This adaptation would make the lymphocytes of this high line less susceptible to the effects of environmental stressors. The high base levels of corticosteroids will favor fat deposition over protein gain, resulting in lower growth rate and small, fat birds with a relatively low feed efficiency.
- If, in contrast, the selection is not based on a physiologically functional relation between body weight, metabolic rate and immune responsiveness, the changes in bodyweight between the high and low selection line are based on genetic linkage. The effects of stressors or high temperature on antibody production would be the same in either line.

Thesis

This thesis is made up of eight papers which describe a series of experiments in which genotype x environmental temperature interactions on the humoral immune response and metabolism of two chicken lines were studied.

The high and low chicken lines, which were developed at the Department of Animal Husbandry, Agricultural University, Wageningen (Van der Zijpp and Nieuwland, 1986) were used throughout these studies. The randombred control line was not included in these studies.

The occurrence of the interaction on antibody production was studied after acute heat stress, implicating the stimulation of the hypothalamo-pituitary-adrenal axis, and at continuously high temperatures, providing enough time for acclimation.

In these studies acute heat stress and high environmental temperature were used as treatments. An important reason for this is the international character of poultry industry. Chicken lines developed under temperate conditions should be able to perform under tropical conditions as well. Large concentrations of poultry production are found in hot areas of the world (central U.S.A., middle east, south America). Furthermore, acute thermal stress can occur during transport, during ventilation failure, or lack of wind when natural ventilation is used. Finally, considering thermal influences also a model for other stressors, temperature is an experimentally accurately regulatable stressor, with a number of well documented effects in poultry.

Metabolism studies were performed before and during the mounting of the immune responses, serving two aims at the same time. Firstly to compare metabolic rate between the lines during the immune response, and secondly to obtain information about the severity of the thermal demand placed upon the birds.

References

Anderson K. and R.E. Kühn, 1988. Elevated environmental temperature overcomes immunosuppression in experimental Chagas' disease. *FASEB j.* 2: A902.(abstract)

Besedovski H.O., A. Del Rey, E. Sorkin, M. Da Prada, R. Burri and C. Honegger, 1983. The immune response evokes changes in brain noradrenergic neurons. *Science* 221: 564-566.

Besedovsky H.O., A. del Rey and E. Sorkin, 1986. Regulatory immune-neuroendocrine feedback signals. pg. 241-249. in: Berczi I. (ed.). *Pituitary function and immunity* (chapter 11). CRC Press inc., Boca Raton, FA.

Biozzi G., D. Mouton, O.A. Sant'Anna, H.C. Passos, M. Gennari, M.H. Reis, K.C.A. Ferreira, A.M. Heumann, Y. Bouthillier, O.M. Ibanez, C. Stiffel and M. Siqueira, 1979. Genetics of immunoresponsiveness to Natural Antigens in the Mouse. *Curr. Topics Microbiol. Immunol.* 85: 31-98.

Davison T.F. and B.H. Misson, 1987. Effect of corticosterone on antibody responses to a T-dependent and T-independent antigen in domestic fowl. *Med. Sci. Res.* 15: 967-968.

Davison T.F., B.H. Misson, R.A. Williamson and J. Rea, 1988. Effect of increased circulating corticosterone in the immature fowl on the blastogenic responses of peripheral blood lymphocytes. *Dev. Comp. Immunol.* 12: 131-144.

Edens F.W., P. Thaxton, G.W. Morgan and C.R. Parkhurst, 1983. Grouping in japanese quail. 2. Suppression of humoral immunity. *Poultry Sci.* 62: 2479-2485.

Gause W.C. and J.A. Marsh, 1985. Differential effects of thyroxine on immune development and autoimmune thyroiditis in the obese strain chicken. *Dev. Comp. Immunol.* 9: 465-475.

Glick B., 1984. Interrelation of the avian immune and neuroendocrine systems. *J. Exp. Zoöl.* 232: 671-682.

Gross W.B., 1986. Effect of dose of antigen and social environment on antibody response of high and low antibody response chickens. *Poultry Sci.* 65: 687-692.

Gross W.B. and G. Colmano, 1969. The effect of social isolation on resistance to some infectious diseases. *Poultry Sci.* 48: 514-520.

Gross W.B. and H.S. Siegel, 1965. The effect of social stress on resistance to infection with *Escheria coli* or *Mycoplasma gallisepticum*. *Poultry Sci.* 44: 998-1001.

Gross W.B. and P.B. Siegel, 1973. Effect of social stress and steroids on antibody production. *Avian Dis.* 17: 807-815.

Gross W.B., P.B. Siegel and R.T DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poultry Sci.* 59: 516-522.

Henken A.M. and H.A. Brandsma, 1982. The effect of environmental temperature on immune response and metabolism of the young chicken. 2. Effect of the immune response to sheep redblood cells on energy metabolism. *Poultry Sci.* 61: 1667-1673.

Henken A.M., G.R. Kleinhout and W. van der Hel, 1982. The effect of environmental temperature on immune response and metabolism of the young chicken. 1. Effect of intramuscular injection on heat production. *Poultry Sci.* 61: 503-509.

Henken A.M., A.M.J. Groote Schaarsberg and M.G.B. Nieuwland, 1983. The effect of environmental temperature on immune response and metabolism of the young chicken. 3. Effect of environmental temperature on the humoral

immune response following injection of sheep red blood cells. *Poultry Sci.* 62: 51-58.
Hillman P.E., N.R. Scott and A. van Tienhoven, 1984. Physiological responses and adaptations to hot and cold environments. pg. 1-71 in: M.K. Yousef (ed.): *Stress physiology in livestock. Vol. 3. Poultry.* CRC Press, Inc. Boca Raton, FA, USA.
Kai O., M. Imada, Y. Imada and K. Sato, 1987. Effects of the thyroid status on the immune system of chickens. *JPN. Poult. Sci.* 24: 150-159.
Kai O., H. Nagase, N. Ishikawa, M. Suzuki, T. Kakegawa and K. Sato, 1988. Effects of propylthiooouracil (PTU) on the immunological status of the chicken. *Dev. Comp. Immunol.* 12: 145-156.
Marsh J.A., W.C. Gause, S. Sandhu and C.G. Scanes, 1984. Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. *Proc. Soc. Exp. Biol. Med.* 175: 351-360.
Marsh J.A., T.J. Lauterio and C.G. Scanes, 1984. Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. *Proc. Soc. Exp. Biol. Med.* 177: 82-91.
Mashaly M.M., S.L. Youtz and R.F. Wideman Jr., 1983. Hypothyroidism and antibody production in immature chickens. *Immunol. Commun.* 12: 551-563.
Munck A. and P.M. Guyre, 1986. Glucocorticoid hormones in stress: Physiological and pharmacological actions. *News in Physiol. Sci.* 1: 69-72.
Powell P.C. and T.F. Davison, 1986. induction of Marek's disease in vaccinated chickens by treatment with betamethasone or corticosterone. *Isr. J. Vet. Med.* 42: 73-78.
Scott T., A. van der Zijpp and B. Glick, 1985. Effect of thiouracil-induced hypothyroidism on the humoral immunity of New Hampshire chickens. *Poultry Sci.* 64: 2211-2217.
Siegel H.S., 1980. Physiological Stress in Birds. *Bio Science* 30: 529-534.
Siegel H.S. and W.B. Gross, 1965. Social grouping, stress and resistance to coliform infection in cockerels. *Poultry Sci.* 44: 1530-1536.
Siegel P.B. and W.B. Gross, 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Sci.* 59: 1-5.
Siegel H.S. and J.W. Latimer, 1975. Social interactions and antibody titres in young male chickens (Gallus domesticus). *Anim. Behav.* 23: 323-330.
Siegel H.S., A.M. Henken, M.W.A. Verstegen and W. van der Hel, 1982. Heat production during the induction of an immune response to sheep red blood cells in growing pullets. *Poultry Sci.* 61: 2296-2300.
Subba Rao D.S.V. and B. Glick, 1970. Immunosuppressive action of heat in chickens. *Proc. Soc. Exp. Biol. Med.* 133: 445-448.
Subba Rao D.S.V. and B. Glick, 1977. Effect of cold exposure on the immune response of chickens. *Poultry Sci.* 56: 992-996.
Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. *Poultry Sci.* 49: 202-205.
Thaxton P. and H.S. Siegel, 1972. Depression of secundary immunity by high environmental temperature. *Poultry Sci.* 51: 1519-1526.
Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Sci.* 52: 618-624.
Trout J.M., M.M. Mashaly, H.S. Siegel, 1988. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Dev. Comp. Immunol.* 12: 331-346.
Yam D., D. Heller and N. Snapir, 1981. The effect of the thyroidal state on the immunological state of the chicken. *Dev. Comp. Immunol.* 5: 483-490.

Zijpp A.J. van der, 1982. *Genetic control of immune responsiveness in the chicken*. Dissertation, Agricultural University, Wageningen, The Netherlands.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterisation of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris)* : 211-215.

Zijpp A.J. van der, J.J. Blankert, E. Egberts and M.G.J. Tilanus, 1988. Advances in genetic disease resistance in poultry. pg. 131-138. in: S. Korver, H.A.M. van der Steen, J.A.M. van Arendonk, H. Bakker, E.W. Brascamp and J. Dommerholt (compilers). *Advances in animal breeding*. Pudoc, Wageningen, The Netherlands.

Chapter I

Heat stress in chicken lines selected for high and low immune responsiveness.

1. Antibody production.

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Abstract

The effect of heat stress on antibody production to sheep red blood cells (SRBC) was investigated in three experiments using chicken lines selected for six generations on high (H) or low (L) plasma antibody titer to SRBC after primary intramuscular (i.m.) immunization. Chickens were immunized 24 h after a heat stress treatment (HS) of four periods each of 30 min, at a temperature of 42°C with an intervening 30 min period at a temperature of 22°C. The control treatment (CT) involved the same handling of chicks, but at a temperature of 22°C. Antibody titers were measured on 3, 5, 7, 10 and 14 days post immunization. Intramuscular immunizations of .25 ml SRBC were given in all three experiments and an additional intravenous immunization of .5 ml 14% SRBC in Experiment 2 and .5 ml 5% SRBC in Experiment 3. A significant effect of HS treatment on antibody titers ($P<.05$ on days 3, 5, 7 and 10 after immunization) was found in Experiment 1 only. Titers were decreased in the H line only. Differences between the H and L lines were significant ($P<.001$) in all three experiments and after both intramuscular and intravenous immunizations. Heat stress was found to have little or no effect on antibody production in these lines.

Keywords: antibody production, selection lines, heat stress, SRBC
Running title: Heat stress and antibody production.

Introduction

There is accumulating evidence to suggest that not only immune responsiveness is affected by stressors, but also that antigenic stimulation evokes hormonal and neural reactions in the central nervous system (Ader *et al.*, 1987; Siegel, 1987). Thus physiological reactions to a stressor may be altered by immunological stimulation along this pathway. For example, selection for antibody production might alter stress susceptibility as a related effect, and thus in turn alter the effect of stress on immune responses.

Stress effects on immune responses have been well documented (Kelley 1985; Siegel 1987). In many experiments concerning immunosuppressive effects on humoral responses in poultry, heat stress has been applied (Thaxton, 1978). Although suppressive effects of intermittent heat periods (at approximately 42°C) on antibody production to sheep red blood cells (SRBC) have been demonstrated in several experiments (Subba Rao and Glick, 1970, 1977; Thaxton *et al.*, 1968; Thaxton and Siegel, 1970, 1972, 1973), considerable variation

in responses has been found. In some experiments, clear immunosuppressive action was found, but the same stressor used on stock of different genetic origin, or with another antigen was not found to be immunosuppressive and in some cases was even immuno-stimulating (Heller *et al.*, 1979; Regnier *et al.*, 1980; Kelley, 1985; Siegel, 1987). Regnier *et al.*(1980) could not demonstrate immunosuppressive action of the heat stress treatments in chickens of the same breeds but of different genetic origin, as used by Subba Rao and Glick (1970, 1977), Thaxton *et al.* (1968) (New Hampshires) or Thaxton and Siegel (1970, 1972, 1973) (Athens randombreds).

Because there are apparent differences in susceptibility to heat stress, the effects of heat stress on chicken lines of different genetic capacity for antibody production need to be investigated. Demonstrable differences in stress susceptibility may have implications for the applicability of selection programs on immune responsiveness. Gross (1986) studied the effects of social environment on immune responses in chicken lines bred selectively for high and low humoral response after intravenous SRBC immunization. No significant interactions between genotype and environment were found.

In the present study, the interaction between genotype and heat stress was investigated in chicken lines bred for humoral immune responsiveness. Short-term heat stress was applied to lines selected for six generations for high and low antibody response to SRBC (Van der Zijpp and Nieuwland, 1986). These lines were selected based on antibody titer after intramuscular (i.m.) immunization. As most of the studies cited used animals that had been immunized intravenously (i.v.), both immunization routes were used in order to investigate possible route differences. Siegel *et al.*(1983) and Siegel and Latimer (1984) demonstrated interactions between heat stress and antigen dose on antibody production in experiments using *Salmonella pullorum* antigen. Immunosuppression occurred only when low antigen doses were used. In our experiments, one antigen dose was administered i.m. and two doses i.v.: one .5 ml 14% SRBC comparable to doses given in other studies, and the other a lower dose of .5 ml 5% SRBC.

Material and Methods

Chicks and pre-experimental conditions

Three experiments were conducted, using male and female chickens from lines selected for high (H) and low (L) antibody titer five days post primary i.m. immunization with .25 ml packed SRBC. Chicks were vaccinated intramuscular against Mareks Disease on day of hatch and intra ocularly against infectious bronchitis at two days of age, infectious bursal disease at 15 days of age and Newcastle Disease at 22 days of age. Chicks were kept

in groups in wire battery cages, and provided with commercial starter feed and water *ad libitum*. The two sexes were kept separately and H and L lines were randomly mixed. The light regime was gradually altered from 23 h light and 1 h darkness at one day of age to 8 h light (8^{00}-16^{00} h) and 16 h darkness at 16 days of age. Over the same period ambient temperature was decreased from 32°C to approximately 22°C.

Heat stress treatment

Chicks were randomly subjected to heat stress (HS) or control treatment (CT). They were transferred from their battery cage in portable cages. At about 9^{00} h the cage with chicks undergoing HS treatment was placed in a climate-respiration chamber approximately 1 * .8 * 1 m in size (Verstegen *et al.*, 1987) in which the temperature was 42°C and relative humidity approximately 50%. At the same time, CT chicks were placed in an identical chamber, in which the temperature was controlled at 22°C and relative humidity approximately 50%. Treatments started randomly with either cockerels or hens. After 30 min, the cages were removed from the chambers and treatment started for the other sex. This procedure was repeated until both sexes had been in the respective chambers for four 30-min periods. In the intervening periods, the cages containing the chickens were placed in an adjoining room, in which the temperature varied between 20 to 25°C and relative humidity was approximately 50%. After the temperature treatment, all chicks were returned to their original battery cage.

Immunizations

Twenty-four hours after the beginning of the first HS/CT episode chicks were immunized with SRBC. Immunization at this time has been demonstrated to result in the most pronounced depression of antibody production (Subba Rao and Glick, 1970; Thaxton *et al.*, 1968). In the first experiment, the chickens were immunized i.m. with 1 ml of a 25% SRBC in phosphate buffered saline (PBS) solution in two equal portions in the chick's thighs. In the two subsequent experiments, the same i.m. immunizations were given, and additional chickens were given intravenous immunization. These immunizations were given via the brachial vein (*vena cutanea ulnaris*) by injecting .5 ml of a 14% SRBC in PBS solution (Exp. 2) and .5 ml 5% SRBC in PBS solution (Exp. 3). The day of immunization is hereafter referred to as day 0.

Blood sampling and hemagglutinin assay

Blood samples were taken from the brachial vein with a syringe rinsed with a heparin solution. The blood samples of approximately 1.5 ml each, were centrifuged to harvest the plasma, which was stored at -20°C until assayed. Total (TO) and 2-Mercaptoethanol (2ME) resistant antibody titers were assayed

by hemagglutinin assay (Van der Zijpp and Leenstra, 1980). Titers were expressed as $\log_2$ of the highest dilution giving total agglutination.

Experimental protocol

The uniformity of antibody titers recorded within an experimental group can be enhanced by 'socialization' and thus increase discrimination between experimental groups (Gross, 1986). Special attention was given in these experiments to handling of the chicks ('socialization').

In Experiment 1 the 72 chicks, equally distributed over both H and L line and both sexes, were handled daily from 26 days of age (Fig. 1). They were subjected to either HS or CT treatment on day 34 of age and were immunized i.m. 24 h later. Blood samples were taken from all chicks immediately before and after temperature treatment and on day 3, 5, 7, 10 and 14 post immunization (Fig. 1).

In Experiment 2 the 78 chicks, including both lines and sexes, were used. They were handled daily from 25 days of age. Temperature treatments were performed at 35 days of age (Fig. 1), and 24 h later 39 chickens were immunized i.m., and the remainder immunized i.v. with .5 ml 14% SRBC solution. Blood samples were drawn immediately before and after temperature treatment and on day 3, 5, 7, 10 and 14 post immunization.

A total of 408 chicks, also equally distributed over males, females and across H and L lines, was used in the Experiment 3. For socialization, each chick was removed daily from the cage, placed in a box, and then replaced in the cage. This procedure was carried out from 14 days of age. The chicks were divided into three groups. Group I received the temperature treatment on 32 days of age, group II on 35 days of age, and group III on 42 days of age (Fig. 1). Antigen injection, 24 h after commencing temperature treatment, was given by both immunization routes (i.v. with .5 ml 5% SRBC or i.m. with 1 ml 25% SRBC) in each group. The distribution of chicks was full factorial: 3 groups * 2 lines * 2 sexes * 2 treatments * 2 immunization routes * 4 replications. In addition within each group, line, sex and treatment, 9 extra chickens were immunized i.v. Three of these were killed on each of day 3, 4 and 5 each post immunization for assay of the number of plaque-forming-cells in the spleen. The plaque test results are included in a separate manuscript (Donker *et al.*, submitted-b), results on antibody titer assays are included here. Blood samples were taken on day 7, 4 and 1 before immunization and day 0, 1, 2, 3, 5, 7, 10 and 14 after immunization. On the days before immunization and on days 0, 1 and 2 post immunization, samples were taken between 08^{30}-09^{30} h and between 13^{30}-14^{30} h.; on the temperature treatment day (-1), this was immediately before and after the treatment. Each chick was bled only once before immunization, once on either day 1 or 2 post immunization, once on day 3, 4 or 5 post immunization. All chicks were bled

on days 7, 10 and 14 post immunization. The blood sampling schedule is given in Table 1.

Figure 1. Experimental layout.

V: Vaccinations
▒: Daily handling
T: Treatment (heat stress or control)
I: Immunization with SRBC, (intravenous or intramuscular)
B: Blood sampling

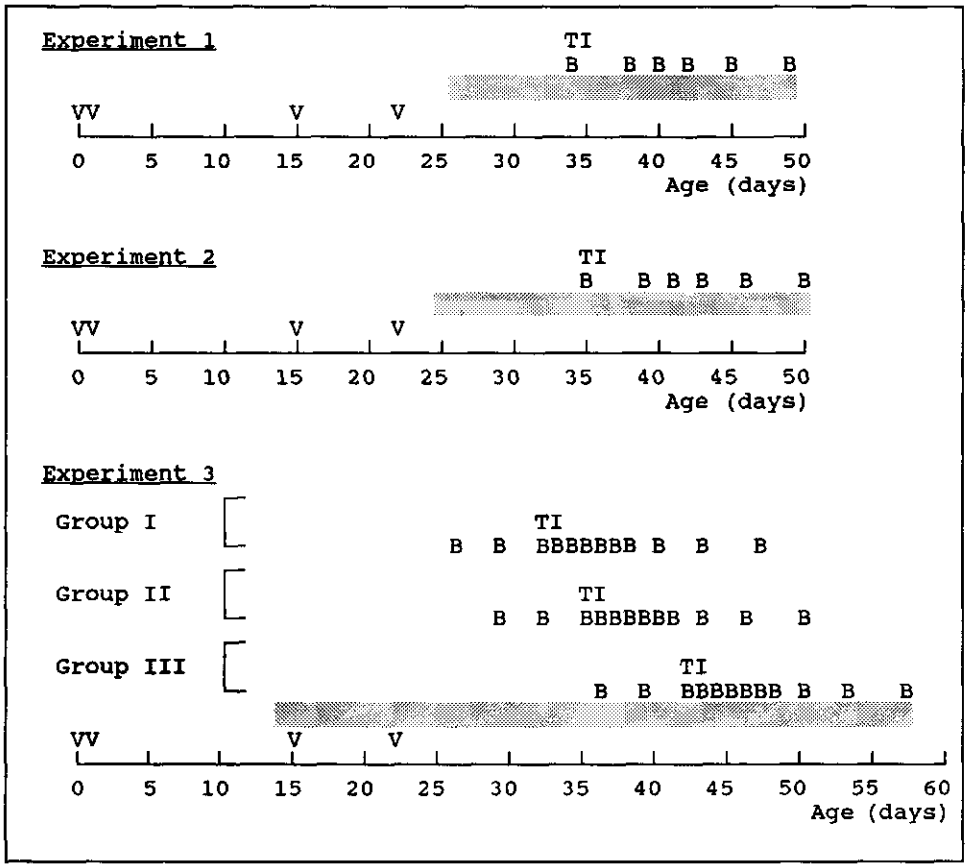

Table 1. Number of blood samples[1)] taken per group in Experiment 3.

Days post immunization	time 08^{30}-09^{30}	13^{30}-14^{30}
-7	8	8
-4	8	8
-1	8	16
0	16	32
1	24	24
2	24	24
3	32 + 24[2)]	
4	24[2)]	
5	32 + 24[2)]	
7	64	
10	64	
14	64	

[1)] The number of chicks bled at any time is composed as follows:
8 = 2 lines * 2 sexes * 2 chicks
16 = 2 lines * 2 sexes * 2 treatments * 2 chicks
24 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 1.5 chicks
32 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 3 chicks
64 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 4 chicks
N.B. 1: 1.5 chicks means randomly 1 or 2 within a line-sex-treatment-route group
N.B. 2: before the temperature treatment, no treatment groups were distinguished; before immunization, no immunization route groups were distinguished.

[2)] These 24 chicks were used for plaque-test assay; were i.v. immunized; plasmas were also tested for antibody titers.

Statistical analysis

Wherever possible, each of the groups (lines, sex, temperature treatment, etc.) contained equal number of chicks. Data were examined for normality of distribution (Snedecor and Cochran, 1969). Values of antibody titers (To) before day 3 and 2ME- titers before day 5 in Experiment 1 and 2, and before day 7 in Experiment 3 showed skewed distributions, because of values of mainly 0 and 1 on these days.

In the analysis of variance (SAS, 1985) performed within sampling day, the factors included in the models were line (H, L), sex (male, female), temperature treatment (HS, CT), route of antigen (i.m., i.v.; in Experiment 2

and 3 only) and group number (I, II, III; in Experiment 3 only). Day of blood sampling was included also for Experiment 3, when independent groups of chicks were bled on consecutive days (for example, day 3, 4 and 5 post immunization). Data from these days were incorporated in one analysis. Two- and three-way interactions were tested and those that were significant ($P<.05$) were included in the model. Pooled data of all three experiments were analyzed together to test also for temperature treatment effect.

Results

Both total and 2ME-resistant antibody titers were higher in the H line than in L line (Tables 2, 3, 4; Fig. 2, 3, 4) in all three experiments, and after both i.m. and i.v. immunization. Higher titers were found in females than in males (Tables 2, 3, 4), which is not unusual in these lines (unpublished results).

In the analysis on pooled data, there were significant differences between experiments ($P<.001$), and route of immunization within experiment ($P<.001$), but no "overall" temperature treatment effect was found.

The significance of effects for antibody titers in Experiment 1 is presented in Table 2. No significant temperature treatment effect was found in L line, but there was immunosuppression in H line (Fig. 2), as indicated by significant line * treatment interactions and the results of the T-test for temperature treatment within H line (Table 2, Fig. 2). However, mean titers in the heat stressed chicks from the H line were still higher than mean titers in both heat stressed and control chicks from the L line.

In Experiment 2, no significant effects of sex, temperature treatment or significant interactions of factors were found (Table 3). Total and 2ME-resistant antibody titers were higher after intravenous than after intramuscular immunization (Table 3, Fig. 3).

Temperature treatment also had no significant effect on total and 2ME-resistant antibody titers after immunization in Experiment 3 (Table 4, Fig. 4). Total and 2ME-titers increased with age of the chicks, as titers in group 3 were higher than those in group 2, which were higher than those in group 1. Intravenous injection of antigen resulted in higher antibody titers in both lines. Significant interactions (Table 4) were caused by differences in magnitude within factors, not by differences in ranking.

Figure 2. Total antibody titer (——) and 2ME-resistant titer (– –) per line and treatment after intramuscular immunization in Experiment 1. Least squares means from a model including line, sex, treatment and line-treatment interaction.

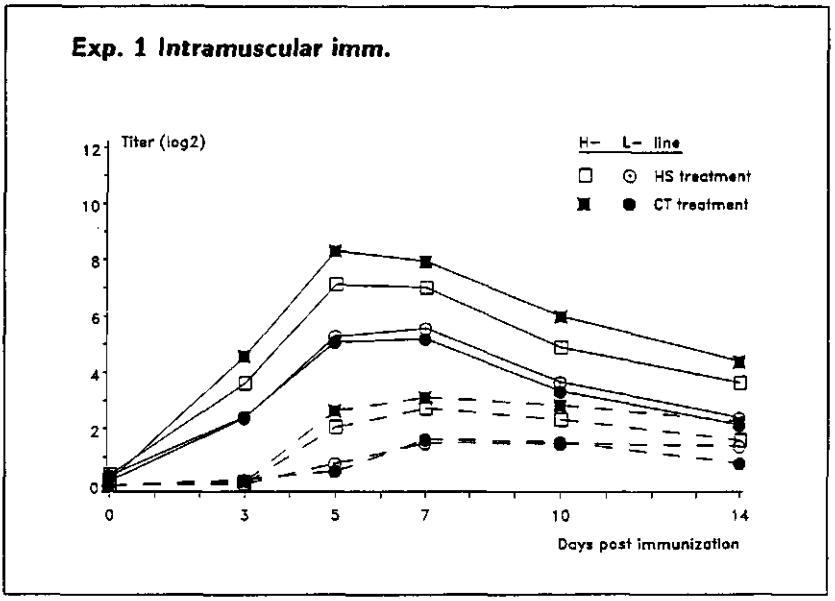

Table 2. Significance[1)] of effects in the analysis of total (TO) and 2ME-resistant (2ME) antibody titers (Exp. 1)

Day	TO/2ME	Sex	Line	Treatment	Line-Treatment interaction	T-test for treatment within: H-line	L-line
0	TO	-	-	-	-	-	-
	2ME	-	-	-	-	-	-
3	TO	ns	***	ns	ns	*	ns
	2ME	-	-	-	-	-	-
5	TO	***	***	ns	*	**	ns
	2ME	***	***	ns	*	*	ns
7	TO	*	***	ns	*	*	ns
	2ME	***	***	ns	ns	ns	ns
10	TO	ns	***	ns	*	**	ns
	2ME	***	***	ns	ns	+	ns
14	TO	ns	***	ns	+	+	ns
	2ME	ns	***	ns	**	+	ns

1) -: not tested because of skewed distribution of values.
ns: not significant (P>.10);
+: P<.10; *: P<.05; **: P<.01; ***: P<.001

Figure 3. Mean total antibody titer (——) and mean 2ME-resistant titer (– –) per line and treatment after intramuscular immunization and intravenous immunization in Experiment 2.

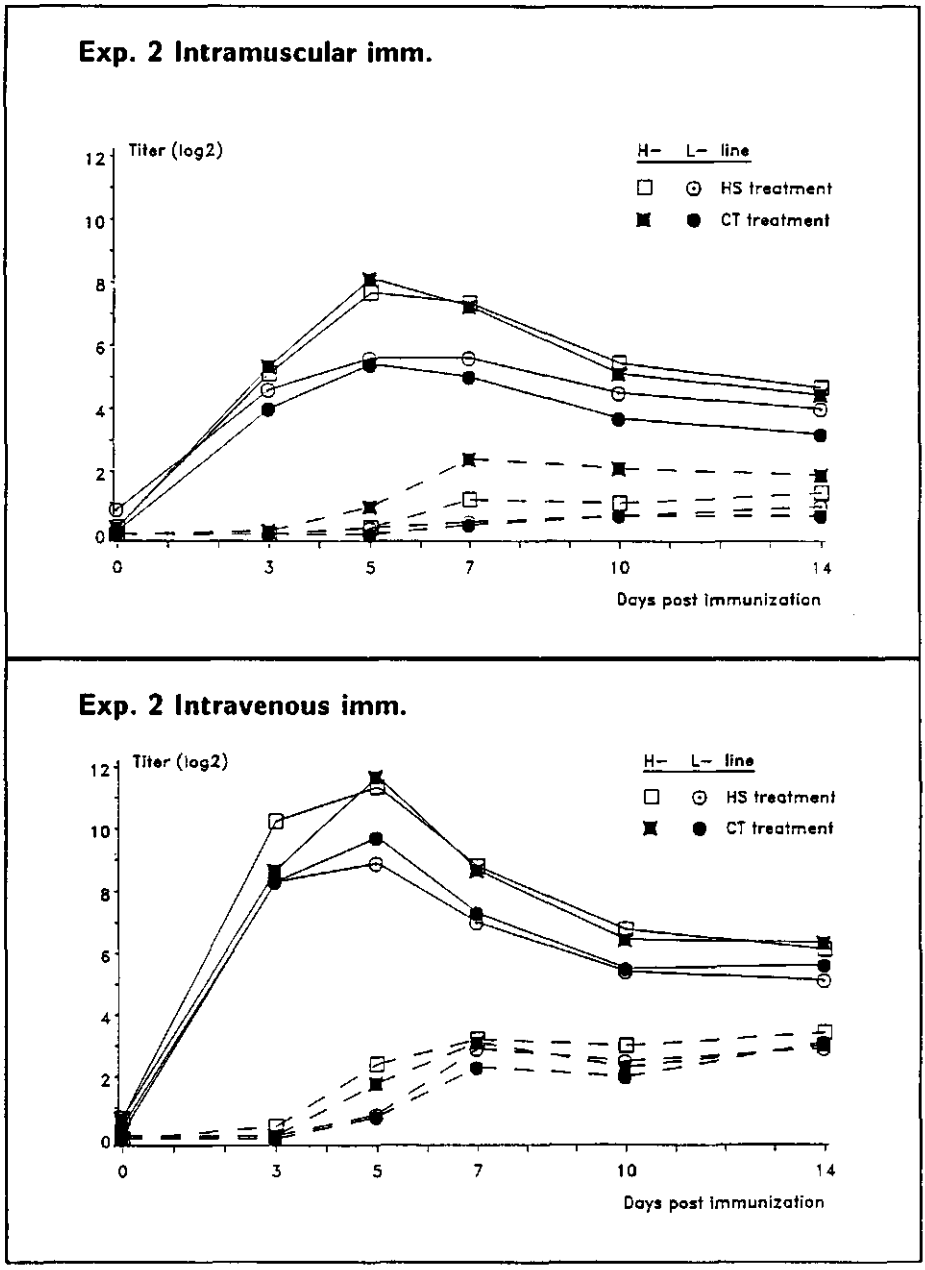

Table 3. Significance[1)] of effects in the analysis of total (TO) and 2ME-resistant (2ME) antibody titers (Exp. 2)

Day	TO/2ME	Sex	Line	Treatment	Immunization route
0	TO	-	-	-	-
	2ME	-	-	-	-
3	TO	*	**	ns	***
	2ME	-	-	-	-
5	TO	ns	***	ns	***
	2ME	ns	***	ns	***
7	TO	ns	***	ns	***
	2ME	ns	***	ns	***
10	TO	ns	***	ns	***
	2ME	ns	**	ns	***
14	TO	ns	***	ns	***
	2ME	ns	**	ns	***

1) -: not tested because of skewed distribution of values.
ns: not significant (P>.10);
+: P<.10; *: P<.05; **: P<.01; ***: P<.001

Figure 4. Total antibody titer (——) and 2ME-resistant titer (– –) per line and treatment after intramuscular immunization and intravenous immunization in Experiment 3. Least squares means from a model including group, line, sex, route and treatment.

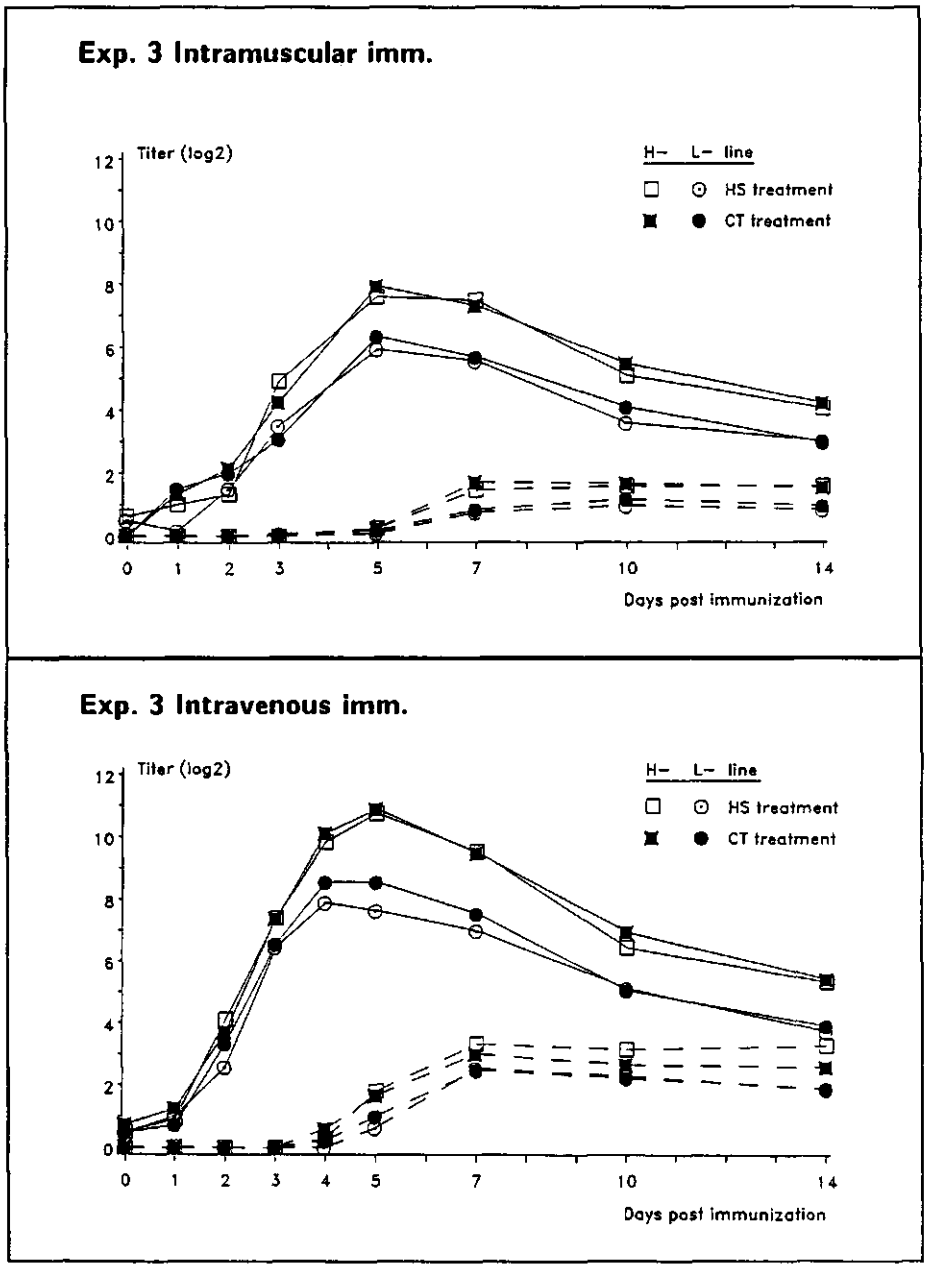

Table 4. Significance[1] of effects in the analysis of total (TO) and 2ME-resistant (2ME) antibody titers (Exp. 3).

Days[2] post Imm.	TO/2ME	Group[3]	Sex	Line	Treatm.	Imm. route	Day[4]
0	TO	-	-	-	-	-	.
	2ME	-	-	-	-	-	.
1+2	TO	-	-	-	-	-	-
	2ME	-	-	-	-	-	-
3+4+5	TO	***	+	***	ns	***	***[5]
	2ME	-	-	-	-	-	-
7	TO	***	*	***	ns	***	.
	2ME	ns	ns	***	ns	***	.
10	TO	***	***	***	*	***	.
	2ME	ns	ns	***	ns	***	.[6]
14	TO	***	***	***	ns	***	.
	2ME	+	**	***	ns	***	.[6]

1) .: not applicable;
-: not tested because of skewed distribution of values;
ns: not significant (P>.10);
+: P<.10; *: P<.05; **: P<.01; ***: P<.001

2) Day 0 included all samples taken before immunization

3) Group I, II, III

4) Day of sampling: 1 or 2; 3, 4, or 5. (day 4 i.v. only)

5) Significant (P<.05) Line * Day interaction

6) Significant (P<.05) Group * Line interaction

Discussion

Despite evidence that chicks in these experiments were under conditions experienced as "stressful" (Donker *et al.*, submitted-a), no consistent suppression of circulating antibodies to SRBC was found. Moderate suppression in heat stressed chicks from the H line was found in Experiment 1 only. A number of factors may be responsible for the considerable variation in immunosuppression after heat stress as found in these experiments and the various studies cited.

Genetics

There may well be considerable genetic differences in stress susceptibility. Thaxton and Siegel (1970, 1972, 1973) found suppressive actions of heat stress on humoral immune responses in chicks from an Athens randombred line selected for high stress susceptibility (Regnier *et al.*, 1980; Thaxton, personal communication). Similar results were obtained by Thaxton *et al.* (1968) and Subba Rao and Glick (1970, 1977) in chicks from a New Hampshire line selected for high bursa weight. Regnier *et al.* (1980) also used Athens randombreds and New Hampshires. These Athens randombreds, however, were from the outbred population and not the inbred line. The New Hampshires were obtained commercially and thus not from the high bursa weight line. Yet in their experiments, no immunosuppression was found after the same heat stress treatment.

Siegel (1987) demonstrated that, after application of a stressor, more corticosteroids were bound in thymus cells from a line selected for high stress response than in the low response line. Immunosuppression is, at least partially, mediated by corticosteroid binding in lymphoid cells (Thaxton and Siegel, 1972; Siegel, 1987). If, as a consequence of selection for other traits, elevated corticosteroid binding in lymphoid cells occurs, increased stress susceptibility can be expected. However, both lines used in our experiments might bind few corticosteroids in lymphoid cells and thus be relatively unsusceptible to stress effects.

Antigen presentation

Antigen dose and route of immunization may have led to differences in results obtained. Siegel *et al.* (1983) and Siegel and Latimer (1984) demonstrated differences in suppressive action which were related to antigen concentration. They demonstrated a interaction between *Salmonella pullorum* antigen dose and heat stress or ACTH injection in White Rock chicks. Heat or ACTH was found to have a suppressive effect only at low antigen doses. In the experiments cited, SRBC was administered intravenously. The dose was, at least

in Thaxton's experiments, the lowest that gave an optimal response (Thaxton, pers. comm., 1987). In our experiments, several intramuscular immunizations were given, with a dose that could be considered high (.25 ml packed SRBC). Results obtained after intravenous immunization, however, were comparable to those obtained after i.m. immunization. The i.v. dose injected in Experiment 2 (.5 ml 14% SRBC) was the same as used in many of the experiments cited (1 ml 7%) (Regnier *et al.*, 1979; Subba Rao and Glick, 1970, 1977; Thaxton *et al.*, 1968; Thaxton and Siegel, 1970, 1972). The i.v. dose used in Experiment 3 can be considered to be "lowest optimal dose" for these lines; doses lower than this showed a rapid decline in antibody titers (unpublished results). Yet, the only significant suppression in antibody titers was found in chicks after i.m. immunization (Exp. 1). Both routes of antigen injection and all doses used showed clear differences between the two selection lines.

Socialization

Socialization or adapting chicks to handling reduces the inter-treatment variation in immune responses (Gross and Siegel, 1979, 1982; Gross, 1986), leading to more consistent results between experimental groups. Beuving and Vonder (1978, 1986) demonstrated a rise in plasma corticosterone after handling. Freeman *et al.* (1979) found that after several daily injections of corticotrophin, a rise in plasma corticosterone still occurs, together with hyperglycemia, but neutralization of these responses is quicker, thus demonstrating habituation. The same phenomenon might occur after daily handling, leading to increased capacity to deal with a stressor. Indications of this were found by Bowen and Washburn (1985), who found a reduced rise in plasma corticosterone levels after an 1 h heating episode, when chickens were extensively handled on four preceding days. Thus in our experiments, the increased attention given to handling may have had the opposite effect than we intended. Gross (1986) found consistent differences in antibody titers between two lines selected for high and low response to SRBC. He kept chicks under various social environments. Results with different antigens were essentially the same in all environments, but most pronounced when chicks had been socialized.

Differences between the high and low selection lines were consistent throughout the experiments, both for total and 2ME-resistant antibody titers. Apart from the moderate suppression found in Experiment 1 in H line, no evidence was found for increased or decreased stress susceptibility in one of these lines.

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References

Ader R., N. Cohen and D.L. Felten, 1987. Brain, behaviour and immunity. *Brain Behav. Immunol.* 1: 1-6.

Beuving G. and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 35: 153-159.

Beuving G. and G.M.A. Vonder, 1986. Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and plasma baseline levels of corticosterone. *Gen. Comp. Endocrinol.* 62: 353-358.

Bowen S.J. and K.W. Washburn, 1985. Thyroid and adrenal response to heat stress in chickens and quail differing in heat tolerance. *Poultry Sci.* 64: 149-154.

Donker R.A., M.G.B. Nieuwland, A.J. van der Zijpp and C.A. Makkink, (*submitted-a*). Heat stress in chicken lines selected for high and low immune responsiveness 2. Physiological parameters.

Donker R.A., A.J. van der Zijpp and M.G.B. Nieuwland, (*submitted to Vet. Immunol. Immunopathol.*). Plaque-forming-cell assay and lymphoid organ development in chicken lines selected for high and low immune responsiveness.

Freeman B.M., A.C.C. Manning and I.H. Flack, 1979. Habituation by the immature fowl in response to repeated injections of corticotrophin. *Br. Poult. Sci.* 20: 391-399.

Gross W.B., 1986. Effect of dose of antigen and social environment on antibody response of high and low antibody response chickens. *Poultry Sci.* 65: 687-692.

Gross W.B. and P.B. Siegel, 1979. Adaptation of chickens to their handler and experimental results. *Avian Dis.* 23: 708-714.

Gross W.B. and P.B. Siegel, 1982. Socialization as a factor in resistance to infection, feed efficiency, and response to antigen in chickens. *Am. J. Vet. Res.* 43: 2020-2012.

Heller E.D., D. Ben-Nathan and M. Perek, 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathol.* 8:195-203.

Kelley K.W., 1985. Immunological consequences of changing environmental stimuli. pages 193-223 in: G. Moberg (ed): *Animal Stress*. American Physiological Society, Bethesda, ML, U.S.A.

Regnier J.A., K.W. Kelley and G.T. Gaskins, 1980. Acute thermal stressors and synthesis of antibodies in chickens. *Poultry Sci.* 599: 985-990.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, N.C.: SAS Institute Inc.

Siegel H.S., 1987. Effects of behavioral and physical stressors on immune responses. pages 39-54 in: P.R. Wiepkema and P.W.M. van Adrichem (eds.). *Biology of stress in farm animals: an integrative approach*. Martinus Nijhoff publ., Dordrecht/Boston/Lancaster.

Siegel H.S. and J.W. Latimer, 1984. Interaction of high temperature and *Salmonella pullorum* antigen concentrations on serum agglutinin and corticosteroid response in white rock chickens. *Poultry Sci.* 63: 2483-2491.

Siegel H.S., J.W. Latimer and N.R. Gould, 1983. Concentration of Salmonella pullorum antigen and the immunosuppressive effect of adrenocorticotropin in growing chickens. *Poultry Sci.* 62: 897-903.

Snedecor G.W. and W.G. Cochran, 1967. *Statistical methods.* Iowa State University, Iowa, U.S.A.

Subba Rao D.S.V. and B. Glick, 1970. Immunosuppressive action of heat in chickens. *Proc. Soc. Exp. Biol. Med.* 133: 445-448.

Subba Rao D.S.V. and B. Glick, 1977. Effect of cold exposure on the immune response of chickens. *Poultry Sci.* 56: 992-996.

Thaxton P., 1978. Influence of temperature on the immune response of birds. Poultry Sci. 57: 1430-1440.

Thaxton P., C.R. Sadler and B. Glick, 1968. Immune response of chickens following heat exposure or injections with ACTH. *Poultry Sci.* 47: 264-266.

Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. Poultry Sci. 49: 202-205.

Thaxton P. and H.S. Siegel, 1972. Depression of secondary immunity by high environmental temperature. *Poultry Sci.* 51: 1519-1526.

Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Sci.* 52: 618-624.

Verstegen M.W.A., W. v.d. Hel, H.A. Brandsma, A.M. Henken and A.M. Bransen, 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. In: M.W.A. Verstegen and A.M. Henken (eds.). *Energy metabolism in farm animals.* Martinus Nijhoff publ., Dordrecht/Boston/Lancaster.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of white leghorn chicks. *Poultry Sci.* 59: 1363-1369.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterization of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris)* : 211-215.

Chapter II

Heat stress in chicken lines selected for high and low immune responsiveness.

2. Physiological parameters.

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Abstract

Physiological responses were measured during studies to investigate the influence of heat stress on humoral immune response in chicken lines selected for immune responsiveness (Donker *et al.*, submitted). During four consecutive periods of 30 min each at 42°C, cloacal temperatures and plasma corticosterone levels were increased and decreased again in intervening 30 min periods. Plasma corticosterone levels also rapidly decreased after the total heating episode. Leukocyte counts (heterophils, lymphocytes and the heterophil/lymphocyte ratio) did not change during or after heat stress treatment, nor did hematocrit percentage. Weight loss during the treatment period was greater in heat stressed chickens than in control chickens. Changes in albumin and fibrinogen levels did not differ between heat stressed or control chickens.

After immunization, given 24 h after the beginning of the heat or control treatment, marked changes in leukocytes counts were detected. The initial decrease in heterophils and heterophil/lymphocyte ratio, which was more pronounced after intramuscular than after intravenous immunization was followed by an increase in levels 24 h after immunization. Plasma levels of albumin and fibrinogen were increased three, four and five days after intravenous immunization but not after intramuscular immunization.

Keywords: selection lines, heat stress, corticosterone, body temperature heterophil/lymphocyte ratio, acute phase proteins, SRBC

Running title: Heat stress and physiology.

Introduction

Heat stress is a frequently occurring factor which can have a negative effect on poultry production. It can directly influence production by reducing feed intake and growth, or in extreme situations, increased mortality may occur. Indirectly, heat stress can reduce immune responsiveness (Thaxton, 1978; Siegel, 1987). Genetic differences in stress responses (Edens and Siegel, 1975, Gross and Siegel, 1985) and heat tolerance occur (Bowen and Washburn, 1984). A number of studies have been carried out on the effect of heat stress on antibody production in two chicken lines that genetically differ in immune responsiveness (Donker *et al.*, submitted). To assess the severity of heat stress in these studies, and possible differences in heat tolerance between these lines, physiological data were gathered during these experiments, which are described in this paper.

Because chickens are homeothermic they will attempt to maintain body temperature within their comfort zone. Therefore measurements of hyperthermia can serve as assessment of the degree of heat stress encountered. In addition to convection, the almost sole mode of heat loss during hyperthermia in birds is through evaporation (Barnas and Rautenberg, 1987). Weight loss, due to evaporative losses could therefore be another simple indicator of heat stress. More generally, increased plasma corticosterone levels are used as an estimate of stress of various kinds, both in mammals and poultry (Siegel, 1987).

Changes in blood leukocyte counts have been frequently found after stressful situations. In poultry, lymphopenia (Ben Nathan *et al.*, 1976; Heller *et al.*,1979), heterophilia and increased heterophil/lymphocyte ratios (Chancellor and Glick, 1960; Gross *et al.*, 1980; Gross and Siegel, 1983) have been reported after heat stress.

In this study two "acute phase proteins" were also measured, to evaluate their value as a possible additional measurement of stress. The acute phase response is a complex physiological reaction, found in animals after a variety of injuries, including infection and mechanical or thermal trauma (Koj and Gordon, 1985; Kushner, 1982; Pindyck *et al.*, 1983). During such a response both local and systemic changes occur. Plasma levels of several "acute phase proteins" are affected during these responses.

In the experiments described in this paper heat stress periods of 30 min at a temperature of 42°C were given. The following were considered as parameters of heat stress: cloacal temperature, weight loss, leukocyte numbers and plasma corticosterone, albumin and fibrinogen levels.

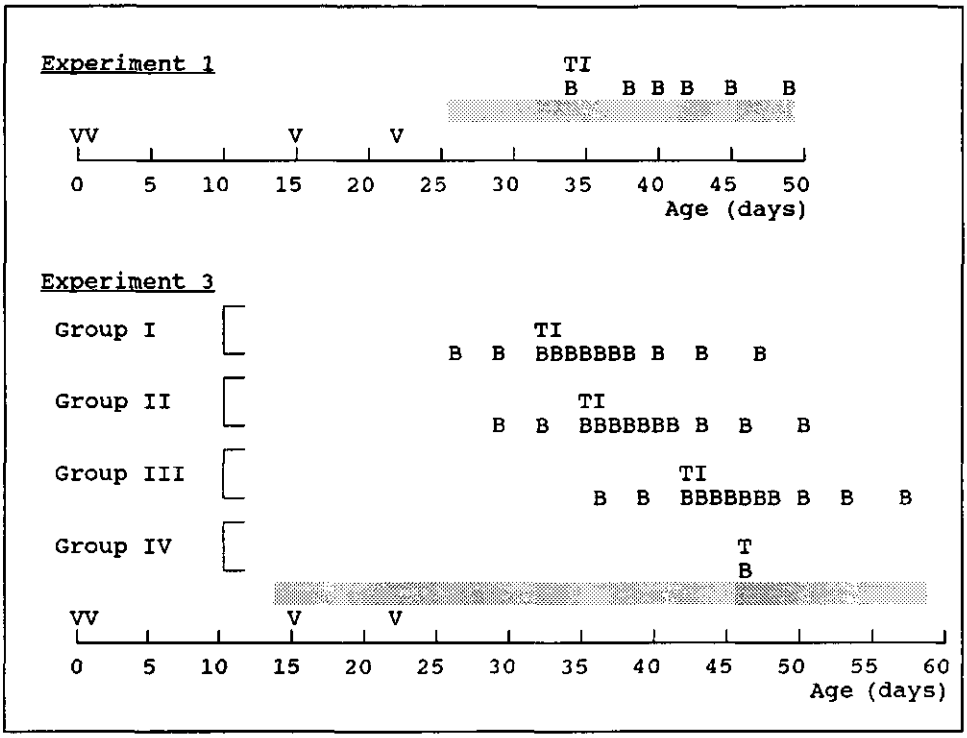
Material and methods

Chicks from the sixth generation of lines selected for high (H) and low (L) antibody production to SRBC (Van der Zijpp and Nieuwland, 1986) were used in three experiments. These chickens were vaccinated against Mareks Disease on day of hatching and against infectious bronchitis, Newcastle Disease and Gumboro on 2, 15 and 22 days of age, respectively (Donker *et al.*, submitted).

After a period of daily handling for socialization purposes (Fig. 1), the chicks were subjected to a heat stress (HS) or control (CT) treatment. Those undergoing heat stress treatment were placed in a portable cage in a climate respiration chamber (Verstegen *et al.*, 1987) at a temperature of 42°C. After 30 min the cages were removed from the chamber and set in an adjoining room at an ambient temperature of approximately 22°C for 30 min After four of these 30 min periods at 42°C, with 30 min intervals, the chicks were returned to the battery. The control group was subjected to the same procedure but at a temperature of approximately 22°C in the respiration

Figure 1. Experimental layout of Experiment 1 and 3.

V: Vaccinations
▒: Daily handling
T: Treatment (heat stress or control)
I: Immunization with SRBC, (intravenous or intramuscular)
B: Blood sampling



chamber. Twenty-four hours after beginning the treatment chicks were immunized with sheep red blood cells (SRBC) intramuscularly (i.m., 1 ml 25% SRBC, in both experiments) or intravenously (i.v., .5 ml 5% SRBC, Exp. 3 only) (Donker *et al.*, submitted). The day of immunization is hereafter referred to as day 0; thus day of treatment is day -1.

Experiment 1

All 72 chicks (equally distributed over H and L line and both sexes) in Experiment 1 were bled from the brachial vein (*vena cutanea ulnaris*) with a heparinized syringe immediately before (approximately 9^{00} h) and after

treatment (approximately 14^{00} h). A further 36 chicks of both lines and sexes remained in the battery cage and were bled at the same times.

Chicks in both treatments were bled again on day 3 and 5 post immunization. Fibrinogen and albumin concentrations were estimated in all plasma samples as amounts relative to a reference plasma, which was included in every assay. Plasma corticosterone concentrations were estimated in plasmas from the day of treatment. For corticosterone assay, plasmas were pooled per three chicks from the same sampling time, line, sex and treatment.

Experiment 3[1)]

The 408 chicks in Exp. 3 were divided into three groups (I, II and III). Each group was treated alike, but at different ages (Fig. 1). Chicks were bled several days before and after treatment (Fig. 1, Table 1). A chick was bled on only one day in each of the following periods: day 7, 4, 1 or 0 before immunization; day 1 or 2 post immunization and day 3, 4 or 5 post immunization. Before immunization and days 1 and 2 after immunization samples were taken at approximately 9^{00} h, that is before treatment on day -1 and before immunization on day 0, and at approximately 14^{00} h, that is after treatment on day -1. The number of samples taken from each group is given in Table 1. In all blood samples taken on days -7, -4, -1, 0, 1 and 2 post immunization, hematocrit percentages were measured and number of heterophils and lymphocytes counted. Relative amount of albumin and fibrinogen was measured in all plasmas. All chicks were weighed immediately before and after treatment.

An extra group (IV) of 68 pullets was treated in the same way as groups I-III, but not bled on days before treatment. These pullets received treatment at 46 days of age (Fig. 1), but were not immunized afterwards. Before treatment, two pullets of each line were bled and cloacal temperature measured (to .1°C precision; Point of time 1, see Table 2). After removal from the battery and immediately before the first heat episode, two additional pullets of each line were bled and temperature measured (point of time 2). Just before the end of the first heat episode two pullets from each line and treatment group were removed individually from the climate-respiration chamber, bled and cloacal temperature measured (point of time 3). This procedure was continued for each of the treatment episodes (Table 2). When the chicks were returned to the battery, two pullets from each line, which had not been removed from the battery, were bled and cloacal temperature measured (point of time 10).

1) During Experiment 2 no physiological data were gathered. The experiment numbers 1 and 3 are maintained in this paper to facilitate comparison with Donker et al. (submitted).

Table 1. Number of blood samples[1)] taken per group (groups I, II and III) in Experiment 3.

Days post immunization	time 08^{30}-09^{30}	13^{30}-14^{30}
-7	8	8
-4	8	8
-1	8	16
0	16	32
1	24	24
2	24	24
3	32 + 24[2)]	
4	24[2)]	
5	32 + 24[2)]	
7	64	
10	64	
14	64	

[1)] The number of chicks bled at any time is composed as follows:
8 = 2 lines * 2 sexes * 2 chicks
16 = 2 lines * 2 sexes * 2 treatments * 2 chicks
24 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 1.5 chicks
32 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 3 chicks
64 = 2 lines * 2 sexes * 2 treatments * 2 imm. routes * 4 chicks
N.B. 1: 1.5 chicks means randomly 1 or 2 within a line-sex-treatment-route group
N.B. 2: before the temperature treatment, no treatment groups were distinguished; before immunization, no immunization route groups were distinguished.

[2)] These 24 chicks were used for plaque-test assay, were i.v. immunized

In this experiment each pullet was bled only once from the brachial vein, and care was taken to complete the sampling procedure within 45-60 sec, the period thought to be necessary to induce adreno-cortical activation by handling (Beuving and Vonder, 1981). Hematocrit percentage was measured in all blood samples, and the number of heterophils and lymphocytes in one of two samples in each line/treatment combination per sampling time was counted.

Table 2. Sampling schedule during HS/CT treatment in Experiment 3, group IV. Number of chickens is shown within each line / treatment / sampling time, used for temperature measurements and bleeding.

Line:	H		L	
Treatment:	CT	HS	CT	HS
Point of time:				
1: at battery cage[1)]	2		2	
2: before HS/CT period 1	2	2	2	2
3: after HS/CT period 1	2	2	2	2
4: before HS/CT period 2	2	2	2	2
5: after HS/CT period 2	2	2	2	2
6: before HS/CT period 3	2	2	2	2
7: after HS/CT period 3	2	2	2	2
8: before HS/CT period 4	2	2	2	2
9: after HS/CT period 4	2	2	2	2
10: at battery cage[1)]	2		2	

[1)] Have not been removed from battery cage

Assays

Corticosterone

Plasma corticosterone levels were estimated by a radioimmunoassay method (Beuving and Vonder 1981).

Acute phase proteins

Albumin and fibrinogen were assayed in duplicate using the rocket-electrophoresis method described by Grieninger *et al.* (1979). The assays were modified as follows. Gels were prepared using agarose immunoelectrophoretic tablets (1% agarose, .025 M tricine, pH 8.6; Biorad, Richmond, California, USA). A 44 mM tris buffer (pH 8.6) was used as the electrophoresis buffer. Gels were made on a plastic support film between two glass plates, using an U-shaped mould to obtain homogeneous .8 mm thick gels. After electrophoresis, the gels were dyed in a solution containing 5 g/l Coomassie blue in 45% ethanol, 45% aqua dest, 10% acetic acid. Peak height was

measured and the amounts expressed in relation to a reference plasma, which was included in every assay.

Heterophil/Lymphocyte ratio

A 1:50 dilution of blood in Natt and Herrick (1952) solution was made. The number of heterophils and lymphocytes present in a volume of 10^{-4} ml was counted in a Börker hemocytometer. A duplicate subsample was counted from each blood sample.

Statistical analyses

Data were evaluated by means of analysis of variance. The general linear models procedure of the SAS package was used (SAS, 1985). Factorial designs were used with 2- and 3-way interactions among factors. Interactions or factors that were not significant (P>.05) were dropped from the model. Levels of significance evaluated were: P<.05 (*), P<.01 (**), P<.001 (***). In the analyses P values >.05 were considered to be not significant (n.s.). Pre-planned comparisons (for example, treatment difference within a day or time) were tested with a Student t-test.

In Experiment 1 analysis was performed within time of sampling:

$$Y_{ijkl} = \mu + L_i + T_j + S_k + e_{ijkl} \quad (1)$$

in which:

Y_{ijkl} = characteristic measured (corticosterone, albumin, fibrinogen)
μ = mean within sampling time
L_i = line effect (i = H, L line)
T_j = treatment effect (j = HS, CT or battery[1])
S_k = sex effect (k = male, female)
e_{ijkl} = residual error

In Experiment 3 weight loss during treatment was analyzed with a model:

$$Y_{gijkl} = \mu + G_g + L_i + T_j + S_k + e_{ijkl} \quad (2)$$

in which:

G_g = group effect (g = I, II, III):

and other factors were as mentioned for model (1)

1) Battery group only included on day of HS/CT treatment

The other characteristics (albumin and fibrinogen, heterophil and lymphocyte count, heterophil/lymphocyte ratio) were analyzed in models containing:

μ, G_g, L_i, T_j, S_k, D_d, M_m, R_r (3)

in which:

D_d = effect of sampling day (d = -7, -4, -1, 0; d = 1, 2; d = 3, 4, 5; where appropriate)

M_m = effect of sampling moment (m = a.m., p.m; where appropriate)

R_r = effect of route of antigen injection (r = i.m., i.v.; after immunization)

In Experiment 3, data on cloacal temperature and corticosterone (group IV) were analyzed with a model:

$\underline{Y}_{ijk} = \mu + L_i + Time_j + \underline{e}_{ijk}$ (4)

in which:

L_i = Line effect (i = H,L)

$Time_j$ = Point of time effect (j = 0, 1,....., 10)

As no significant line effects were found, a model without L_i was also used for these data.

Results and Discussion

Temperature

Ambient temperature during treatment was kept within 1°C of the set point temperature (22°C for CT and 42°C for HS treatment).

Cloacal temperature during treatment in group IV are presented in Figure 2A. In the statistical analysis, time-treatment interaction was significant at $P<.001$. Cloacal temperature was higher immediately after a HS treatment than after a CT treatment period. Significance of a T-test between HS and CT treatment is indicated in Figure 2A. The increase in cloacal temperature indicate that the chicks under HS treatment could not handle the excessive heat in order to maintain homeothermy. In all three experiments they panted heavily during both the heat periods and the intervals between. They tended to "overshoot" their thermoneutral set point after a heat period, that is during the intervening periods (Figure 2A). Similar findings have been recorded by Siegel and Gould (1982) in chicks subjected to heat periods of one hour, with eight hour intervals.

Weight loss

As ruffling of feathers and panting are intended to increase evaporative heat loss, for cooling purposes, weight loss during treatments was expected to be highest in HS treated chicks. Weight loss, relative to live weight,

Figure 2. Cloacal temperature (A) and plasma corticosterone (B) values during four 30 min heat stress (HS) or control (CT) periods. (Exp. 3, group IV). LSM ± s.e.m. HS/CT periods are indicated with a bar at the bottom of the figure. Point of time as in Table 1.

"B control" was battery control
Significance of difference HS-CT:
+: P<.10; *: P<.05; **: P<.01; ***: P<.001

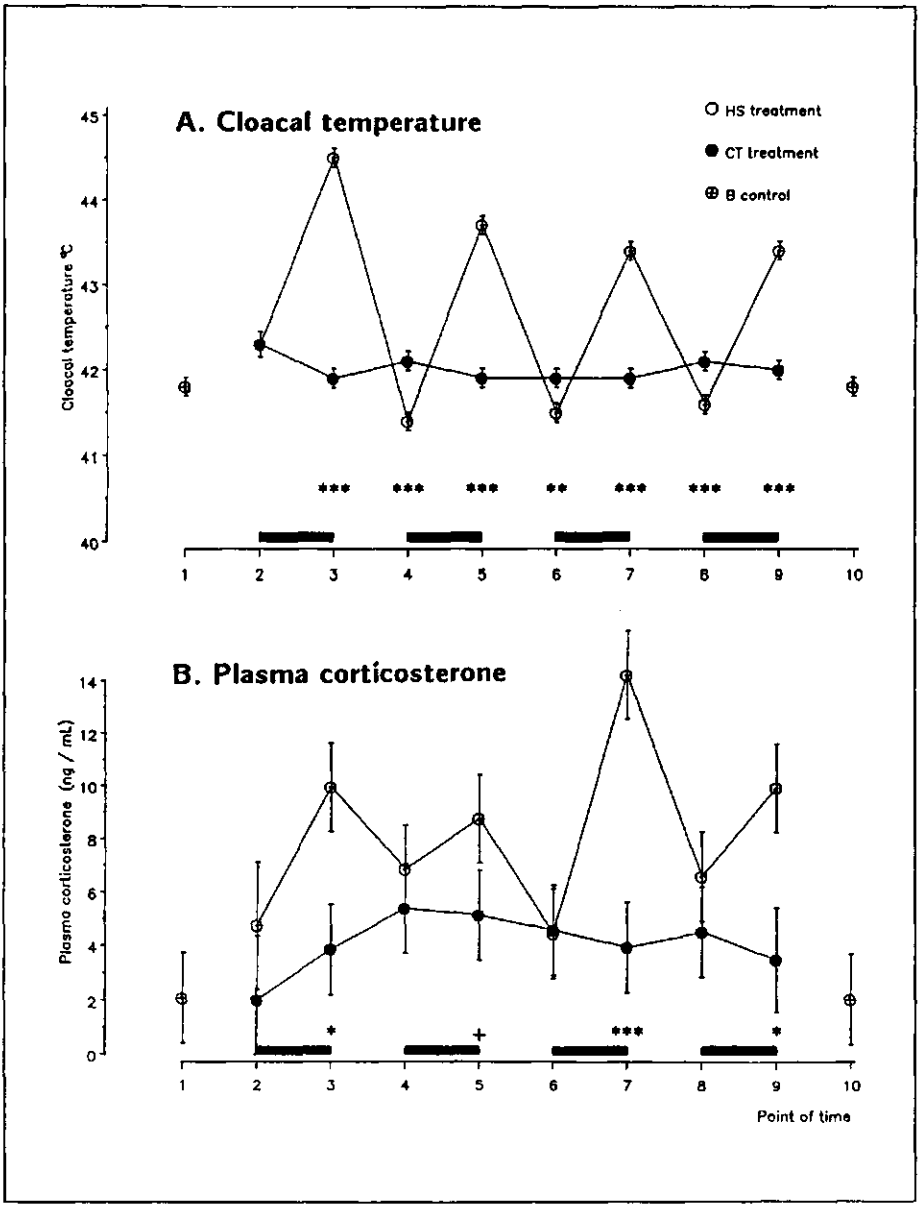
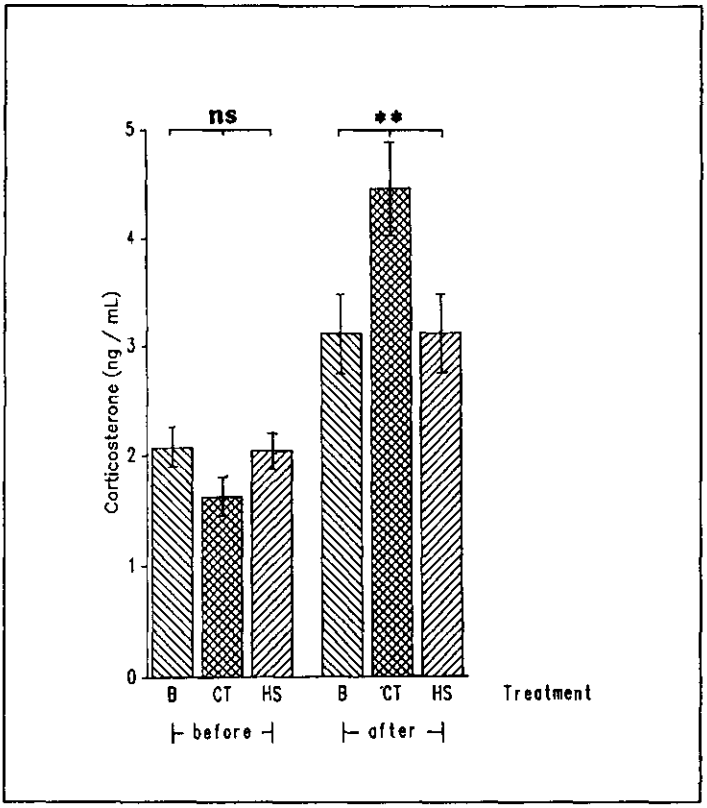


Figure 3. Plasma corticosterone levels (LSM ± s.e.m.) before and after four 30 min heat (HS) or control (CT) periods in respiration chambers. (Exp. 1)

"B"-chicks were battery control.

Significance of difference: ns: P>.05; **: P<.01



recorded during HS treatment in Experiment 3 (LSM ± s.e.) was 7.5 ± .2%, 8.4 ± .2% and 7.5 ± .2% for group I, II and III, respectively. For CT treatments these values were 8.1 ± .2%, 7.8 ± .2% and 5.9 ± .2% respectively. Thus, greater weight loss after HS treatment was found in groups II and III ($P<.05$), but not in group I. The difference between HS and CT treatment increased with age or live weight, as the difference was smallest in group I and greatest in group III. The amount of droppings may account for a considerable variation in weight loss recorded. It cannot be excluded that this amount differed between treatments or groups.

Corticosterone

In Experiment 1 plasma corticosterone levels before treatment did not differ between experimental groups (HS, CT or battery control) (Fig. 3). Levels after treatment were higher than before in all experimental groups. After treatment levels in CT treated chicks were higher than HS treated or battery control chicks ($P<.01$). Morning values were higher in cockerels (LSM = 2.17) than in pullets (LSM=1.65, $P<.05$). The H and L line did not differ significantly at any time.

Plasma corticosterone levels measured in Experiment 3, group IV, during treatment (Fig. 2B) showed a pattern comparable to that found in cloacal temperature. Time-treatment interaction was significant only at $P<.10$. After a heat episode plasma corticosterone values where higher in HS than CT treated chicks ($P<.05$), and decreased again during intermittent periods (Figure 2B). Significances for differences between CT and HS treatment in a T-test within sampling time are indicated in Figure 2B.

The decline in corticosterone levels was apparently rapid, since levels in Experiment 1 at the time of returning HS chicks to their battery cage were the same as in the chicks left in the battery (Fig. 3). These relatively low levels in HS treated chicks at this time may also indicate depletion of corticosterone in the adrenals. During continuous heat stress at a temperature of 43°C for a period of several hours, Edens and Siegel (1975, 1976) recorded a rise in plasma corticosterone levels in chicks followed by a decrease after 60 min. This decrease coincided with a depletion of adrenal corticosterone (Edens, 1978). This acute adrenal cortical insufficiency was accompanied by decreased levels of plasma glucose, cholesterol, Na^+, Ca^{2+} and P. When returned to the battery cage, CT chicks in Experiment 1 had corticosterone levels comparable with those during CT treatment in Experiment 3 (group IV; Figure 2B). This indicated some increase caused by the continuously handling, but this was apparently not as stressful as HS treatment.

Hematocrit values

No consistent influences on hematocrit values were found, neither in groups I-III, nor group IV in Experiment 3. Variations in hematocrit percentages (in the range of 29-33 %) were not found to be related to group, line, treatment or days post or pre-immunization.

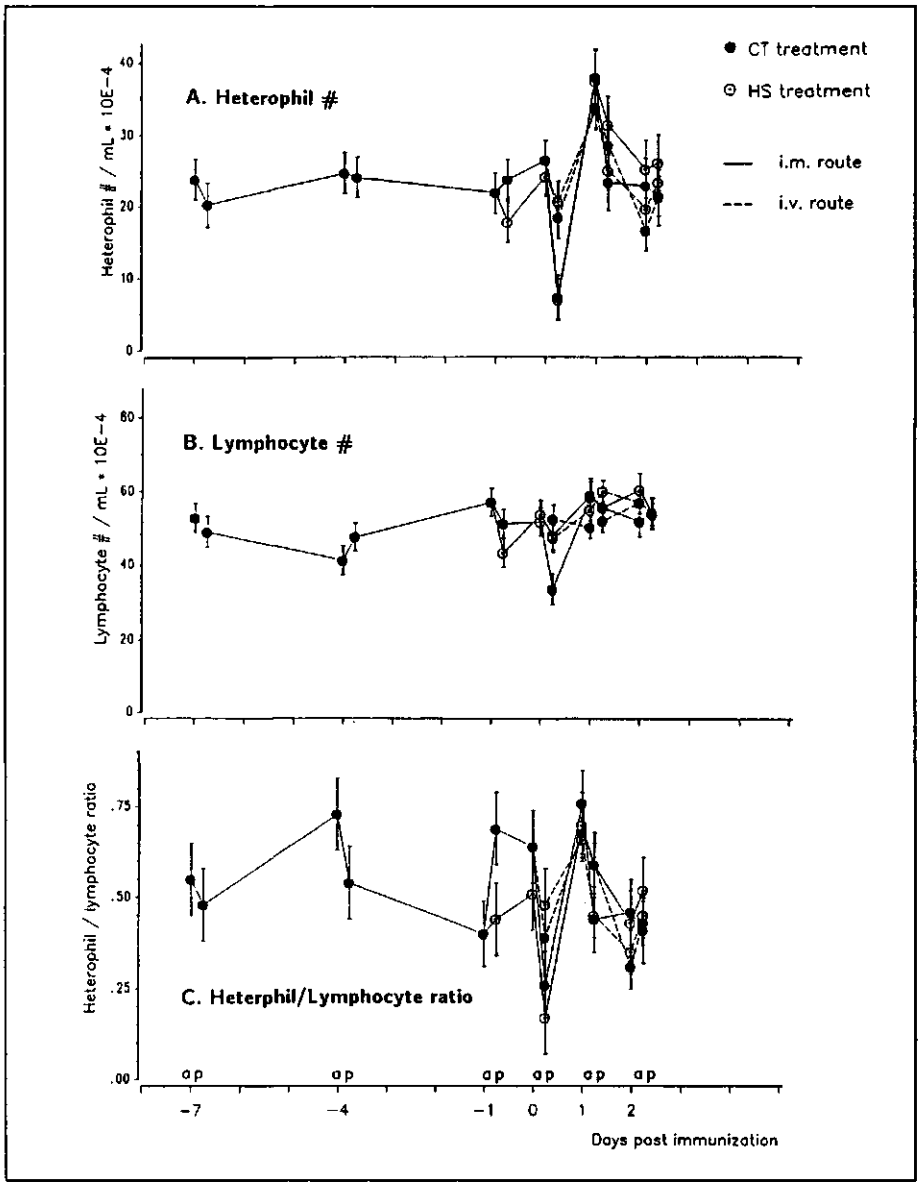
Leukocyte counts

The course in time of numbers of heterophils, lymphocytes and the heterophil/lymphocyte ratio in groups I-III from Experiment 3 are presented in Figure 4. Pronounced variations were found in heterophil numbers, causing the variations found in heterophil/lymphocyte ratios. The a.m.-p.m. differences in heterophils and heterophil/lymphocyte ratio found on days -7

Figure 4. Number of heterophils (A), lymphocytes (B) counted in blood and heterophil/lymphocyte (C) ratio in experiment 3. LSM ± s.e.m. from models including group effects.

Heat stress (HS) or control (CT) treatment was given on day -1, immunization with SRBC was given on day 0.

"a" indicates a.m. samples, "p" indicates p.m. samples.
Before treatment all samples are indicated as "CT";
before immunization all samples are indicated as "i.m.".



and -4 was not significant. Although after treatment (day -1) the rise in heterophil counts and decrease in lymphocyte numbers were not significant, the changes resulted in an increase in the heterophil/lymphocyte ratio ($P<.05$). However, heterophil and lymphocyte counts were not influenced differently by HS or CT treatment. Also, in group IV leukocyte counts (heterophils, lymphocytes, heterophil/lymphocyte ratio) did not show a significant relation with sampling time or treatment. Thaxton *et al.* (1967) also did not find influences of heat stress on leukocyte counts immediately after treatment. This is in contrast, however, with the results of Chancellor and Glick (1960), Ben Nathan *et al.* (1976) and Heller *et al.* (1979), who found significant changes in these values immediately after heat stress treatments.

Leukocyte counts were affected by immunization with SRBC. Influences were different in i.m. and i.v. immunized chicks. On the afternoon after immunization (day 0), heterophil count was significantly lower ($P<.001$) in chicks immunized intramuscularly. Heterophil/lymphocyte ratio decreased accordingly ($P<.05$). Differences between immunization route were significant ($P<.001$ for heterophils; $P<.01$ for the ratio) at this time. This reduction in blood counts might indicate a mobilization of these cells with phagocytic and antigen presenting abilities from the blood to the site of antigen injection. The morning of day 1 post immunization heterophil counts were increased in all treatment/immunization route groups. Trout *et al.* (1988) found a comparable rise in blood heterophils after immunizations with *Brucella abortus*, which peaked 12 hours after immunization.

Acute phase proteins

In Experiment 1 plasma albumin and fibrinogen levels were higher in the samples before treatment than thereafter (Fig. 5A, 5B). A similar difference between morning and afternoon levels was found in chicks left in the battery cage. No significant differences were found between treatments in either plasma albumin or fibrinogen levels. A similar diurnal pattern in albumin and fibrinogen levels was found in Experiment 3, on days before treatment (Fig. 6A, 6B). On days -7 and -4 albumin levels were higher in the morning than afternoon ($P<.05$), but the opposite was found on day of treatment (day -1; $P<.05$). On the morning of the day after treatment (day 0) values were lower ($P<.01$) but subsequent were constant on days 1 and 2. Higher albumin levels were found in chicks immunized i.v. than i.m. on days 3, (4) and 5. Changes in fibrinogen levels were comparable to those in albumin levels. On day -7 and -4 values were higher in the morning than afternoon ($P<.05$), but on the day of treatment no significant differences were found between morning and afternoon levels. Levels were elevated on the morning of day 1 after immunization, thereafter they declined again. Values were higher in chicks

Figure 5. Plasma albumin (A) and fibrinogen (B) levels (LSM ± s.e.m.) before and after four 30 min heat (HS) or control (CT) periods in respiration chambers and 3, 5 days after immunization. (Exp. 1).

"B"-chicks were battery control
"a" indicates a.m. samples, "p" indicates p.m. samples

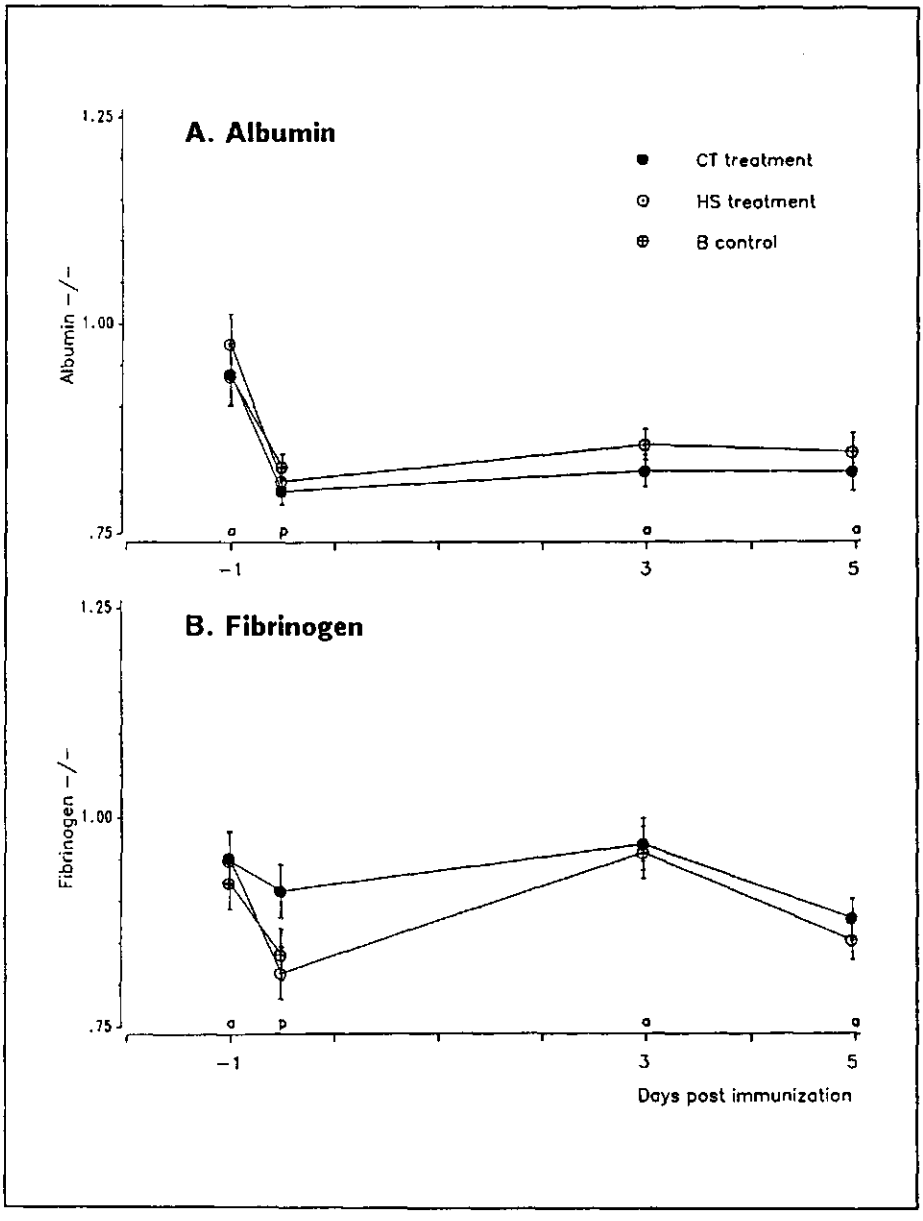
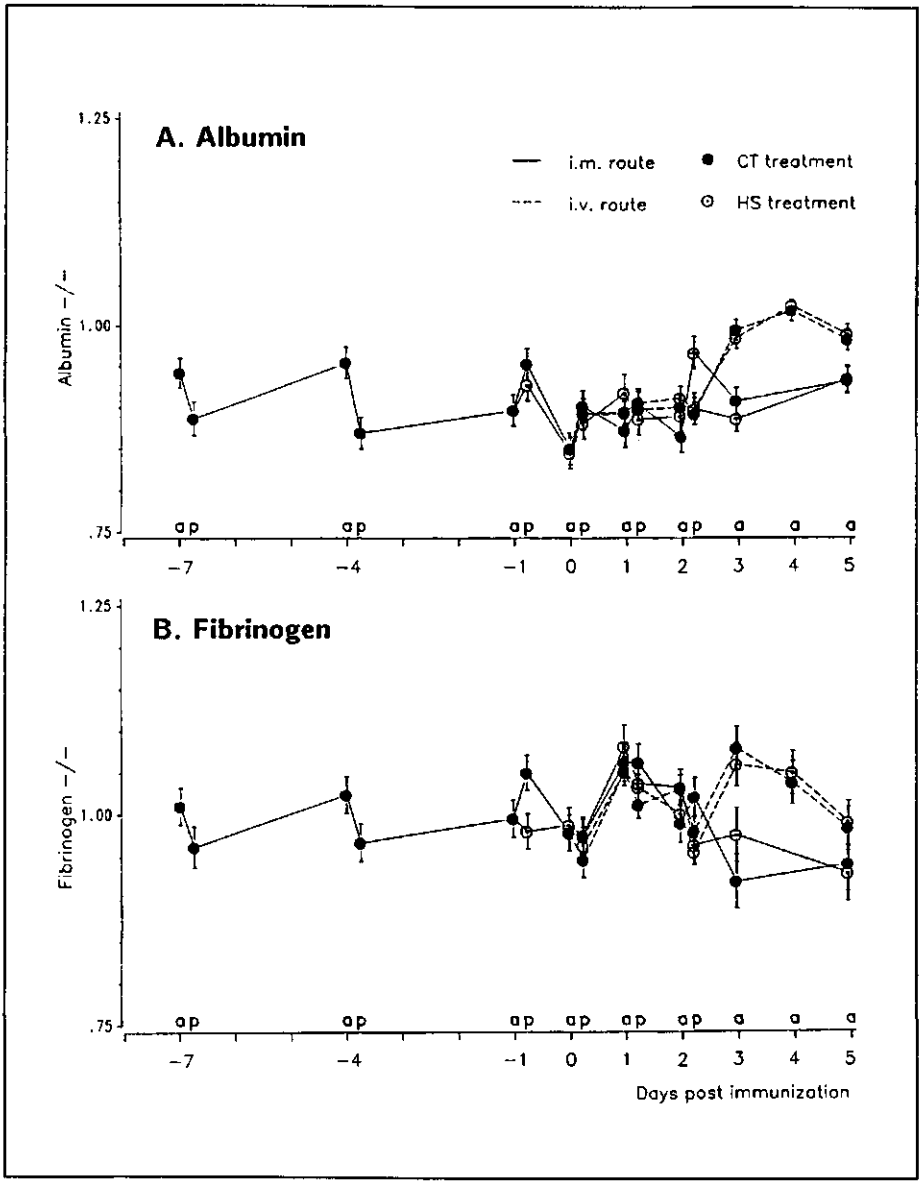


Figure 6. Plasma albumin (A) and fibrinogen (B) levels in Experiment 3. LSM ± s.e.m. from models including group effects.

Heat stress (HS) or control (CT) treatment was given on day -1, immunization with SRBC was given on day 0.

"a" indicates a.m. samples, "p" indicates p.m. samples.
Before treatment all samples are indicated as "CT";
before immunization all samples are indicated as "i.m.".



immunized i.v. than in those immunized i.m. on days 3, (4) and 5 ($P<.001$).

Thus no significant differences were found in albumin and fibrinogen levels between morning and afternoon samples, on day of treatment in Experiment 3, in contrast to the days before treatment. These changes were found, either after HS or CT treatment. Therefore it is concluded that these changes were not induced by the HS treatment, but rather by the absence of food or water during the HS and CT treatment. Levels of both proteins increased significantly on days 3 and 5 after i.v. immunization compared to i.m. immunized chicks. These higher values coincide with higher SRBC antibody levels on these days (Donker *et al.*, submitted). Changes in these proteins therefore do not seem indicative for (heat) stress, but may reflect metabolic changes, during the mounting of an humoral immune response. The reaction pattern found in albumin and fibrinogen levels differed from that usually found during an acute phase response. In these experiments changes occurring during treatment and after immunization showed a similar pattern for albumin and fibrinogen levels. This is the reverse of changes found during the usual acute phase reaction, in which albumin levels decrease and fibrinogen levels rise (Kushner, 1982; Koj, 1985).

In conclusion the physiological responses of H and L selection line to heat stress and immunization were similar. It may be concluded that heat stress affected chickens because both body temperature and plasma corticosterone increased considerably during this treatment. The changes in leukocyte counts in blood and plasma albumin and fibrinogen concentrations in plasma were not indicative of heat stress. The relevance of the diurnal rhythm and changes in leukocyte counts and acute phase proteins observed after immunization need further study.

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References.

Barnas G.M. and W. Rautenberg, 1987. Temperature control. pages 131-153 in: T.J. Seller (ed.). *Bird Respiration (Vol. 1)*. CRC Press, Inc. Boca Raton, FL, U.S.A.

Ben Nathan D., E.D. Heller and M. Perek, 1976. The effect of short heat stress upon leucocyte count, plasma corticosterone level, plasma and leucocyte ascorbic acid content. *Br. Poult. Sci.* 17: 481-485.

Beuving G. and G.M.A. Vonder, 1981. The influence of ovulation and oviposition on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 44: 382-388.

Bowen S.J. and K.W. Washburn, 1984. Genetics of heat tolerance in Japanese quail. *Poultry Sci.* 63:430-435.

Chancellor L. and B. Glick, 1960. Effect of temperature as a stressor on white blood cells, adrenals and bursae of Fabricius of chicks. *Am. J. Physiol.* 198: 1346-1348.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp. (*submitted*). Heat stress in chicken lines selected for high and low immune responsiveness 1. Antibody production.

Edens F.W., 1978. Adrenal cortical insufficiency in young chickens exposed to a high ambient temperature. *Poultry Sci.* 57: 1746-1750.

Edens F.W. and H.S. Siegel, 1975. Adrenal responses in high and low ACTH-response lines of chickens during acute heat stress. *Gen. Comp. Endocrinol.* 25: 64-73.

Edens F.W. and H.S. Siegel, 1976. Modification of corticosterone and glucose responses by sympatholitic agents in young chickens during acute heat exposure. *Poultry Sci.* 55: 1704-1712.

Grieninger G., J. Pindyck, K.M. Herzberg and M.W. Mosesson, 1979. Application of electroimmunoassay to the study of plasma protein synthesis in cultured hepatocytes. *Ann. Clin. Lab. Sci.* 9: 511-517.

Gross W.B. and H.S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* 27: 972-979.

Gross W.B. and P.B. Siegel, 1985. Selective breeding of chickens for corticosterone response to social stress. *Poultry Sci.* 44:2230-2233.

Gross W.B., P.B. Siegel and R.T. DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poultry Sci.* 59: 516-522.

Heller E.D., D. Ben-Nathan and M. Perek, 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathol.* 8: 195-203.

Koj A., 1985. Definition and classification of acute-phase proteins. p. 139-144 In: A.H. Gordon and A. Koj (Eds.) *The acute-phase response to injury and infection*. Elsevier, Amsterdam, New York, London.

Koj A. and A.H. Gordon, 1985. Introduction. p. XXI-XXXI In: A.H. Gordon and A. Koj (Eds.) *The acute-phase response to injury and infection*. Elsevier, Amsterdam, New York, London.

Kushner I., 1982. The phenomenon of the acute phase response. *Ann. N.Y. Acad. Sci.* 389: 39-48.

Natt M.P. and C.A. Herrick, 1952. A new blood diluent for counting the erythrocytes and leukocytes of the chicken. *Poultry Sci.* 31: 735-738.

Pindyck J., G. Beuving, K.M. Herzberg, T.J. Liang, D. Amrani and G. Grieninger, 1983. Control of fibrinogen synthesis by glucocorticoids in the acute phase response. *Ann. N.Y. Acad. Sci.* 408: 660-661.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC: SAS Institute Inc.

Siegel H.S., 1987. Effects of behavioral and physical stressors on immune responses. pages 39-70 in: Wiepkema P.R. and P.W.M. van Adrichem (eds.): *Biology of stress in farm animals: A integrative approach*. Martinus Nijhoff publ., Dordrecht, Boston, Lancaster.

Siegel H.S. and N.R. Gould, 1982. High temperature and corticosteroid in the lymphocytes of domestic fowl (*Gallus domesticus*). *Gen. Comp. Endocrinol.* 48: 348-354.

Thaxton P., 1978. Influence of temperature on the immune response of birds. *Poultry Sci.* 57: 1430-1440.

Thaxton P., C.R. Sadler and B. Glick, 1967. The physiological response of New Hampshires to high temperatures. *Poultry Sci.* 46: 1598-1599.

Trout J.M., M.M. Mashaly and H.S. Siegel, 1988. Changes in the profiles of circulating white blood cells, corticosterone, T3 and T4 during the

initiation of humoral immunity in immature chickens. *Dev. Comp. Immunol.* 12: 331-346.

Verstegen M.W.A., W. v.d. Hel, H.A. Brandsma, A.M. Henken and A.M. Bransen, 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. in: M.W.A. Verstegen and A.M. Henken (eds.). *Energy metabolism in farm animals.* Martinus Nijhoff publ., Dordrecht/Boston/Lancaster.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterization of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris)* : 211-215.

Chapter III

The effect of environmental temperature on antibody production and some physiological parameters in chicken lines selected for humoral immune responsiveness.

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The effect of environmental temperature on antibody production and some physiological parameters in chicken lines selected for humoral immune responsiveness.

Abstract

1. To study genotype x environmental temperature interactions on antibody production, chickens from lines, divergently selected for antibody production to sheep erythrocytes (SRBC), were kept at different temperatures. Temperatures were either constant at 25°C, 35°C or fluctuating diurnally between 15-25°C or 25-35°C.
2. A primary immunization with SRBC was given at 35 d of age. At the time of primary immunization, some of the chickens were exchanged between different temperatures. A secondary immunization was given at 63 d of age.
3. Higher cloacal temperature, decreased growth and decreased lymphoid organ weights *post mortem* indicated a major stress influence in high temperature environments.
4. Higher antibody titres were found in the high temperature environments, but moving chickens from 25°C to 35°C at the time of immunization caused immunosuppression during primary immunization.
5. Differences between the two selection lines in antibody production and physiological characteristics measured were constant in all environments and treatments, indicating the absence of environment x genotype interactions.

Introduction

Antibody production is one of a number of important responses in an animals defence against pathogens. For a given antigen and dose, differences in antibody production can occur because of variation in nutrition, genetics and environment (Kelley, 1985; Siegel, 1987). Genetic differences can be utilized to develop selection lines that differ in antibody production capacity, as was demonstrated in mice (Biozzi *et al.*, 1979) and chickens (Siegel and Gross, 1978; Van der Zijpp and Nieuwland, 1986).

The most studied environmental influence on antibody production has been environmental temperature. In particular immunosuppression as a consequence of sudden changes in temperature has been reported (Subba Rao and Glick, 1970, 1977; Thaxton and Siegel, 1970, 1972, 1973). This immunosuppressive effect of acute thermal stress was associated with stimulation of the hypothalamo-pituitary-adrenal axis.

The influence of moderate but prolonged heat has been described by Blecha and Kelley (1979), Henken *et al.* (1983a, b) and Beard and Mitchell (1987). After adaptation to temperatures just outside the thermoneutral zone, immunoenhancement can occur (Henken *et al.*, 1983a; Beard and Mitchell, 1987; Anderson and Kühn, 1988).

The occurrence of interactions between different environmental temperatures and antigen type and dose have been reported (Siegel and Latimer, 1984; Beard and Mitchell, 1987). However, no results are available concerning genotype x environment interactions with respect to antibody production. These interactions would be of importance for the practical application of selection on immunological traits in breeding programmes.

To study this interaction, chickens from lines selected for high (H) and low (L) antibody titres after immunization with sheep erythrocytes (SRBC) were kept under different environmental conditions. Temperatures ranging from 15 - 35°C were used.

To study the effect of acute thermal stress at the moment of immunization, compared to the effect of prolonged stress, birds were exchanged between the different temperatures.

To monitor metabolic changes which could be associated with alterations in the environment, the cloacal temperature and growth were measured frequently.

As indicators of stress, measurements of cloacal temperature and the heterophil/lymphocyte ratio were made; lymphoid organ development and liver weight were checked at the end of the experiment.

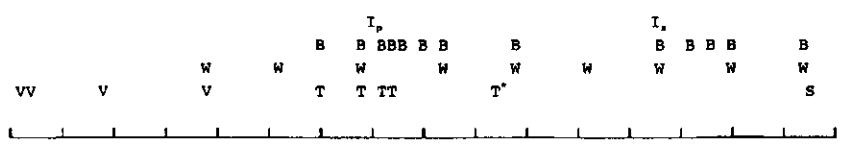
Material and methods

In this experiment a total of 396 chickens was used. These were genetically identical to the sixth generation of the H and L selection lines for antibody titre to sheep red blood cells (SRBC). These lines are selected on antibody titre five days after an intramuscular (i.m.) immunization with 1 ml 25% SRBC in phosphate buffered saline (PBS) (Van der Zijpp and Nieuwland, 1986).

After hatching the birds were individually wingbanded and vaccinated against Marek's disease, infectious bronchitis, Gumboro's disease and Newcastle disease (Fig. 1). They were placed in wire cages and provided with commercial layer starter feed and water *ad libitum* throughout the experiment. The two sexes were housed separately, but the two lines were intermingled.

The temperature in all chambers was gradually lowered from 32°C at d 1 to 25°C at 25 d of age, and during the following six days gradually changed to the final temperatures for the specific treatment. One control chamber (C) was

Figure 1. Experimental layout.



I Immunization: I_p primary immunization
I_s secundary immunization
exchange between chambers for CH, HC and C_fH_f groups on day 35
B Blood sampling
W Weighing of birds
V Vaccinations: d 1 Marek's disease
d 2 infectious bronchitis
d 9 Gumboro's disease
d 19 Newcastle disease
T Body temperature measurement
(T* measured at maximum and minimum chamber temperature)
S Slaughter and dissection

continuously kept at 25°C, one chamber kept at the high (H) temperature of 35°C, one chamber fluctuated daily between 15 and 25°C (fluctuating Control; C_f) and one chamber fluctuated between 25 and 35°C (fluctuating Hot; H_f). Temperatures were regulated by a microcomputer and kept within setpoint temperature ± 1°C. Relative humidity was approximately 70% in all chambers. Fluctuating temperatures followed a sinusoid with the highest temperature at 4^{00} h and the lowest at 16^{00} h. The light regime was 12 h light, 12 h dark.

Seven treatment groups were used. In each of the four chambers one group stayed there throughout the experiment (CC, HH, C_fC_f, H_fH_f). Immediately after primary immunization, one group was moved from the 25 to the 35°C environment (CH), one group from the 35 to the 25°C environment (HC) and one group was moved from the fluctuating control to the fluctuating hot environment (C_fH_f). Chickens from both lines and both sexes were equally distributed over treatment groups at 13 d of age. Thus a total of 28 line-sex-treatment combinations were distinguished.

From 19 d of age chickens were weighed weekly (Fig. 1). At 35 d of age chickens were immunized i.m. with 1 ml 25% (v/v) SRBC in PBS. Chickens were bled randomly 5 d or 1 d before immunization (referred to as day -5, -1) and 1 or 2 d post immunization. All chickens were bled 3, 5, 7 and 14 d after immunization. At 65 d of age all chickens were bled again just prior to a secondary immunization with 1 ml 50% (v/v) SRBC in PBS. They were bled again 3, 5, 7 and 14 d post secondary immunization (Fig. 1).

Five and 1 d before primary immunization (-5, -1) and 1 and 2 d after immunization cloacal temperature was measured with .1°C precision prior to bleeding. These measurements were made between 9^{00} and 12^{00} h. Cloacal temperature was measured 12 d after primary immunization, either between 4^{00} and 6^{00} h or between 16^{00} and 18^{00} h. At this time the chamber temperature was the highest (4^{00}-6^{00} h) or the lowest (16^{00}-18^{00} h) in the environments with fluctuating temperatures.

At 78 d of age, chickens were killed by cervical dislocation and from every treatment 24 chickens (6 of every line-sex-treatment combination) were dissected. The liver, spleen, bursa of Fabricius and thymus were excised and weighed.

Assays

All blood samples were taken between 9^{00} and 12^{00} h from the brachial vein (*vena cutanea ulnaris*) with a syringe which was rinsed with a heparin solution.

Smears were made from 112 blood samples (4 in every line-sex-treatment group), that were taken on 5 and 1 d before and 1 and 2 d after the primary immunization. These were dyed with May-Grünwald-Giemsa stain and a differential count was made of the number of heterophils and lymphocytes. A total number of 100 leukocytes was counted in each smear to estimate the heterophil/lymphocyte ratio.

The packed cell volume (PCV) was measured in 112 blood samples (4 per line-sex-treatment combination) taken before and on d 1, 2, 3, 7 and 14 after primary immunization.

After centrifugation, plasma was collected and stored at -20°C until assayed. Total (TO) and 2-Mercapto ethanol (2ME-) resistant antibody titres were assayed in all plasmas by hemagglutinin assay (Van der Zijpp and Leenstra, 1980). Titres were expressed as $\log_2$ of the highest dilution giving total agglutination.

Statistical analysis

Analyses of variance were performed within day of sampling, with full factorial designs, using the GLM procedure of the SAS package (SAS, 1985). Main factors included in the models were line, sex and treatment. Two- and three- way interactions between the factors were analyzed in the initial full factorial model, but left from the model if not significant ($P > .05$). The sex effect was also left from the model if not significant. Student-Newman-Keuls multiple range tests were used to test for differences among treatments. T-tests on least squares means were performed to test a number of pre-planned comparisons between environments, e.g. comparison of CC with HH or CH.

Results

Cloacal temperature

Treatment differences in cloacal temperature are presented in Table 1. Elevated cloacal temperatures were found in chickens in high temperature environments (35°C) at all times. In the environment with fluctuating high temperature (H_f) cloacal temperature was not increased on d -5, -1, 1 and 2 (between 9^{00} and 12^{00} h). In this environment the cloacal temperature was increased during the 35°C period (day 12 high), but not during the 25°C period (day 12 low) (Table 1). Cloacal temperatures of chickens in the C_f environment were comparable to those in the C environment at all times. No significant differences between lines and sexes were found.

Table 1. Cloacal temperature (°C). Least squares means for each treatment[1)]

	Days after immunization					
Treatment	-5	-1	1	2	12 low[2)]	12 high[3)]
CC	41.5^{b}	41.5^{c}	41.5^{b}	41.3^{b}	41.3^{b}	41.2^{b}
CH	41.5^{b}	41.8^{bc}	42.0^{a}	41.9^{a}	42.1^{a}	42.3^{a}
HC	41.9^{a}	42.4^{a}	41.3^{b}	41.2^{b}	41.3^{b}	41.2^{b}
HH	41.8^{a}	42.0^{ab}	41.8^{ab}	41.8^{a}	41.9^{a}	42.0^{a}
C_fC_f	41.5^{b}	41.6^{c}	41.4^{b}	41.5^{b}	41.4^{b}	41.2^{b}
C_fH_f	41.5^{b}	41.5^{c}	41.5^{b}	41.4^{b}	41.3^{b}	42.1^{a}
H_fH_f	41.6^{b}	41.6^{c}	41.5^{b}	41.4^{b}	41.1^{b}	42.0^{a}

1) Least squares means in the same column bearing different superscripts differ significantly ($P<.05$)

2) Measured during the low temperature period in fluctuating temperature environments

3) Measured during the high temperature period in fluctuating temperature environments

Body weight

Average body weights for each treatment are presented in Table 2. Higher body weights were found in the L line from 26 d of age. Males were heavier from 19 d of age. Decreased growth was found in high temperature environments; differences in body weights were significant from 34 d of age. Chickens in control environments (C and C_f) grew fastest, and chickens kept in the continuous hot (H) environment grew slowest. The chickens in the fluctuating high temperature group (H_f) showed intermediate growth. After transfer from

one environment to another (d 0), the chickens adapted rapidly to the new environment with regard to growth rate.

Table 2. Body weight (g). Least squares means for each treatment[1)].

	Age (days)								
Treatment	19	26	34	42	49	56	63	70	77
CC	188	273	401^{a}	550^{a}	714^{a}	866^{b}	1020^{b}	1137^{b}	1287^{b}
CH	186	270	389^{a}	466^{c}	560^{d}	621^{d}	686^{e}	761^{e}	836^{e}
HC	183	260	336^{b}	460^{c}	626^{c}	760^{c}	784^{d}	910^{d}	1055^{d}
HH	190	269	348^{b}	426^{d}	535^{d}	624^{d}	708^{e}	777^{e}	852^{e}
C_fC_f	192	278	391^{a}	560^{a}	730^{a}	903^{a}	1066^{a}	1196^{a}	1362^{a}
C_fH_f	186	262	397^{a}	529^{b}	672^{b}	784^{c}	884^{c}	986^{c}	1127^{c}
H_fH_f	182	265	387^{a}	513^{b}	662^{b}	776^{c}	900^{c}	999^{c}	1137^{c}

1) Least squares means in the same column bearing different superscripts differ significantly ($P<.05$)

Antibody production

Antibody titres after primary immunization are presented in Figure 2 and those after secondary immunization in Figure 3. Significance levels of the factors line, environment and sex are shown in Table 3. No significant interactions were found.

Higher antibody titres were found in the H line, both in total and 2ME resistant titres.

After primary immunization total antibody titres in chickens in the HH treatment were significantly increased, and those in CH chickens significantly decreased, compared to CC chickens. HC chickens showed an increase on d 5, and a decrease on d 7, compared to CC chickens, but these differences were significant only in a direct comparison using a T-test ($P<.05$).

From the chickens in fluctuating temperature environments, those in C_fH_f treatment showed higher antibody titres compared to C_fC_f (and CC) treatment. Levels in the other treatments in environments with fluctuating temperatures (C_fC_f and H_fH_f) were comparable to the CC treatment.

Differences between the H and L line and between treatments in 2ME-resistant titres were comparable to those found in total titres, but titers were lower and with lower levels of significance.

Figure 2. Antibody production after primary immunization with SRBC. Least squares means for each treatment.
——— total titre – – – 2ME resistant titre.

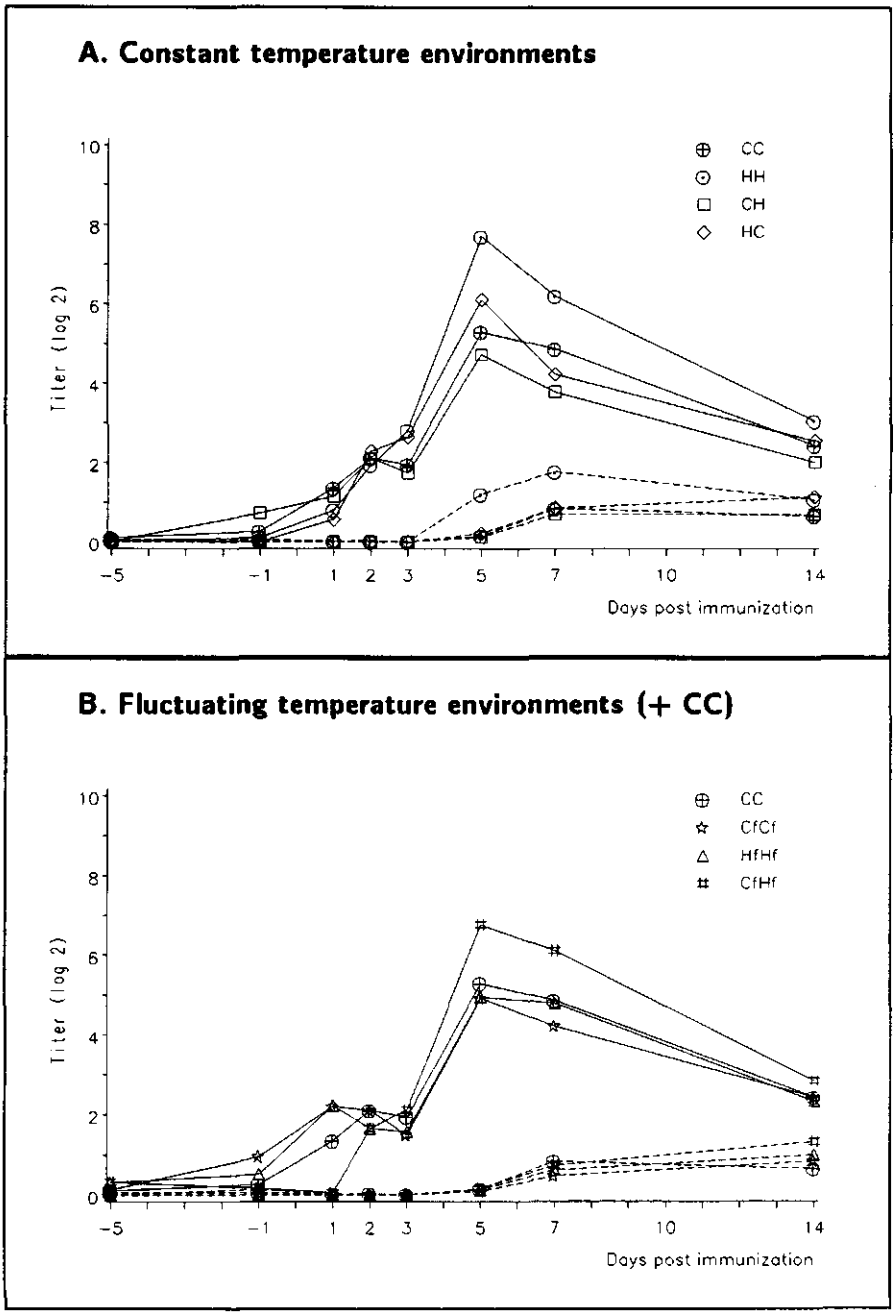


Figure 3. Antibody production after secondary immunization with SRBC. Least squares means for each treatment.
——— total titre – – – 2ME resistant titre.

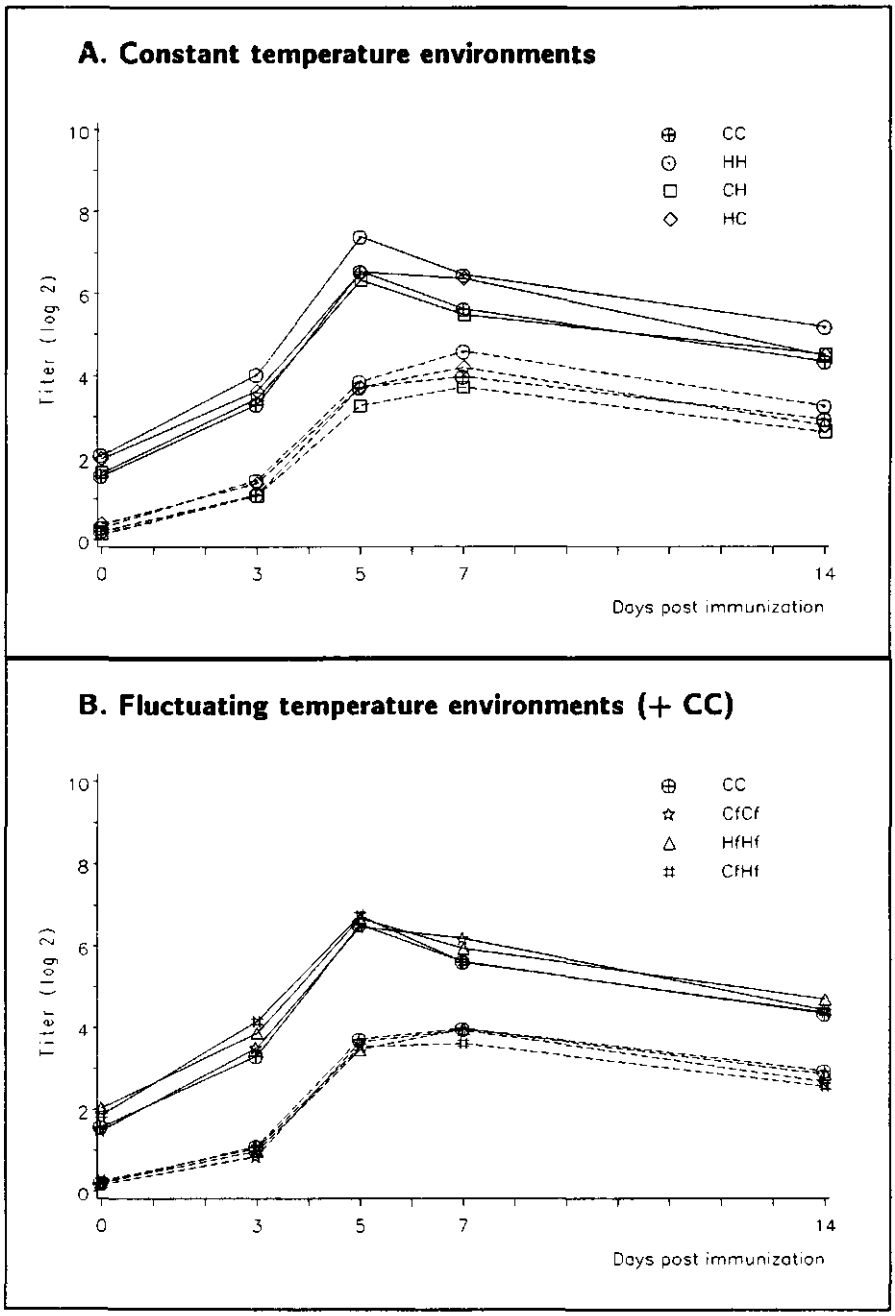


Table 3. Significance[1] of line, treatment and sex in analysis of total (TO) and 2ME-resistant (2ME) antibody titres.

Days after immunization	Primary immunization								Secondary immunization				
	-5	-1	1	2	3	5	7	14	0	3	5	7	14
TO:													
Line	-	-	ns	***	***	***	***	***	***	***	***	***	***
Treatment	-	-	***	ns	***	***	***	***	***	***	**	***	***
Sex	-	-	ns	ns	ns	*	*	*	ns	ns	ns	ns	ns
2ME:													
Line	-	-	-	-	-	-	***	***	-	***	***	***	***
Treatment	-	-	-	-	-	-	***	**	-	**	*	***	***
Sex	-	-	-	-	-	-	**	ns	-	ns	ns	ns	ns

Differences between treatments[2] (Figures 3 and 4):	Primary immunization								Secondary immunization				
	-5	-1	1	2	3	5	7	14	0	3	5	7	14
TO:													
CC	-	-	ab	a	bc	cd	b	bc	b	c	b	b	b
HH	-	-	bc	a	a	a	a	a	a	ab	a	a	a
CH	-	-	b	a	bc	d	c	c	ab	bc	b	b	b
HC	-	-	bc	a	a	bc	bc	abc	a	abc	b	a	b
C_fC_f	-	-	a	a	c	d	bc	bc	b	bc	b	a	b
H_fH_f	-	-	a	a	bc	d	b	bc	a	abc	b	ab	b
C_fH_f	-	-	c	a	b	b	a	ab	ab	a	b	b	b
2ME:													
CC	-	-	-	-	-	-	b	b	a	abc	ab	bc	ab
HH	-	-	-	-	-	-	a	ab	a	a	a	a	a
CH	-	-	-	-	-	-	b	b	a	abc	b	c	b
HC	-	-	-	-	-	-	b	ab	a	ab	ab	b	b
C_fC_f	-	-	-	-	-	-	b	b	a	c	ab	bc	b
H_fH_f	-	-	-	-	-	-	b	ab	a	bc	ab	bc	b
C_fH_f	-	-	-	-	-	-	b	a	a	abc	ab	c	b

1) -: not tested because of skewed distribution of values.
ns: not significant (P>.05);
*: P<.05; **: P<.01; ***: P<.001

2) Treatments bearing different superscripts within a column differ significantly in a Student-Newman-Keuls multiple range test (P<.05).

After secondary immunization the chickens in HH environment had higher total titres again than all other treatment groups. The other treatment groups were not significantly different from each other. Only the HC group showed higher titres at d 7, at the same level as the HH group.

After secondary immunization the 2ME-resistant titres were highest at d 7. The 2ME-titres in the HH treated group were increased, while some decreased titres were found in the CH and C_fH_f groups compared to CC and C_fC_f. However, these differences were significant only when compared in a direct T-test.

Packed cell volume (PCV)

The PCV values were always higher in the H line than the L line, but the difference was only significant ($P<.01$) on d -5, -1 and 1. The difference between the two sexes was not consistently significant. Differences between treatments (Table 4) were significant at all days ($P<.01$ on d -5, -1; $P<.001$ on other days). Values were very similar in the treatment groups kept at the same temperature before immunization (e.g. CH and CC), or after transfer to another temperature (e.g. HC and CC). The day after immunization an increase was found in some groups (CC, CH, C_fH_f), whereas in the others there was a decrease. On d 2 and 3 after immunization all groups had lower PCV values than before. These differences later disappeared, and the HH and HC group showed a relative rise on d 14. The changes observed were the greatest in groups kept at constant temperatures.

Table 4. Packed cell volume (%). Least squares means for each treatment[1)].

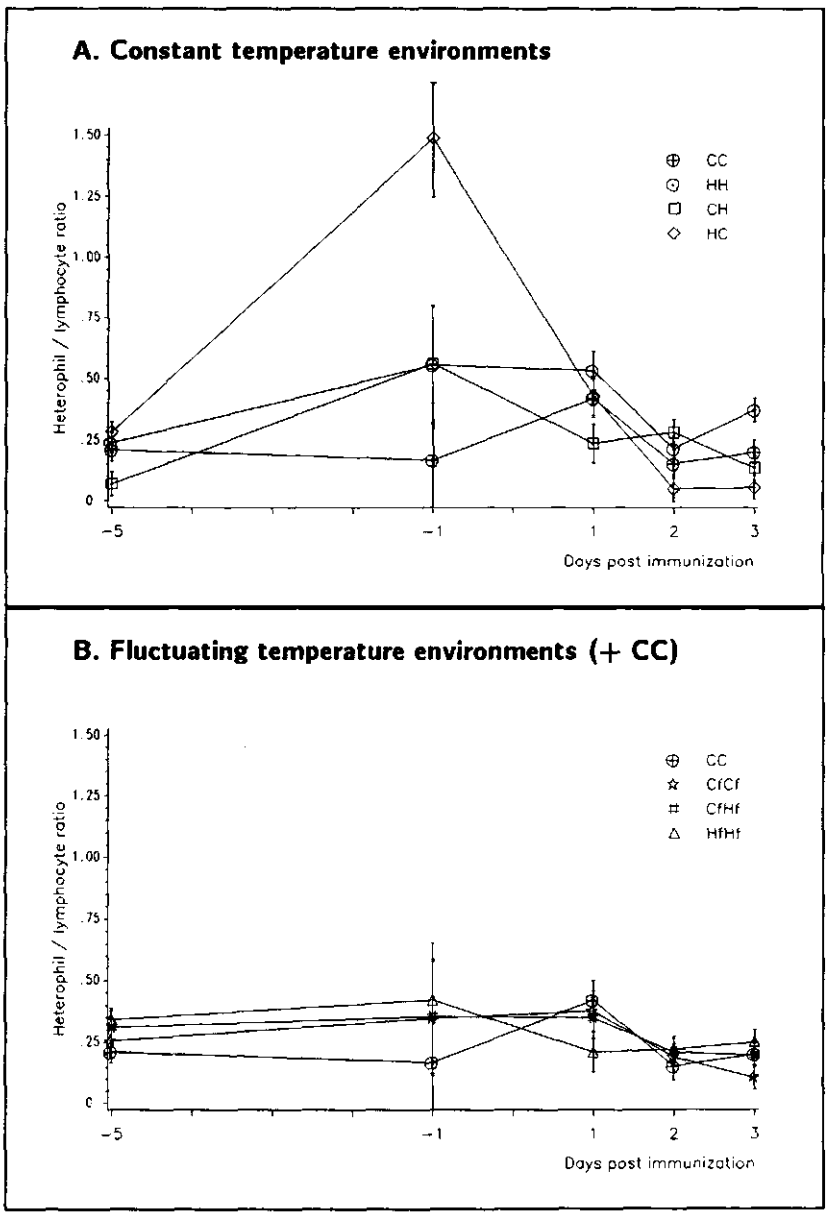
Treatment	Days after immunization						
	-5	-1	1	2	3	7	14
CC	29.8^{ab}	28.9^{bc}	31.5^{a}	27.8^{b}	27.6^{a}	30.3^{ab}	29.8^{ab}
CH	30.2^{ab}	28.2^{c}	29.2^{b}	27.6^{b}	27.2^{ab}	27.3^{cd}	27.7^{b}
HC	28.6^{b}	28.4^{bc}	25.3^{c}	24.0^{d}	24.7^{c}	28.8^{bc}	32.1^{a}
HH	29.8^{ab}	29.3^{ab}	27.9^{b}	25.7^{c}	25.3^{bc}	26.4^{d}	29.6^{ab}
C_fC_f	29.5^{ab}	30.3^{ab}	28.5^{b}	28.4^{b}	28.2^{a}	31.0^{a}	31.3^{a}
C_fH_f	32.0^{a}	30.9^{a}	31.9^{a}	30.4^{a}	28.4^{a}	27.9^{cd}	29.9^{ab}
H_fH_f	29.2^{b}	29.0^{b}	28.0^{b}	28.1^{b}	27.1^{ab}	28.7^{bc}	30.2^{a}

1) Least squares means in the same column bearing different superscripts differ significantly ($P<.05$)

Heterophil / lymphocyte ratio

The variation in heterophil/lymphocyte ratio (H/L; Fig. 4)) were such that no consistent influence of line, sex or treatment could be found on d -5, -1 and 1. A decrease in the ratio was observed in all environments 2 d after immunization ($P<.01$) compared to the days before. Despite reduced variation on d 2 and 3, no significant differences were found between treatments.

Figure 4. Heterophil/lymphocyte ratio. Least squares means for each treatment.



Relative organ weight

Relative organ weights of liver, spleen, thymus and bursa were, like body weight, influenced significantly by the environmental temperatures. Significance of factors and least squares means are given in Table 5.

Table 5. Significance[1] of line, treatment and sex in analysis of relative organ weight[2] of liver, spleen, thymus and bursa. Least squares means for each line and treatment.

Significance:

	Relative weight of: liver	spleen	thymus	bursa
Factor:				
Line	+	***	ns	ns
Treatment	***	***	***	***
Sex	+	***	***	ns

Least squares means:

	Relative weight of: liver	spleen	thymus	bursa
Treatment:				
CC	1.53^{ab}	0.18^{a}	0.43^{a}	0.16^{abc}
HH	1.37^{c}	0.13^{b}	0.22^{b}	0.08^{d}
CH	1.36^{c}	0.13^{b}	0.23^{b}	0.08^{d}
HC	1.59^{a}	0.17^{a}	0.43^{a}	0.15^{bc}
C_fC_f	1.63^{a}	0.18^{a}	0.45^{a}	0.19^{ab}
H_fH_f	1.46^{bc}	0.16^{a}	0.37^{a}	0.13^{c}
C_fH_f	1.46^{bc}	0.15^{ab}	0.37^{a}	0.21^{a}
Line:				
H	1.46^{x}	0.18^{x}	0.35^{x}	0.15^{x}
L	1.50^{x}	0.14^{y}	0.36^{x}	0.14^{x}

[1] ns: not significant (P>.10);
+: P<.10; *: P<.05; **: P<.01; ***: P<.001

[2] Organ weight / live bodyweight * 100 %.
Least squares means in the same column bearing different superscripts differ significantly (P<.05)

The environment where the chickens were kept after the immunization was of most influence on the weight of these organs, because no differences were found in relative weight between CC and HC; HH and CH; H_fH_f and C_fH_f treatments. Only one exception was found: bursa weight in C_fH_f was as high as in C_fC_f chickens, and different from H_fH_f. All relative organ weights were depressed in chickens kept in hot environments. Most significant suppression was found in the continuous hot environments: HH and CH. Reductions were also found in the fluctuating high temperature environments (H_fH_f and C_fH_f) for

liver, spleen and bursa (only in H_fH_f); although the reductions in spleen and thymus weights were not significant. A significant difference between lines was found only in spleen weight. Significant sex influences were found on spleen and thymus weight.

Discussion

Cloacal temperature

The environmental temperature markedly influenced the cloacal temperature of the chickens, since homeothermia was lost at 35°C. In the environment with fluctuating high temperature, chickens passed daily through homeothermic and hyperthermic conditions. Elevated cloacal temperatures found at 35°C are in agreement with results found by others (Sykes and Fataftah, 1985, 1986; Meltzer, 1987).

No indications were found of differences in cloacal temperatures between the H and L line in the various treatments. Thus, apparently, no difference between the lines in adaptation to the different temperatures is evident.

Growth

Growth was dramatically affected by the high temperature. A main factor in maintaining homeothermia in chickens is regulation of feed intake (Farrell and Swain, 1977; Henken *et al.*, 1983b), with an associated higher feed conversion ratio at high temperatures (Henken *et al.*, 1983b). Although not measured in this experiment, feed intake was obviously decreased at high temperatures. Therefore chickens decreased their feed intake to minimize metabolic heat production in an effort to maintain homeothermia.

In those treatments in which chickens were moved from one environment to another, growth rates rapidly approached those of the chickens that were already present in that environment. Existing differences in body weight between chickens that stayed in the same room and those which were placed there after immunization either did not disappear or did so only very slowly.

Like the effects on cloacal temperature, no line x treatment (or line x sex x treatment) interactions were found for body weight and growth rate. Therefore, the reduction in growth caused by the temperature differences was comparable for H and L line, indicating a comparable level of thermal stress in both lines.

Antibody production

Differences in antibody production between H and L line were as expected. Higher antibody titres in the H line were consistent in these lines from the first generation (Van der Zijpp *et al.*, 1988). This has been demonstrated

under different conditions (Donker *et al.*, submitted: a). Higher titres in females than in males are usual in these lines (unpublished data).

After primary immunization treatment effects were found on antibody titres. Immunosuppression was found in the CH treatment when compared to those in CC or HH treatment. This is consistent with results found by Subba Rao and Glick (1970, 1977) and Thaxton and Siegel (1970, 1972, 1973). This contradicts, however, previously reported results by Donker *et al.* (submitted: a), who could not demonstrate immunosuppression by means of short term acute heat stress in the same selection lines. A crucial difference in the present experiments could be the absence of daily handling, which Donker *et al.* (submitted: a) used to 'socialize' their birds. In those experiments, immunosuppression was only found in the first experiment, carried out with not very intense daily handling. Daily handling might give frequent stimulation of the hypothalamo-pituitary-adrenal axis, which through negative feed-back could reduce the number of free receptors for corticosteroids on lymphocytes and thus decrease susceptibility for stressors (Beuving and Vonder, 1978; Axelrod and Reisine, 1984; Munck *et al.*, 1984).

Birds in the HH treatment had higher antibody titres than those in CC treatment, and thus showed an immuno stimulation. A comparable difference was found by Henken *et al.* (1983a) in commercial chickens. They discussed a possible relation between the metabolic rate above thermoneutral levels and antibody production levels. Immuno-stimulatory effects were also found by Anderson and Kühn (1988) in mice. They found that immunosuppression on antibodies to SRBC antigen by *T. cruzi* in infected mice could be overcome by high environmental temperature, and they also found immuno-stimulation in mice that were not infected. They postulated alterations in T-helper cell populations caused by the high temperature environment.

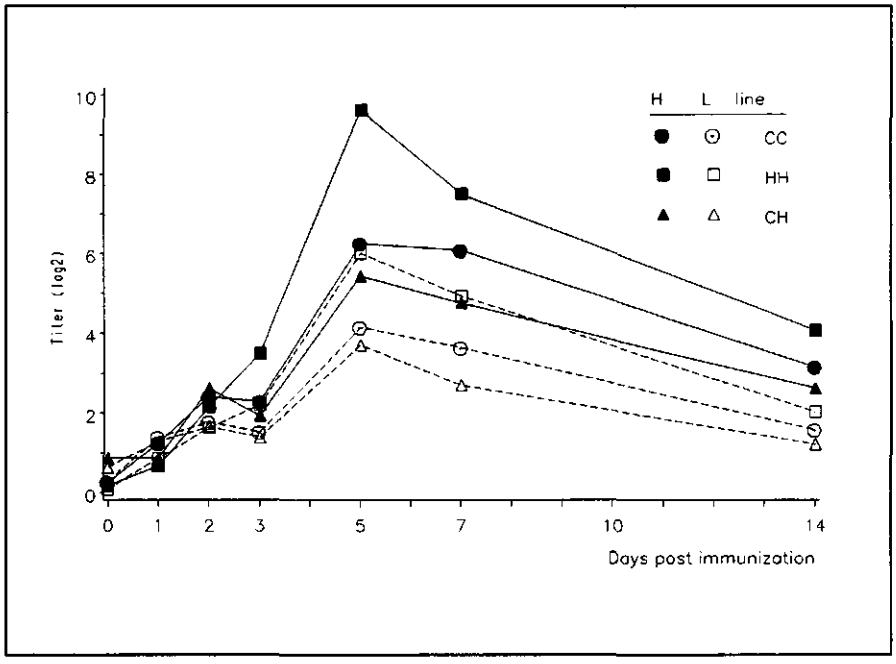
In the treatment groups in fluctuating temperatures only the C_fH_f was different from the other treatments with fluctuating temperatures and from CC. Antibody titres were increased in this group, as in HH. Because of improved acclimation to the high temperature, induced by daily fluctuations before immunization this treatment could possibly adapt to high temperatures as the HH treatment did. Higher antibody production at fluctuating high temperature was also reported by Beard and Mitchell (1987), using Newcastle disease virus. The question remains, however, why this was not found in H_fH_f treatment in this experiment.

The differences between treatments at fluctuating temperatures were smaller than those found at constant temperatures. This could be explained by the less extreme temperatures used, but also by better acclimation of the birds to changes in temperature. Henken *et al.* (1983a) also found less profound influences of fluctuating temperatures on antibody production compared to constant temperatures.

The differences in antibody titres between the treatments after secondary immunization were comparable to those after the primary immunization, but were less pronounced. Higher titres were found only in HH treatment after secondary immunization; all other treatments showed very comparable levels. This indicates adaptation to the environmental temperature at the time of secondary immunization.

An important finding for the practical application of breeding for immune responsiveness was the absence of genotype x treatment interactions in this experiment. For the primary response in the HH, CC and CH treatments this is illustrated in Figure 5. All treatment influences on antibody titres were in the same direction and of the same magnitude in both lines.

Figure 5. Antibody production in CC, HH and CH treatment after primary immunization with SRBC. Least squares means for each line-treatment.



Packed cell volume

No clear relations were found between PCV and treatment or environmental temperature. Differences in the PCV in chickens kept at different temperatures, or which were exchanged between these temperatures were reported previously (Huston, 1965; Deaton *et al.*, 1969; Moye *et al.*, 1969). However,

higher packed cell volumes were found only about two or three weeks after the high temperature was set.

A decreased PCV was found after immunization. No other results are known which show this effect. Only Panigrahy *et al.* (1986) found a comparable decrease in PCV, erythrocyte count and hemoglobin concentration 5 d after an infection with infectious bursal disease. However, the repeated bleeding may have caused reductions in PCV values (Gildersleeve *et al.*, 1985).

Heterophil / lymphocyte ratio

H/L ratios have been used as a measure of stress (Chancellor and Glick, 1960; Gross *et al.*, 1980; Gross and Siegel, 1983). However, no consistent changes in the H/L ratio were found in relation to the treatment or environmental temperature. Either the differences in temperature between the treatments were apparently not of a sufficient magnitude to influence the ratio, or temperature does not act on these ratios in the same way as do other stressors.

On d 2 after immunization a decrease in the ratio was found in all treatments, which is probably related to the immunization with SRBC. Donker *et al.* (submitted: b) found a comparable decrease after immunization with SRBC. Also Trout *et al.* (1988) found comparable changes in the H/L ratio after immunization with *Brucella abortus*. However, the lowest values of the ratio in those experiments was found about 12 - 24 h after the immunization.

The high variation in the H/L ratio on the day before immunization was unexpected, and no sound explanation could be found.

HC treatment

Chickens in the HC treatment showed somewhat different results from the other treatments.

In particular, on d -1 a very high cloacal temperature was found for chickens in this treatment, higher than for the HH treatment. Also on d -1, the heterophil/lymphocyte ratio was considerably higher in the HC group than in any other group. These two observations could be an indication of some unknown stressor.

The reasons for these findings are unknown. However, they might also have influenced antibody production. The antibody titres in the HC treatment group were comparable to HH treatment at 3 and 5 d after immunization, but much lower values were found on 7 and 14 d after immunization, comparable to CC treatment.

Relative organ weight

The differences between treatments in relative organ weights illustrate the detrimental effects of the hot environments. The decreased spleen-, bursa- and thymus- weight in the hot environments were remarkable since these chickens had shown the highest antibody titres. All three organs, but particularly the spleen, play an essential role in the initiation of an immune response. As found in this experiment and earlier work (Donker *et al.*, submitted: c), higher spleen weights were found in H line chickens than in L line chickens, indicating a positive correlation between spleen weight and antibody production. Thus a higher spleen weight might be related to higher antibody producing capacity, as is the case in the difference between H and L line. However, the higher antibody production initiated by the high temperature was not associated with an high spleen weight.

The different treatments in the climate chambers provoked a number of differences in the chickens. Influences on cloacal temperature, growth, lymphoid organ development and antibody titres were observed. No consistent influence was found on packed cell volume and heterophil/lymphocyte ratios. Most important is the observation that all changes induced by the temperature treatments were the same in the H and L selection line, and did not interfere with existing differences between the lines. It is concluded that no genotype x environmental temperature interactions are evident in these selection lines.

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References

Anderson K. and R.E. Kühn, 1988. Elevated environmental temperature overcomes immunosuppression in experimental Chagas' disease. *FASEB Journal* 2: A902. (Abstract)

Axelrod J. and T.D. Reisine, 1984. Stress hormones: their interaction and regulation. *Science* 224: 452-459.

Beard C.W. and B.W. Mitchel, 1987. Influence of environmental temperatures on the serologic responses of broiler chickens to inactivated and viable Newcastle disease vaccines. *Avian Diseases* 31: 321-326.

Beuving G. and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *General and Comparative Endocrinology* 35: 153-159.

Biozzi G., D. Mouton, A.M. Heumann, Y. Bouthillier, C. Stiffel and J.C. Mevel, 1979. Genetic analysis of antibody response to sheep erythrocytes in crosses between lines of mice selected for high or low antibody synthesis. *Immunology* 36: 427-438.

Blecha F. and K.W. Kelley, 1979. Influence of cold and weaning stressors on the humoral immune system of swine. *Abstracts of American Society of Animal Science*, 1979 Meeting. nr 96: pg ?.

Chancellor L. and B. Glick, 1960. Effect of temperature as a stressor on white blood cells, adrenals and bursae of Fabricius of chicks. *American Journal of Physiology* 198: 1346-1348.

Deaton J.W., F.N. Reece and W.J.Tarver, 1969. Hematocrit, hemoglobin and plasma-protein levels of broilers reared under constant temperatures. *Poultry Science* 48: 1993-1996.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, (*submitted: a*). Heat stress in chicken lines selected for high and low immune responsiveness 1. Antibody production.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, (*submitted: b*). Heat stress in chicken lines selected for high and low immune responsiveness 2. Physiological parameters.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, (*submitted: c*). Plaque-forming-cell assay and lymphoid organ development in chicken lines selected for high and low immune responsiveness.

Farrell D.J. and S. Swain, 1977. Effects of temperature treatment on the energy and nitrogen metabolism of fed chickens. *British Poultry Science* 18: 735-748.

Gildersleeve R.P., J.P. Thaxton, M.J. Galvin and D.I. McRee, 1985. Hematological response of immunized and serially bled Japanese quail. *Poultry Science* 64: 1806-1808.

Gross W.B. and H.S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* 27: 972-979.

Gross W.B., P.B. Siegel and R.T DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poultry Science* 59: 516-522.

Henken A.M., A.M.J. Groote Schaarsberg and W. van der Hel, 1983b. The effect of environmental temperature on immune response and metabolism of the young chicken. 3. Effect of environmental temperature on some aspects of energy and protein metabolism. *Poultry Science* 62:59-65.

Henken A.M., A.M.J. Groote Schaarsberg and M.G.B. Nieuwland, 1983a. The effect of environmental temperature on immune response and metabolism of the young chicken. 5. Effect of environmental temperature on the humoral immune response following injection of sheep red blood cells. *Poultry Science* 62:51-58.

Huston T., 1962. The influence of different environmental temperatures on immature fowl. *Poultry Science* 44: 1032-1036.

Kelley K.W., 1985. Immunological consequences of changing environmental stimuli. pg. 193-223 in: G. Moberg (ed.): *Animal stress*. American Physiological Society.

Meltzer A., 1987. Acclimatization to ambient temperature and its nutritional consequences. *World's Poultry Science Journal* 43: 33-44.

Moye R.J., K.W. Washburn and T.M. Huston, 1969. Effects of environmental temperature on erythrocyte numbers and size. *Poultry Science* 48: 1683-1686.

Munck A., P.M. Guyre and N.J. Holbrook, 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine reviews* 5: 25-44.

Panigraphy B., L.D. Rowe and D.E. Corrier, 1986. Haematological values and changes in blood chemistry in chickens with Infectious Bursal disease. *Research in Veterinary Science* 40: 86-88.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC: SAS Institute Inc.

Siegel H.S., 1987. Effects of behavioral and physical stressors on immune responses. pages 39-70 in: Wiepkema P.R. and P.W.M. van Adrichem (eds.): *Biology of stress in farm animals: A integrative approach*. Martinus Nijhoff publishers, Dordrecht, Boston, Lancaster.

Siegel P.B. and W.B. Gross, 1978. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Science* 59: 1-5.

Siegel H.S. and J.W. Latimer, 1984. Interaction of high temperature and *Salmonella pullorum* antigen concentrations on serum agglutinin and corticosteroid response in white rock chickens. *Poultry Science* 63: 2483-2491.

Subba Rao D.S.V. and B. Glick, 1970. Immunosuppressive action of heat in chickens. *Proceeding of the Society for Experimental Biology and Medicine* 133: 445-448.

Subba Rao D.S.V. and B. Glick, 1977. Effect of cold exposure on the immune response of chickens. *Poultry Science* 56: 992-996.

Sykes A.H. and A.R.A. Fataftah, 1985. Effect of a change in environmental temperature on heat tolerance in laying fowl. *British Poultry Science* 27: 307-316.

Sykes A.H. and A.R.A. Fataftah, 1986. Acclimatization of the fowl to intermittent acute heat stress. *British Poultry Science* 27: 289-300.

Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. Poultry Science 49: 202-205.

Thaxton P. and H.S. Siegel, 1972. Depression of secondary immunity by high environmental temperature. *Poultry Science* 51: 1519-1526.

Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Science* 52: 618-624.

Trout J.M., M.M. Mashaly, H.S. Siegel, 1988. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Developmental and Comparative Immunology* 12: 331-346.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poultry Science* 59: 1363-1369.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterization of lines selected for high and low antibody production. *Proceedings of the 7th European Poultry Conference (Paris)* : 211-215.

Zijpp A.J. van der, J.J. Blankert, E. Egberts and M.G.J. Tilanus, 1988. Advances in genetic disease resistance in poultry. pg. 131-138 in: S. Korver, H.A.M. van der Steen, J.A.M. van Arendonk, H. Bakker, E.W. Brascamp and J. Dommerholt (compilers). *Advances in animal breeding*. Pudoc, Wageningen, The Netherlands.

Chapter IV

Plaque-forming-cell assay and lymphoid organ development in chicken lines selected for high and low immune responsiveness.

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Abstract

In two experiments, the number of direct and indirect plaque-forming-cells in spleens of two chicken lines, selected for humoral immune response to sheep erythrocytes (SRBC) was studied. Chickens from the fifth and sixth generation were used. After immunization with SRBC, the high antibody production line showed significantly higher numbers of direct plaque-forming-cells in the spleen. Additionally, relative spleen weight was higher in the high antibody line. Thus, higher antibody titers in the high response line partly can be explained by the occurrence of higher numbers of immunocompetent cells. Differences were consistent after both intravenous and intramuscular immunization, and after both primary and secondary immunization.

In a third experiment, chickens from the sixth generation were dissected at 1, 7, 15, 22, 29, 37 and 50 days of age, without prior immunization. Higher relative spleen weights were found in high line chickens in this experiment. Slightly lower relative weight of bursa of Fabricius was found in high line chickens. No significant differences in live weight and relative thymus weight were found. Thus the higher spleen weight and probably higher antibody production capacity in high line chickens is already initiated during the ontogeny, before immunization. Lower bursa weight apparently does not impair immune function.

Keywords: selection lines, plaque-forming-cells, organ development, SRBC

Introduction

During the mounting of an immune response, a complex mechanism is triggered, in which macrophages, T- and B-cells cooperate. B-cells, which originate in the primary lymphoid organs, differentiate into plasma cells after being primed with antigen and eventually release antibodies. These processes are regulated by interleukins released by macrophages and various subsets of lymphocytes. After immunological stimulation, (potential) plasma cells and macrophages, which bind antigen particles, migrate into secondary lymphoid organs, such as the spleen, to intensify contact between plasma cells and antigen (Roitt *et al.*, 1985).

Selection for humoral immune responsiveness can result in selection lines that differ in antibody production (Biozzi *et al.*, 1979; Siegel and Gross,

1978; Van der Zijpp and Nieuwland, 1986). Such differences could be caused by alterations in several phases of this process. Biozzi *et al.* (1979) described modifications in numbers and activity in populations of macrophages as well as lymphocytes in mice selected for various antigens.

Siegel *et al.* (1982) and Ubosi *et al.* (1985) found that bodyweight and organ development were influenced in chickens, after selection for immune responsiveness. Landreth and Glick (1973) and Yamamoto and Glick (1982) found differences in antibody production in chicken lines which were selected for high or low bursal weight.

To study the changes in the selection lines used in our laboratory (Van der Zijpp and Nieuwland, 1986), occurring as a result of the selection for antibody production to sheep red blood cells (SRBC) a series of experiments were carried out. In combination with antibody titers, the number of plaque-forming-cells in the spleen was measured, in order to estimate the potential number of antibody producing cells. Route of antigen administration, intravenous (i.v.) and intramuscular (i.m.), and age influence were included in the experiments. The ontogeny of lymphoid organ development was studied without immunization in young chickens of various ages.

Material and methods

Three experiments were conducted, using chickens from lines selected for high (H) and low (L) antibody titer five days after a primary i.m. immunization with 1 ml 25% (v/v) sheep red blood cells (SRBC) in phosphate buffered saline (PBS) at 37 days of age (Van der Zijpp and Nieuwland, 1986).

Chicks used in the experiments were vaccinated against Marek's disease and, sexes separately, housed on wire brooder cages. Chicks were provided with starter feed and water *ad libitum*. Vaccination against infectious bronchitis was given at one day of age, against Gumboro's disease at 15 days and Newcastle disease at 21 days of age.

Experiment 1 (route influence)

A total of 143 chickens, genetically identical to the fifth generation of the H and L selection lines, was used. Chickens were immunized with SRBC at 52 days of age. Immunization was given i.m. for half the chickens (1 ml 25% SRBC in PBS) and i.v. (.5 ml 5% SRBC) for the other half. Three, 4, 5 and 6 days after immunization, 16 chicks were weighed, killed by decapitation, blood was collected for antibody titer assay, their spleens removed, weighed and direct and indirect plaque tests were performed on spleen cells. Twenty-nine days after primary immunization, the remaining chicks were re-immunized via the same route as at primary immunization. SRBC-dose was doubled, compared to

primary dose. On 2, 3 ,4 ,5 and 6 days after immunization chicks were weighed, sacrificed and the same procedure was followed as after primary immunization. Experimental design was a full factorial design: 2 lines x 2 sexes x 2 immunization routes x 9 sampling days x 2 replicates. However, because some pullets were incorrectly sexed as cockerels, and because one bird died, this was not completely achieved.

Experiment 2 (age influence)

A total of 216 chickens, genetically identical to the sixth generation of the H and L selection lines, was used. These chickens were involved in an experiment to investigate the influence of heat-stress on antibody production. Details of this experiment are described elsewhere (Donker *et al.*, submitted-a, -b). To habituate chickens to handling, they were handled daily from 16 days of age, until the end of the experiment. To study age differences, chickens were separated into three groups, which were treated alike, but at different ages. Chickens were immunized 24 hours after a heat stress treatment (four 30 min. periods at 42°C with 30 min. intervals) or a control treatment (identical treatment but at 22°C). Immunization was performed with .5 ml 5% SRBC in PBS. Immunizations were given at ages of 33 days (group I), 36 days (group II) and 43 days (group III), respectively. Three, 4 and 5 days after immunization a number of chickens were weighed and sacrificed, blood was collected for antibody titer measurements, spleen and bursa were removed and weighed, and plaque tests were performed on spleen cells. The experimental design was factorial: 3 age-groups x 2 lines x 2 sexes x 2 stress-treatments x 3 days x 3 replicates.

Experiment 3 (ontogeny of organ weight)

A total of 140 chickens, from the same hatch as those in Experiment 2, was used for this experiment. Housing and husbandry were as described for Experiment 2, except that these chickens were not specifically handled daily. On the day after hatching and at 7, 15, 22, 29, 37 and 50 days of age, 5 chicks from each line and sex were weighed and sacrificed. The bursa of Fabricius, thymus, spleen and liver were removed and weighed.

Assays:

Hemagglutinin

Blood samples were collected in a tube containing a drop of a heparin solution. Samples were centrifuged to harvest plasma and stored at -20°C until assayed. Total (TO) and 2-Mercapto ethanol (2ME-) resistant antibody titers were assayed by hemagglutinin assay (Van der Zijpp and Leenstra, 1980). Titers were expressed as $\log_2$ of the highest dilution giving total agglutination.

Plaque test

Hemolytic plaque tests were performed in an assay comparable to that as described by McCorkle and Leslie (1983). After decapitation of the chickens the spleen was quickly excised from the animal and stored in a complete cell medium (RPMI, Flow, Irvine, Scotland, U.K.) which was kept on ice. The spleen was squashed and gently pressed through a nylon mesh to separate cells. Cells were washed twice, centrifuged (7 min., 0°C, 400 g) and resuspended in fresh RPMI solution. The percentage of live and dead cells was estimated by a count with trypan-blue. A dilution of the cells was made so that the number of plaques to be counted in the final plaque slides was expected to be between 50 and 200. For the assay of direct plaque-forming-cells (DPFC, 19S-antibody producing cells), 100 μl cell suspension was mixed with 100 μl SRBC suspension (6 * 10^8 cells/ml) and 25 μl of a pooled serum from unrelated cocks as complement source. Previously this serum had been absorbed in the cold with SRBC to remove all possible agglutinating or lysing antibodies. For the assay of indirect plaque-forming-cells (IPFC, 7S-antibody producing cells) 100 μl cell suspension was mixed with 100 μl SRBC suspension (6 * 10^8 cells/ml), 20 μl RaChIgG solution (Rabbit anti chicken-IgG, heavy and light chain specific, Cappel, USA) and 20 μl Guinea-pig complement solution (Flow, Germany). To obtain a monolayer of cells the suspension was brought in a double slide chamber by capillary action. The volume of each sample was such that three of these chambers were required. Chambers were sealed with paraffin and incubated at 41°C for at least 75 min (DPFC) or 90 min (IPFC) to allow formation of the plaques. A total count of all plaques present in the three chambers was made under a preparation microscope. Plaque numbers were ^{e}log transformed to obtain a distribution of values which resembled a normal distribution.

Statistical analysis

Analysis of variance of data was performed using the SAS-package (SAS, 1985). Full factorial designs were evaluated, with two and three way interactions among factors. Non-significant ($P > .05$) interactions and factors (with the exception of line effect) were removed from the models.

Experiment 1

A statistical model including line, sex, route and sampling day was applied:

$$\underline{Y}_{ijklm} = \mu + L_i + S_j + R_k + D_l \text{ (+ interactions)} + \underline{e}_{ijklm} \quad (1)$$

in which:

$\underline{Y}_{ijklm}$ = characteristic analyzed: antibody titer, DPFC, IPFC, relative spleen weight

μ = overall mean

L_i = line effect (i = H, L)

S_j = sex effect (j = male, female)

R_k = route of immunization effect (k = i.m., i.v.)

D_l = day of sampling (l = 3, 4, 5, 6, 2_s, 3_s, 4_s, 5_s, 6_s)[1)]

$\underline{e}_{ijklm}$ = random error

Experiment 2

A statistical model including line, sex, group, treatment and sampling day was applied:

$$\underline{Y}_{ijklmn} = \mu + L_i + S_j + G_k + D_l + Tm \text{ (+ interactions)} + \underline{e}_{ijklmn} \quad (2)$$

in which:

$\underline{Y}_{ijklmn}$ = characteristic analyzed: antibody titer, DPFC, IPFC, relative spleen and bursa weight

G_k = group effect (k = I, II, III)

D_l = day of sampling effect (l = 3, 4, 5)

T_m = treatment effect (m = heat stress, control treatment)

and μ, L_i, S_j, $\underline{e}_{ijklmn}$ as mentioned in model (1).

Experiment 3

Weight data in Experiment 3 were analyzed in a model including line, sex and age at dissection:

$$\underline{Y}_{ijkl} = \mu + L_i + S_j + A_k \text{ (+ interactions)} + \underline{e}_{ijkl} \quad (3)$$

in which:

$\underline{Y}_{ijk}$ = characteristic analyzed: Body weight, relative spleen, bursa and thymus weight

A_k = age at dissection (k = 1, 7, 15, 22, 29, 37, 50 days)

and μ, L_i, S_j as mentioned in model (1)

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1) Days are relative to primary immunization; days with subscript $_s$ are relative to secondary immunization.

Results

Antibody production

Total and 2ME-resistant antibody titers were consistently higher in H line than in L line (Tables 1, 2; Fig. 1a, 2a, 3a). This was found both after i.m. and i.v. immunization (Fig. 1a, 2a, 3a); and for primary and secondary response (Fig. 1a, 2a). The significant interactions (Tables 1, 2) demonstrated differences in magnitude between days, or route (Fig. 1a, 2a), rather than differences in ranking between the two lines. Titers mounted after i.v. immunization were higher than those after i.m. immunization (Fig. 1a, 2a; Table 1). Secondary responses were characterized by faster increase in titers, and in most cases higher peak values (Fig. 1a, 2a). With increasing age (or group number) antibody titers increased (Fig. 3a; Table 2).

Table 1. Significance[1)] of effects in statistical analysis. Experiment 1.

Factor[2)]:	L_i	S_j	R_k	D_l	LS_{ij}	LR_{ik}	LD_{il}	SR_{jk}	SD_{jl}	RD_{kl}
Antibody titer (total)	***	+	***	***	.	.	.	.	**	*
Antibody titer (2ME resistant)	ns	**	***	***	+	.	.	.	+	**
DPFC (^{e}log direct plaques)	***	ns	***	***	.	+	**	.	***	***
IPFC (^{e}log indirect plaques)	ns	.	***	***	.	.	.	.	.	**
Spleen weight (relative %)	***	***	*	***	.	*	.	.	.	.

1) .: not in model; ns: non significant (P>.10); +: P<.10; *: P<.05; **: P<.01; ***: P<.001

2) L_i = line effect (i = H, L)
S_j = sex effect (j = male, female)
R_k = route of immunization effect (k = i.m., i.v.)
D_l = day of sampling (l = 3, 4, 5, 6, 31, 32, 33, 34, 35)
LS_{ij} = interaction between line and sex; etc.

Plaque-forming-cells

The number of plaque-forming-cells found in the spleen was higher after i.v. immunization than after i.m. immunization (Figure 1b,c, 2b,c; Table 1). Indirect plaques were very low in number after i.m. immunization, especially

Figure 1. Responses after intravenous immunization in Experiment 1. Least squares means ± s.e.m.

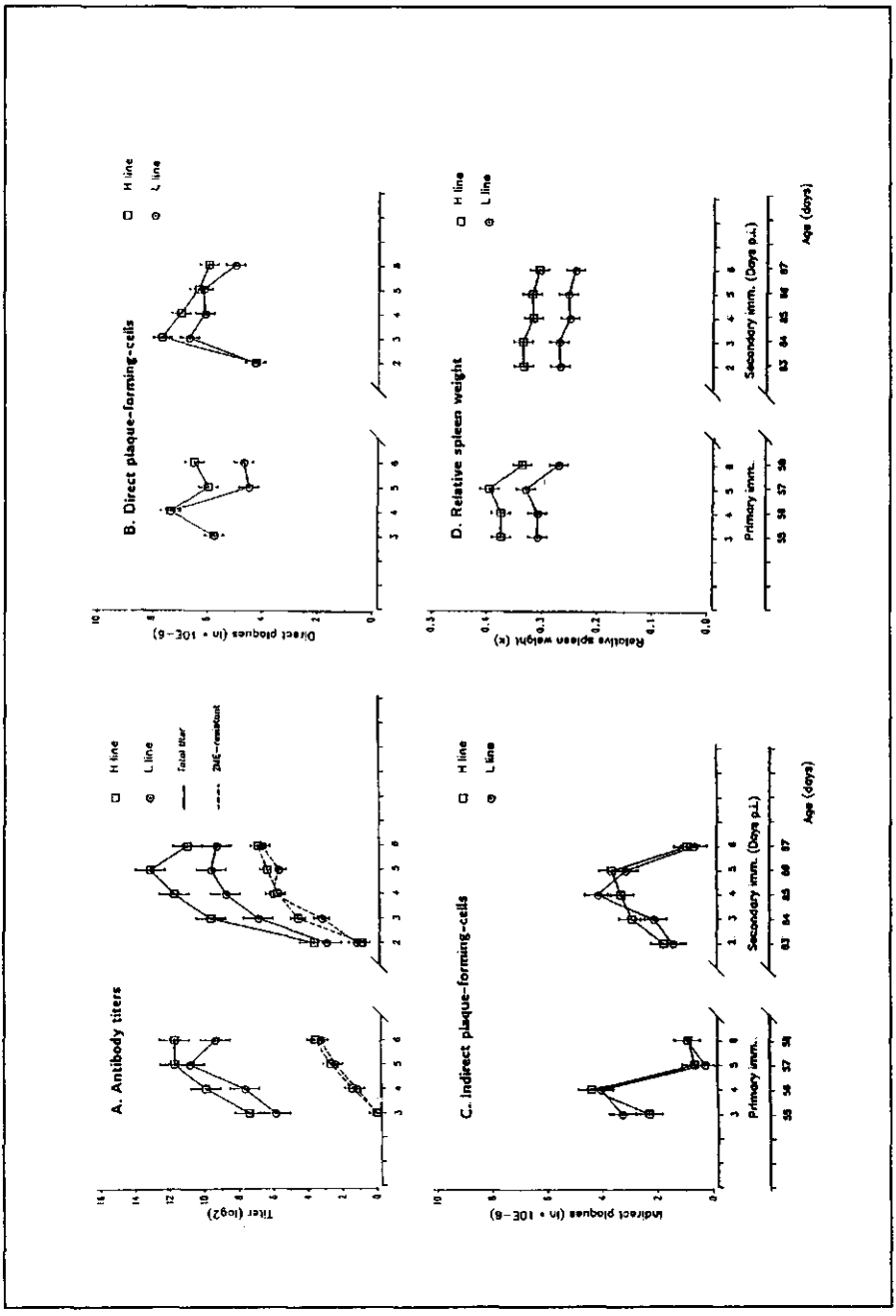
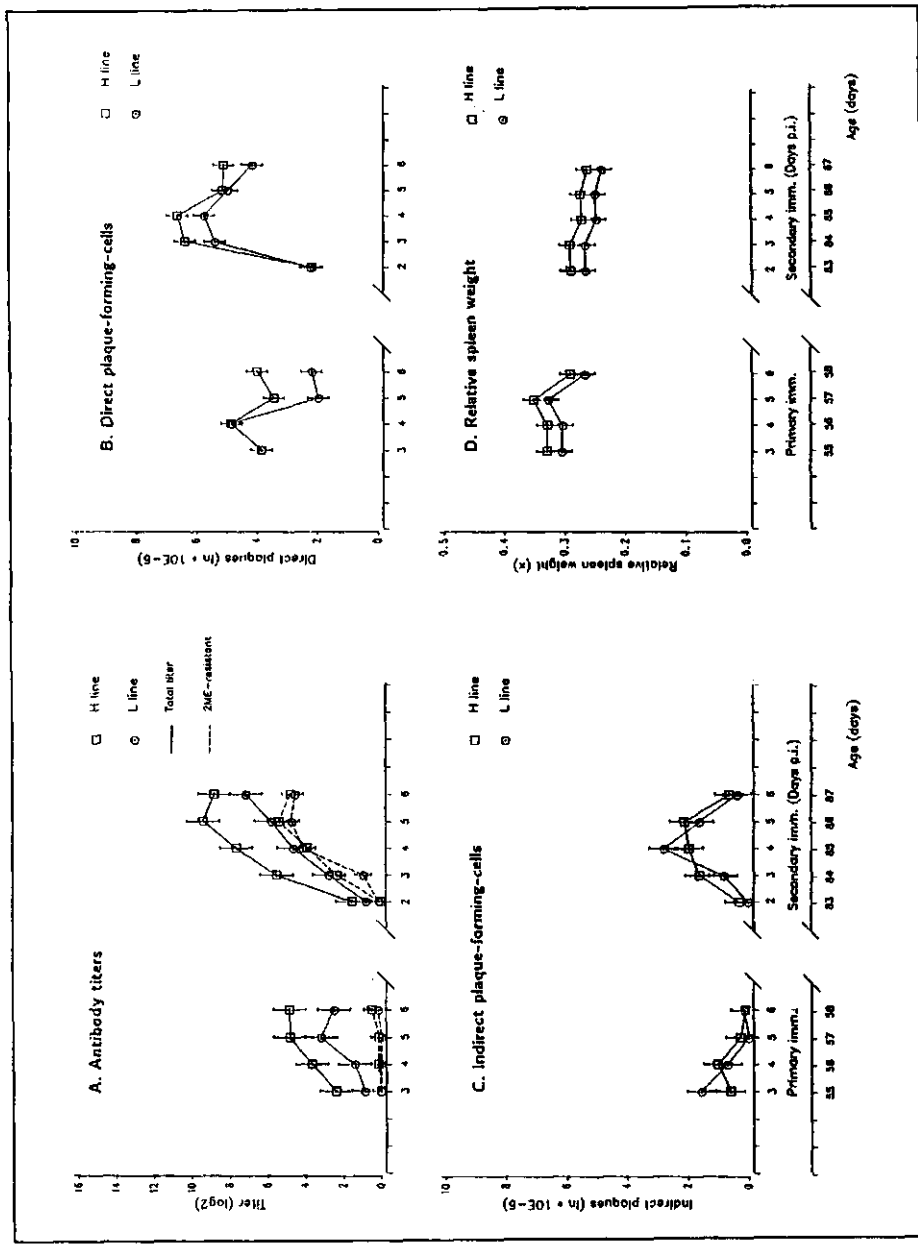


Figure 2. Responses after intramuscular immunization in Experiment 1. Least squares means ± s.e.m.



during the primary response (Fig. 2c). In both experiments significant differences between H and L line were found in the number of direct plaque-forming-cells (Table 1, 2). Higher numbers of plaques were found in the H line (Fig. 1b, 2b). Significant interactions encountered (Table 1, 2) clearly indicated different kinetics between the immunization routes (Fig. 1b, 2b) and age groups (Fig. 3b). In Experiment 1 and 2, no consistent difference in indirect plaques was found between lines, but in Experiment 2, a significant day-line and group-day interaction was found. In all three age groups, the number of indirect plaques on day 4 was highest in the H line (Fig. 3c).

Table 2. Significance[1)] of effects in statistical analysis. Experiment 2.

Factor[2)]:	L_i	S_j	G_k	D_l	LS_{ij}	LG_{ik}	LD_{il}	SG_{jk}	SD_{jl}	GD_{kl}
Antibody titer (total)	***	ns	***	***	.	.	**	.	.	.
Antibody titer (2ME resistant)	***	.	***	***	.	+	***	.	.	***
DPFC (°log direct plaques)	***	.	***	***	.	.	*	.	.	**
IPFC (°log indirect plaques)	ns	.	***	***	.	.	*	.	.	***
Spleen weight (relative %)	***	***	ns	***	*	*	.	.	.	.
Bursa weight (relative %)	ns	+	.	.	.	.	.	.	.	.

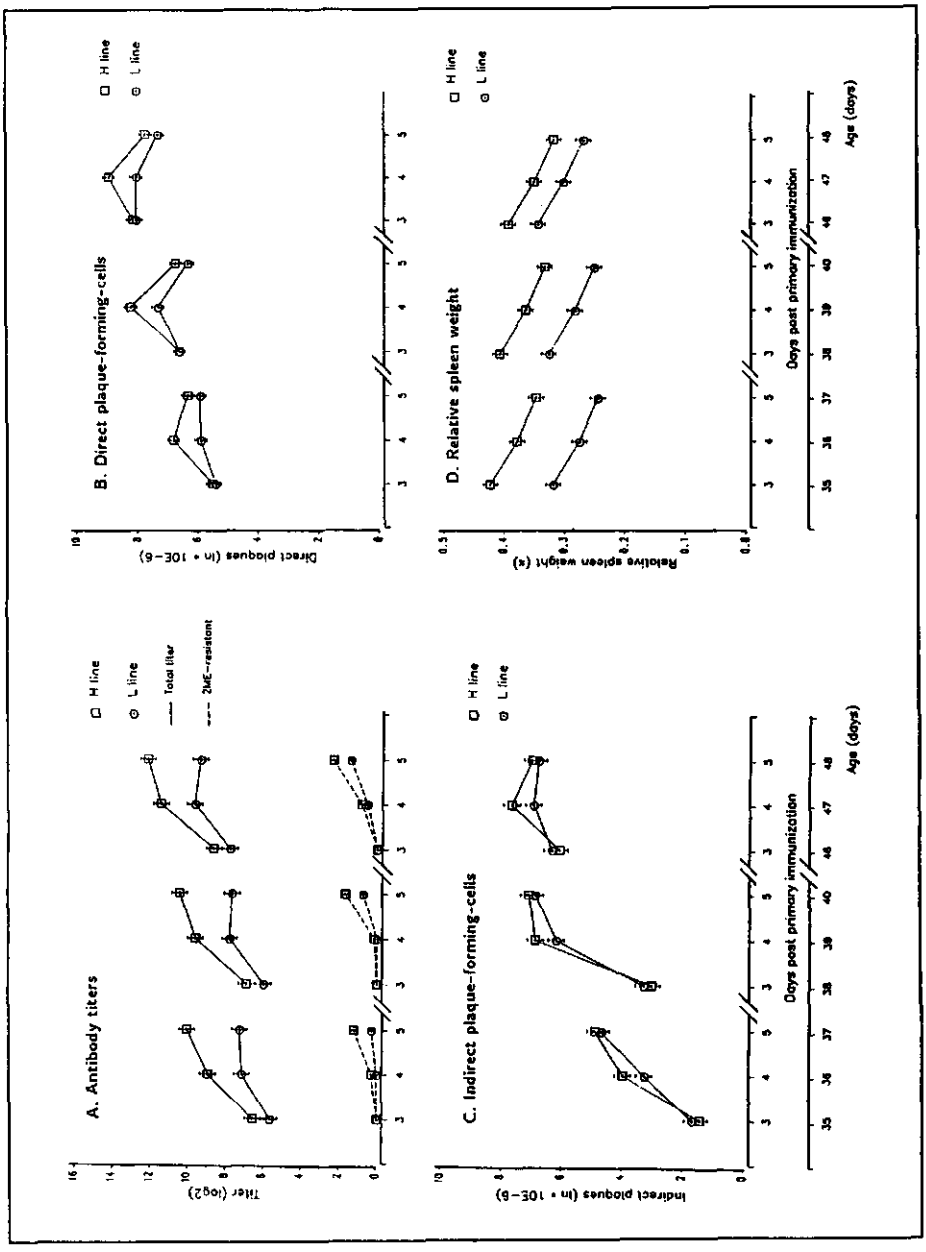
1) .: not in model; ns: non significant (P>.10); +: P<.10; *: P<.05; **: P<.01; ***: P<.001

2) L_i = line effect (i = H, L)
S_j = sex effect (j = male, female)
G_k = group effect (k = I, II, III)
D_l = day of sampling (l = 3, 4, 5, 6, 31, 32, 33, 34, 35)
LS_{ij} = interaction between line and sex; etc.
N.B. Treatment effect (heat stress, control) and interactions were never significant

Spleen weight after immunization

After immunization the spleen quickly increased in size (compare spleen size at the same age: Experiment 2, group II, day 3 (Fig. 3d): with immunization and Experiment 3, day 37 (Fig. 4d): without immunization). Later during the immune response relative spleen weight decreased again (Fig. 1d, 2d, 3d). Spleen weight after i.v. was higher than after i.m. immunization

Figure 3. Responses after intravenous immunization in Experiment 2. Least squares means ± s.e.m.



(Table 1; Fig. 1d, 2d). H line had higher spleen weight than L line, (Tables 1, 2; Fig. 1d, 2d). This difference was bigger after i.v. immunization (Table 1; Fig. 2d) and decreased with age (Table 2; Fig. 3d).

Growth and lymphoid organ development

In Experiment 3, the difference in body weight, and thus growth, between the two non-immunized selection lines was not significant (Table 3, Fig. 4a). Relative spleen weight was consistently higher in the H line (Table 3, Fig. 4b). Relative bursa weight was higher in the L line, but this varied with age (Table 3, Fig. 4c). Differences in relative thymus weight, when present, were not consistent over ages (Table 3, Fig. 4d). No differences were found in relative liver weight between the H and L line, but the weight decreased with age (Table 3). Relative liver weight estimates (g / 100 g body weight) were (age): 3.00 (22 d), 3.11 (29 d), 2.79 (37 d), 2.32 (50 d); s.e.m. = ±.08.

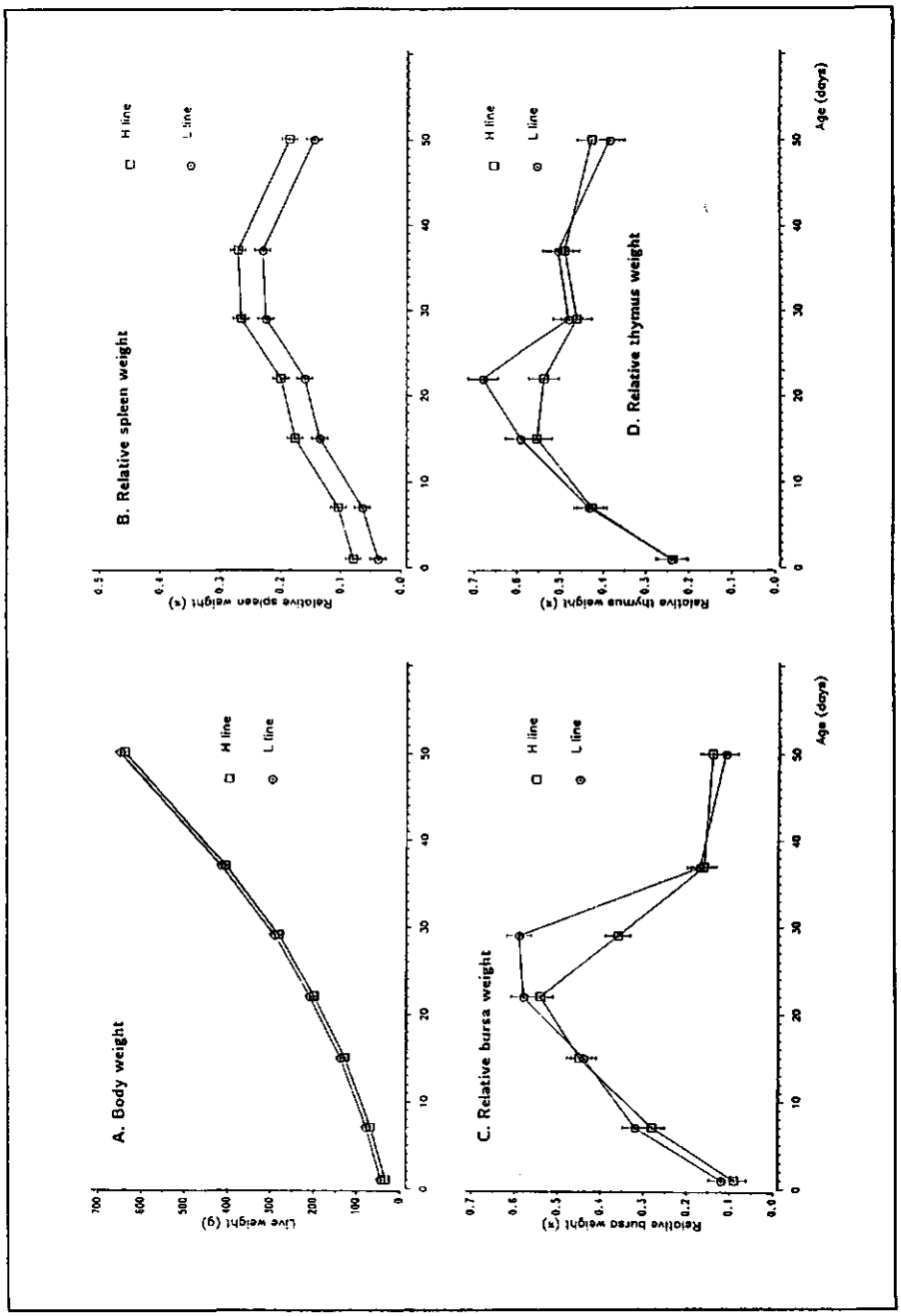
Table 3. Significance[1)] of effects in statistical analysis. Experiment 3.

Factor[2)]:	L_i	S_j	A_k	LS_{ij}	LA_{ik}	SA_{jk}	LSA_{ijk}
Body weight	ns	***	***	.	.	*	.
Spleen weight (relative %)	***	ns	***	*	.	.	.
Bursa weight (relative %)	**	*	***	.	**	.	.
Thymus weight (relative %)	ns	*	***	.	.	*	*
Liver weight (relative %)	ns	**	***	.	.	.	.

1) .: not in model; ns: non significant (P>.10); +: P<.10; *: P<.05; **: P<.01; ***: P<.001

2) L_i = line effect (i = H, L)
S_j = sex effect (j = male, female)
A_k = age effect at dissection (k = 1, 7, 15, 22, 29, 37, 50 d)
LS_{ij} = interaction between line and sex; etc.

Figure 4. Growth and lymphoid organ development of chicks in Experiment 3. Least squares means ± s.e.m.



Discussion

In the two experiments involving immunizations, H line chickens produced consistently more antibodies to SRBC than L line chickens. This is in accordance with our other observations in these lines (Van der Zijpp and Nieuwland, 1986; Donker *et al.*, submitted-a; Donker *et al.*, unpublished data). Biozzi *et al.* (1979) also reported higher antibody production to a number of antigens after selection on antibody production. They also reported differences in kinetics of antibody titers; detectable titers were found at an earlier time after immunization, and persistence was higher in the high selection line.

As well as higher antibody titers, higher numbers of plaque-forming-cells were found. Intravenous immunization resulted in higher titers and a higher number of direct and indirect plaques in the spleen. The H line chickens had relative higher numbers of direct plaque-forming-cells in their spleen than did L line chickens. Indirect plaque-forming-cells showed the same tendency, but the difference between the two selection lines was not significant.

In all three experiments, with or without immunization, relatively higher spleen weights were found in H line chickens. Combined with an increased number of plaque-forming-cells (expressed per 10^6 spleen cells), this means that total number of immunocompetent cells in the spleen is probably much higher in the H line than in the L line. Thus, at least a part of the difference in antibody production between H and L line can be subscribed to different numbers of cells with antibody producing capacity in the spleen.

After immunization, the spleen quickly increased in size. This is probably due to the 'homing' and proliferation of a large number of lymphocytes to this organ during the initiation of the immune response. Later during the immune response, when most antigen is neutralized, these cells enter the peripheral blood again, which explains the decrease in spleen size found later after immunization. In Experiment 1, a relatively low level of plaques was found on day 5, in all experimental groups and in direct and indirect plaques. Day-to-day variation in the assay could account for this, since plaque tests are sensitive to variation in, for example, temperature (Bhogal *et al.*, 1984). However, a marked increase in spleen weight was noted on the same day (Fig. 1d), which was not found either during the secondary response, or in Experiment 2. No obvious factor was known which could have caused these effects.

Average body weight in Experiment 3 was higher in the H line than in the L line, but the difference was not significant. This contrasts with the differences usually found in these lines. Weight recording of the selection lines at 30 days of age revealed higher weights in L line over a number of

generations (Van der Zijpp and Nieuwland, unpublished results). The same relation was reported by Siegel *et al.* (1982) and Ubosi *et al.* (1985) in their selection lines for high and low antibody production after i.v. immunization.

Higher spleen weights in H line were found both with and without immunization, independent of age. Ubosi *et al.* (1985) also reported higher spleen weight in their high selection line. These differences suggest a direct relation between antibody production capacity and spleen weight, already before antigenic stimulation.

Ubosi *et al.* (1985) also reported an increased thymus weight in the high antibody line, which was not found in this study. The thymus is essential for the production of T lymphocytes. The antibody response to SRBC is dependent of T and B lymphocytes. A small difference in bursa weight, which was higher in the L line, was also reported by them. These observations are in line with those made by Landreth and Glick (1973) and Yamamoto and Glick (1982), who observed decreased production of antibodies to SRBC in a line selected for large bursa size compared to the small bursa line. Although the presence of the bursa is essential for development of normal reactivity to antigen, no positive relation with bursa size is apparent. The presence of active bursa follicles is of more importance than bursa size (Ubosi *et al.*, 1985). As they postulated, the number of follicles might be negatively correlated to bursa size.

In conclusion it can be stated that higher antibody production is associated with higher numbers of plaque-forming-cells in the spleen. The difference between lines differing in antibody production capacity can partly be explained by this difference, in combination with higher spleen weight during the ontogeny before antigenic challenge. Thymus and bursa development are not influenced, or only modestly during the ontogeny. T and B cell function are not impaired, even when bursa weight is somewhat reduced, as found in the high selection line.

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References

Bhogal B.S., D.S. Chi and G.J. Thorbecke, 1984. Effect of temperature on suppressor cells in chicken spleen cell cultures. *Proc. Soc. Exp. Biol. Med.* 176: 414-418.

Biozzi G., D. Mouton, O.A. Sant'Anna, H.C. Passos, M. Gennari, M.H. Reis, K.C.A. Ferreira, A.M. Heumann, Y. Bouthillier, O.M. Ibanez, C. Stiffel and M. Siqueira, 1979. Genetics of immunoresponsiveness to natural antigens in the mouse. *Current Topics in Microbiology and Immunology* 85: 31-98.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp (*submitted-a*). Heat stress in chicken lines selected for high and low immune responsiveness. 1. Antibody production.

Donker R.A., M.G.B. Nieuwland, A.J. van der Zijpp and C.A. Makkink (*submitted-b*). Heat stress in chicken lines selected for high and low immune responsiveness 2. Physiological parameters.

Landreth K.S. and B. Glick, 1973. Differential effect of bursectomy on antibodyproduction in a large and small bursa line of New Hampshires. *Proc. Soc. Exp. Biol. Med.* 144: 501-505.

McCorkle F.M. and G.A. Leslie, 1983. IgM - plaque - forming cells in the peripheral blood of chickens. *Dev. Comp. Immunol.* 7: 391-396.

Roitt I.M., J. Brostoff and D.K. Male, 1985. *Immunology*. Gower Medical Publishing, London, New York.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC: SAS Institute Inc.

Siegel P.B. and W.B. Gross, 1978. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Sci.* 59: 1-5.

Siegel P.B., W.B. Gross and J.A. Cherry, 1982. Correlated responses of chickens to selection for production of antibodies to sheep erythrocytes. *Anim. Blood Gr. Biochem. Gen.* 13: 291-297.

Ubosi C.O., W.B. Gross and P.B. Siegel, 1985. Divergent selection of chickens for antibody production to sheep erythrocytes: Age effect in parental lines and their crosses. *Avian Dis.* 29: 150-158.

Yamamoto Y. and B. Glick, 1982. A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of Fabricius. *Poult. Sci.* 61: 2129-2132.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterization of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris)* : 211-215.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poult. Sci.* 59: 1363-1369.

Chapter V

The effect of corticosterone infusion on plasma corticosterone concentration, antibody production, circulating leukocytes and growth in chicken lines selected for humoral immune responsiveness.

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The effect of corticosterone infusion on plasma corticosterone concentration, antibody production, circulating leukocytes and growth in chicken lines selected for humoral immune responsiveness.

Abstract

1. The effect of corticosterone on antibody production in chicken lines selected for humoral immuneresponse was studied.
2. Twelve cockerels (33 days old) from lines selected for high and for low antibody response after immunization with sheep red blood cells (SRBC) were implanted with mini-infusion pumps delivering corticosterone or vehicle continuously for 14 d.
3. Three days after implantation, the chickens were immunized intramuscularly with 0.25 ml packed SRBC. Blood samples were taken before implantation, before immunization and 3, 5, 7 and 11 days after immunization.
4. Corticosterone infusion induced higher plasma corticosterone concentrations ($P<.001$) and heterophil/lymphocyte ratios ($P<.05$) than infusion of vehicle only. Growth was considerably depressed ($P<.01$) and relative weights of the thymus, bursa of Fabricius and spleen were less ($P<.001$) in the corticosterone infused chickens.
5. An effect of corticosterone on antibody production could not be demonstrated, and differences between selection lines were unaffected.

Introduction

The avian hypothalamo-pituitary-adrenal axis can be activated by either external or internal stimuli and result in increased concentrations of corticosterone in the plasma, as well as in the cytoplasm and nucleus of thymocytes and the lymphoid cells of the bursa of Fabricius, thymus and spleen (Siegel and Gould, 1982; Siegel, 1987). Immunosuppression has been reported after stress or ACTH injections (Thaxton *et al.*, 1968; Subba Rao and Glick, 1970; Thaxton and Siegel, 1970, 1973; Siegel, 1987) and shown to be largely mediated by corticosterone. Injections with cortisone acetate or corticosterone before immunization with sheep red blood cells (SRBC) reduced antibody titres (Sato and Glick, 1970). Corticosterone fed to chickens depressed antibody titres to SRBC in inverse relation to the dose (Gross *et al.*, 1980). Also implantation of pellets containing corticosterone caused a dose-related decrease in antibody titres to SRBC and *Brucella abortus* (Davison and Misson, 1987). Continuous infusion of corticosterone has been reported to

increase the incidence of Marek's disease after challenge (Powell and Davison, 1986).

In this study the effects of corticosterone on antibody titres to SRBC were investigated in chicken lines, selected for high and low antibody titres to SRBC. Application of breeding for immune responsiveness could be of restricted value if genotype-environment interactions are manifest. These lines were used to determine if differences in susceptibility to stress are related to selection for antibody production.

Corticosterone was infused continuously for 14 d by osmotic mini-infusion pumps and the effects on plasma corticosterone and the humoral immune response to SRBC were measured. The effect of corticosterone infusion on the heterophil/lymphocyte ratio was measured and used as an index of stress (Selye, 1976; Gross and Siegel, 1983; Siegel, 1987).

Material & methods

Chickens and pre-experimental conditions

A total of twelve cockerels from the sixth generation of two lines (medium heavy layers) selected for high or low antibody titres after immunization with SRBC (Van der Zijpp and Nieuwland, 1986) were used.

They were kept in wire cages and provided with commercial laying starter feed and water *ad libitum*. Each cockerel was vaccinated against Marek's disease on day of hatching; against infectious bronchitis at 2 d of age, Gumboro's disease at 12 d of age and Newcastle disease (LaSota strain) at 19 d of age. From 26 d of age, all cockerels were handled daily to familiarise them with the blood sampling procedure (Gross and Siegel, 1982; Gross, 1986). Individual weights were recorded at 30 d of age.

Experimental procedure

At 33 d of age the cockerels were bled and anaesthetized with ether. Two infusion pumps (Alzet osmotic minipumps, model 2002; Alza Corp., Palo Alto, CA, USA) were implanted subcutaneously in the neck contralateral to the crop in each chicken. Each implanted minipump delivered a continuous flow of 0.60 ± .03 µl/h polyethyleneglycol for 14 days (PEG, polyethyleneglycol-400, Merck, Darmstadt, Federal Republic of Germany). The experimental group received PEG containing corticosterone (Sigma, St Louis, MO, USA) at an rate of 8.48 µg corticosterone/h. Mini-infusion pumps filled with PEG only were implanted in a control group of chickens. The number of chickens in each (line, treatment) was: (high line, corticosterone): 3, (high line, PEG): 2, (low line, corticosterone): 4, (low line, PEG): 3.

Three days after implantation, the cockerels were bled again and immunized intramuscularly with 0.25 ml packed SRBC in 1 ml phosphate buffered saline (PBS). Hereafter day of immunization is referred to as day 0. The cockerels were bled again after 3, 5, 7 and 11 d.

The chickens were weighed, killed by decapitation on day 11 and the thymus, spleen, liver and bursa of Fabricius were excised and weighed.

Blood sampling

Blood samples (1.5 - 2.0 ml) were obtained from the ulnar vein using heparinized syringes. Samples were taken between 9.00 and 10.00 h, immediately after the chicken had been removed from its cage. The entire procedure did not take longer than 45-60 seconds since prolonged handling can cause increased circulating corticosterone (Beuving and Vonder, 1978).

Assays

Heterophil/lymphocyte count

A smear was prepared from each blood sample and stained with May-Grünwald-Giemsa stain. The heterophil/lymphocyte ratio was estimated by counting a total of 200 leukocytes in each smear. Blood samples were centrifuged and plasma stored at -20°C until assay.

Corticosterone

Plasma concentrations of corticosterone were determined by a radioimmunoassay (Beuving and Vonder, 1981). Specificity of the antiserum (estimated on plasma of laying hens) for corticosterone was high: cross-reactivity with cortisone, cortisol, 21-deoxycortisol, 11-deoxycortisol, testosterone and 18-hydroxycorticosterone was less than 5%. Cross-reactivity with 11-deoxycorticosterone and progesterone was 28.6 and 22.5%, respectively (Beuving and Vonder, 1981). Coefficient of variation (within assay) was 7.4.

Haemagglutinin assay

Total haemagglutinin- and 2-mercapto-ethanol resistant (2ME-) antibody titres against SRBC were determined using a microtitre assay (Van der Zijpp and Leenstra, 1980). Titres were expressed as $\log_2$ of the highest dilution giving total agglutination.

Statistical analysis

The statistical significance of the results was determined in an analysis of variance with the SAS program (SAS, 1985). Initially the following model was used:

$$\underline{Y}_{ijk} = \mu + L_i + T_j + LT_{ij} + e_{ijk}$$

in which:

$\underline{Y}_{ijk}$ = the value for the evaluated characteristic of animal k from line i and treatment j
μ = the overall mean
L_i = the effect of line (i = H, L)
T_j = the effect of treatment (j = CS, CT)
LT_{ij} = the interaction between line and treatment
$\underline{e}_{ijk}$ = random error

Analyses were performed within day of sampling. For most characteristics the interaction between line and treatment was not significant (P>.05) and therefore an analysis with the same model, but omitting the interaction, was performed. If the interaction was significant (Table 2), cell means were compared in a multiple range test, using the Student-Newman-Keuls procedure.

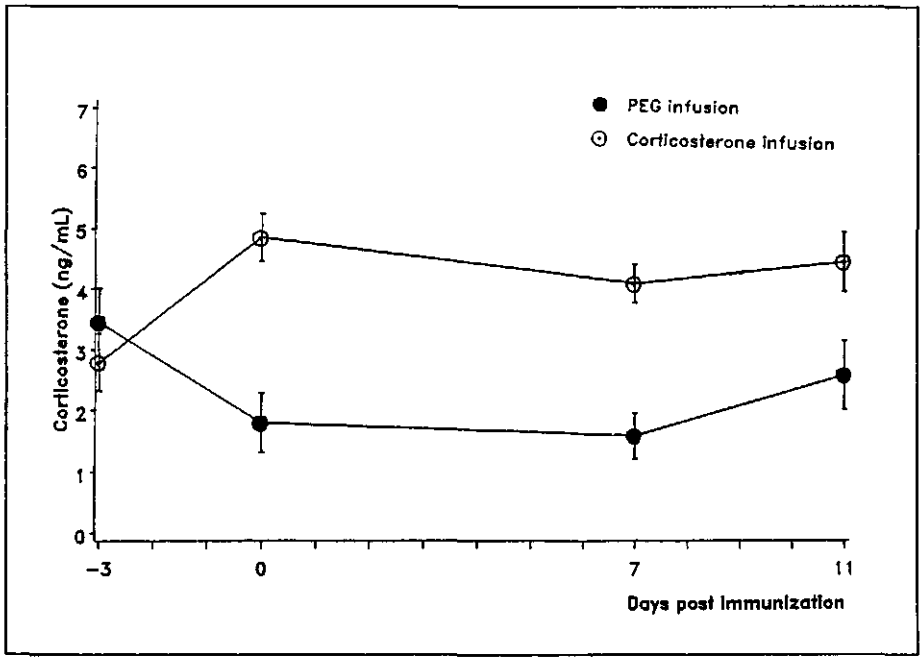
Results

None of the implanted mini-infusion pumps caused visible infection or irritation and none of the minipumps was found to be encapsulated when examined *post mortem*.

Mean plasma concentrations of corticosterone are presented in Figure 1. Before pump implantation (day -3) concentrations were similar in both groups (Table 1). After implantation plasma corticosterone concentration was significantly (P<.001) higher in corticosterone-infused chickens (Fig. 1; Table 1). No differences in plasma corticosterone concentrations between the two lines were evident (Table 1).

Mean total antibody titres and 2ME-resistant antibody titres to SRBC for each line and treatment are plotted in Figure 2. A clear difference for both total and 2ME-resistant titre between high and low line was evident (Fig. 2; Table 1). Corticosterone infusion was found to have a suppressive effect on total antibody production only in the high line on day 3 (P<.05) post immunization (Fig. 2; Table 1). Titres were significantly higher (P<.05) in

Figure 1. Plasma corticosterone levels in chickens implanted with mini-infusion pumps (day -3) delivering corticosterone or polyetheleneglycol (PEG) and immunized with 0.25 ml SRBC in 1 ml PBS (day 0).
Least squares means ± standard error from a statistical model including line and treatment.



corticosterone infused chickens than in controls on day 11 (Fig. 2; Table 1), indicating a more rapid decline in antibody titre in controls.

The 2ME-resistant titres (Fig. 2) showed similar trends but at a lower level. Line differences were evident after day 5; but there was no significant difference between treatments.

In both corticosterone- and PEG infused groups heterophil/lymphocyte ratios increased from day 3 (Fig. 3), but the rise in corticosterone infused birds was significantly greater ($P<.05$). The significant line-treatment interaction and line effect on day 3 (Table 1) was caused by an extremely high value for this ratio in one high line chicken in the corticosterone-treated group.

Weight gain was reduced by 23% in the corticosterone-infused group (Table 2). In the corticosterone-infused chickens the relative weight of the thymus was reduced by approximately 71% ($P<.001$), relative bursa weight and relative spleen weight by approximately 57 and 35% respectively ($P<.001$). Only

the relative weight of the liver was increased by approximately 37% ($P<.05$). The spleen was significantly ($P<.001$) heavier in the high than in the low line (Table 2).

Figure 2. Mean total antibody titres and 2ME-resistant antibody titres to SRBC in chickens implanted with mini-infusion pumps (day -3) delivering corticosterone or polyetheleneglycol (PEG) and immunized with 0.25 ml SRBC in 1 ml PBS (day 0). n=3 (high line, corticosterone; low line, PEG), n=4 (low line, corticosterone), n=2 (high line, PEG).

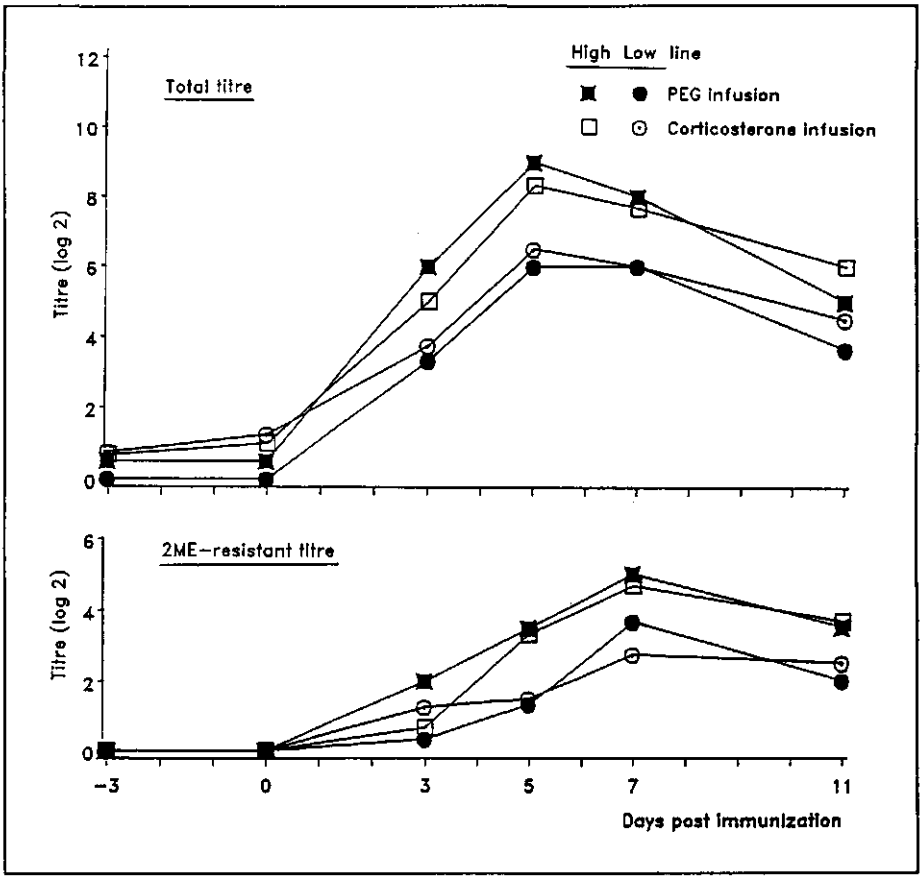


Table 1. Significance[1)] of line and treatment effects in the statistical analysis for blood parameters.

Blood parameter:	Number of days post immunization					
	-3	0	3	5	7	11
Corticosterone						
Line	n.s.	n.s.	.	.	n.s.	n.s.
Treatment	n.s.	***	.	.	***	*
Total titre						
Line	-	-	***[2)]	***[3)]	**	**
Treatment	-	-	n.s.	n.s.	n.s.	*
2ME titre						
Line	-	-	-	**	**	*
Treatment	-	-	-	n.s.	n.s.	n.s.
Heterophil/lymphocyte ratio						
Line	n.s.	n.s.	**[2)]	n.s.	n.s.	n.s.
Treatment	n.s.	n.s.	***	*	*	**

1) n.s.: P>.10; + P<.10; *: P<.05; ** P:<.01; ***: P<.001;
. not measured; -: not tested because of skew distribution of values.

2) In Preceding analysis: line x treatment interaction: P<.05

3) In Preceding analysis: line x treatment interaction: P<.10

Table 2. Relative growth[1)] and relative organ weight[2)]. Least squares means ± standard errors and significance levels[3] from the statistical analysis.

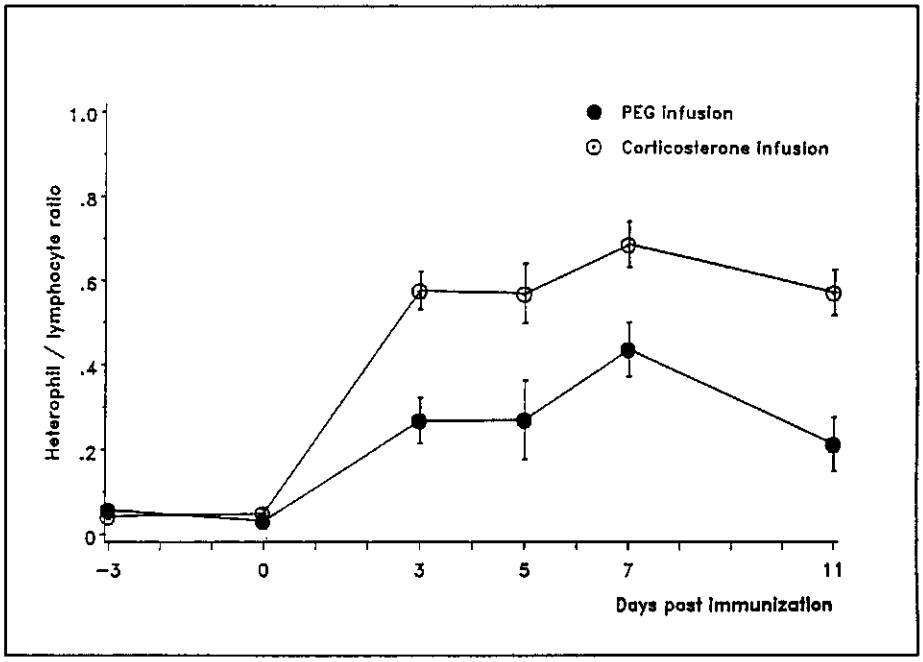
	Treatment			Line		
	control	corticosterone		High	Low	
Relative growth[1)]	121.9 ± 6.2	93.99 ± 5.2	**	108.6 ± 6.2	107.2 ± 5.2	n.s.
Thymus (g/gBW)	0.77 ± 0.06	0.23 ± 0.05	***	0.51 ± 0.05	0.49 ± 0.05	n.s.
Bursa (g/gBW)	0.56 ± 0.03	0.24 ± 0.03	***	0.44 ± 0.03	0.35 ± 0.03	+
Spleen (g/gBW)	0.18 ± 0.01	0.12 ± 0.01	***	0.18 ± 0.01	0.12 ± 0.01	***
Liver (g/gBW)	2.80 ± 0.23	3.84 ± 0.20	*	3.11 ± 0.23	3.52 ± 0.20	n.s.

1) = $\frac{\text{(Live weight at killing - live weight at 30 days)}}{\text{Live weight at 30 days}} \times 100$

2) = Organ weight / Body weight x 100

3) n.s.: P>.10; + P<.10; *: P<.05; ** P:<.01; ***: P<.001;

Figure 3. Heterophil/lymphocyte ratio in chickens implanted with mini-infusion pumps (day -3) delivering corticosterone or polyetheleneglycol (PEG) and immunized with 0.25 ml SRBC in 1 ml PBS (day 0).
Least squares means ± standard error from a statistical model including line and treatment.



Discussion

Throughout the experiment the plasma corticosterone concentrations in corticosterone-infused chickens were higher than in the control group. In a similar experiment with corticosterone infused adult hens, plasma corticosterone concentrations were highest 1-2 d after implantation and then plateaued at a higher level than in the controls infused with PEG (Beuving, unpublished results). Davison *et al.* (1985) observed a similar pattern with continuous infusion of ACTH in young chickens. The corticosterone concentrations in the corticosterone infused group were close to the upper limits of the normal physiological range (Beuving, 1983; Webb and Mashaly, 1985; Beuving and Vonder, 1986).

The severe effect of infused corticosterone on body weight and on the relative weight of the thymus, bursa and spleen is consistent with the view that stressors and corticosterone, have catabolic effects on lymphoid organs (Selye, 1976). Sato and Glick (1970) reported decreased growth rate and bursa, spleen and thymus development in chickens given daily injections of cortisone acetate or corticosterone. Siegel and van Kampen (1984) made similar observations in broilers after five daily injections of corticosterone. In their experiment metabolic measurements indicated higher protein catabolism and increased fat deposition. Similar reductions in growth and spleen and bursa development were found in young chickens implanted with either pellets containing corticosterone, or mini-infusion pumps delivering ACTH (Davison *et al.*, 1985).

Heterophil/lymphocyte ratios were increased in both groups of chickens. The increase in corticosterone-infused animals was considerably higher ($P<.05$) and was consistent with the lymphocytosis and eosinopenia (Selye, 1976) and heterophilia (Gross and Siegel, 1983) induced by various stressors. Lymphocytosis and heterophilia, induced by feeding corticosterone was related to the dose fed (Gross *et al.*, 1980). In this experiment, the rise in heterophil/lymphocyte ratio in the control group of chickens may have been caused by the implantation of the minipumps and the PEG infusion. A rise of the same magnitude was recorded in adult hens implanted with minipumps delivering PEG (Jones *et al.*, 1988). Also immunization with SRBC can increase the heterophil/lymphocyte ratio (Donker *et al.*, submitted-a). However, they found a rise in the ratio only on day 2 after immunization. Similar findings have been reported for immunizations with *Brucella abortus* antigen (Trout *et al.*, 1988).

Although corticosterone infusion was found to have marked effects on growth, organ weight and heterophil/lymphocyte ratio there was no evidence of a consistent depression of the antibody production. Only a small difference in the high line was evident on day 3, but this disappeared later. These findings are in agreement with observations in subsequent experiments using heat-stress (Donker *et al.*, submitted-a). Corticosterone and cortisone acetate were found to have suppressive effects on antibody titres to SRBC when injected in chickens daily from 13-28 days of age (Sato and Glick, 1970). When injected from 2-5 days of age, however, only cortisone acetate was demonstrated to be immunosuppressive, indicating that exogenous corticosterone might be a relatively weak immunosuppressant or is bound to serum globulin, inactivating cellular action. Davison and Misson (1987) however, reported dose-dependent depressions of SRBC and *Brucella abortus* titres using subcutaneous implants releasing corticosterone.

The dose of antigen (SRBC) administered in this experiment might be considered high compared with other reports. Siegel *et al.* (1983) and Siegel

and Latimer (1985) could demonstrate immunosuppressive effects of heat or ACTH on the antibody response to *Salmonella pullorum* only at low doses of antigen. Nevertheless, in other experiments (Donker *et al.*, submitted-a; Donker *et al.*, submitted-b), we have been able to demonstrate suppressive effects of heat stress on the antibody response with the same dose of antigen injected by the same route. These effects, however, were much smaller than the differences achieved by selection. Thus, in spite of the effects of heat stress or corticosterone infusion, these lines have a constant response to SRBC, with differences between the lines remaining unaffected. The absence of genotype-environment interaction on antibody production in these lines may be of importance in selection programmes which include breeding for immune responsiveness.

References

Beuving G., 1983. Corticosteroids in welfare research of laying hens. pg.47-53 in: Smidt D. (ed): *Indicators relevant to farm animal welfare*. Martinus Nijhoff publ., Boston, The Hague.

Beuving G. and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *General and Comparative Endocrinology*, 35: 153-159.

Beuving G. and G.M.A. Vonder, 1981. The influence of ovulation and oviposition on corticosterone levels in the plasma of laying hens. *General and Comparative Endocrinology*, 44: 382-388.

Beuving G. and G.M.A. Vonder, 1986. Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and plasma baseline levels of corticosterone. *General and Comparative Endocrinology*, 62: 353-358.

Davison T.F., B.M. Freeman and J. Rea, 1985. Effects of continuous treatment with synthetic ACTH or corticosterone on immature *Gallus domesticus*. *General and Comparative Endocrinology*, 59: 416-423.

Davison T.F. and B.H. Misson, 1987. Effect of corticosterone on antibody responses to a T-dependent and T-independent antigen in domestic fowl. *Medical Science Research*, 15: 967-968.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp (*submitted-a*). Heat stress in chicken lines selected for high and low antibody production. 1. Antibody production.

Donker R.A., A.G.J.C. Swinkels, A.J. v.d. Zijpp, (*submitted-b*). The effect of environmental temperature on antibody production and some physiological parameters in chicken lines, selected for humoral immune responsiveness. Submitted to Br. Poult. Sci.

Gross W.B., 1986. Effect of dose of antigen and social environment on antibody response of high and low antibody response chickens. *Poultry Science*, 65: 687-692.

Gross W.B. and P.B. Siegel, 1982. Socialization as a factor in resistance to infection, feed efficiency, and response to antigen in chickens. *American Journal of Veterinary Research*, 43: 2020-2012.

Gross W.B. and H.S. Siegel, 1983. Evaluation of the heterophil / lymphocyte ratio as a measure of stress in chickens. *Avian Diseases*, 27: 972-979.

Gross W.B., P.B. Siegel and R.T. DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poultry Science*, 59: 516-522.

Jones R.B., G. Beuving and H.J. Blokhuis, 1988. The effects of corticosterone infusion on tonic immobility response and heterophil/lymphocyte ratio's in the domestic fowl. *Physiology and Behaviour*, 42: 249-253.

Powell P.C. and T.F. Davison, 1986. Induction of Marek's disease in vaccinated chickens by treatment with betamethasone or corticosterone. *Israelian Journal of Veterinary Medicine* 42: 73-78.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC, USA: SAS Institute Inc.

Sato K. and B. Glick, 1970. Antibody and cell mediated immunity in corticosteroid-treated chicks. *Poultry Science*, 49: 982-986.

Selye H., 1976. *Stress in health and disease*. Butterworth, Boston, London.

Siegel H.S., 1987. Effects of behavioral and physical stressors on immune responses. pg.39-54. In: P.R. Wiepkema and P.W.M. van Adrichem (Eds): *Biology of stress in farm animals: an integrative approach*. Martinus Nijhoff Publ., Dordrecht, Boston, Lancaster.

Siegel H.S. and N.R. Gould, 1982. High temperature and corticosteroid in the lymphocytes of domestic fowl (*Gallus domesticus*). *General and Comparative Endocrinology*, 48: 348-354.

Siegel H.S. and M. van Kampen, 1984. Energy relationship in growing chickens given daily injections of corticosterone. *British Poultry Science*, 25: 477-485.

Siegel H.S. and J.W. Latimer, 1985. Interaction of high temperature and *Salmonella pullorum* antigen concentration on serum agglutinin and corticosteroid responses in white rock chickens. *Poultry Science*, 63: 2483-2491.

Siegel, H.S., J.W. Latimer and N.R. Gould, 1983. Concentration of *Salmonella pullorum* antigen and the immunosuppressive effect of adrenocorticotropin in growing chickens. *Poultry Science*, 62: 897-903.

Subba Rao D.S.V. and B. Glick, 1970. Immunosuppressive action of heat in chickens. *Proceedings of the Society for Experimental Biology and Medicine*, 133: 445-448.

Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. *Poultry Science*, 49: 202-205.

Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Science*, 52: 618-624.

Thaxton P., C.R. Sadler and B. Glick, 1968. Immune response of chickens following heat exposure or injections with ACTH. *Poultry Science*, 47: 264-266.

Trout J.M., M.M. Mashaly, H.S. Siegel, 1988. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Developmental and Comparative Immunology*, 12: 331-346.

Van der Zijpp A.J. and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poultry Science*, 59: 1363-1369.

Van der Zijpp A.J. and M.G.B. Nieuwland, 1986. Immunological characterization of lines selected for high and low antibody production. *Proceedings of the 7th European Poultry Conference (Paris)* : 211-215.

Webb M.L. and M.M. Mashaly, 1985. Maturation of the diurnal rhythm of corticosterone in female domestic fowl. *Poultry Science*, 64: 744-750.

Chapter VI

Antibody production in chicken lines divergently selected for immune responsiveness, in relation to energy metabolism.

1. Heat production at different ambient temperatures and energy metabolism during the immune response under homeotherm conditions.

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Abstract

Energy metabolism was studied in two lines, divergently selected for humoral immune response after immunization with sheep erythrocytes.

In two experiments the relation between heat production and ambient temperature was studied without immunization. The heat production (kJ kg^{-1}) was higher in chickens of the low response line in one experiment at temperatures below 30°C. Levels were similar for both lines in the other experiment. Relative *ad libitum* feed intake, relative growth rate and cloacal temperature were not different between the two lines. No thermoneutral zone was found in either line.

In two other experiments, metabolism before and during an immune response was studied. Energy utilization was somewhat more efficient in the high line; fat deposition was higher at a comparable metabolized energy intake. During the immune response, fat deposition was decreased in the low response line, but not in the high line.

Levels of metabolism-associated hormones (growth hormone, somatomedine, thyroxine, triiodothyronine (T3)) were not different between the high and low line, except for T3 concentration. T3 levels were higher in the high line, but no direct relation with antibody titers was evident.

Despite profound differences in antibody titers only small differences in metabolic efficiency were found between the two lines. It is not very likely that these contribute to the immunological difference.

Keywords: metabolism, immune response, SRBC, selection lines
Running title: Metabolism and immune response

Introduction

Selection for immune responsiveness possibly is a fruitful way to increase disease resistance in poultry (van der Zijpp *et al.*, 1988). However, when selecting for immune responsiveness, other characteristics may show associated alterations (Siegel *et al.*, 1982a; Ubosi *et al.*, 1985). For example, in the lines divergently selected for immune responsiveness in our laboratory (van der Zijpp and Nieuwland, 1986) bodyweight is obviously influenced by the selection (van der Zijpp and Nieuwland, unpublished data). Average body weight is higher in the low response line.

Important endocrine regulators of growth and body composition are growth hormone, somatomedines and thyroid hormones (Scanes, 1987). These hormones have been reported to act as immuno-modulating agents. Growth hormone has been

described as immuno stimulant (Berczi, 1986) and hypo- and hyper-thyroidism have been found to influence immune responsiveness (Yam *et al.*, 1981; Mashaly *et al.*, 1983; Berczi, 1986; Martin *et al.*, 1988).

It has also been reported that the mounting of an immune response influences the levels of these hormones (Besedovski *et al.*, 1975, 1986; Trout *et al.*, 1988) and metabolic rate and composition of gain (Henken and Brandsma, 1982; Siegel *et al.*, 1982b). Thus as a result of selection for immune responsiveness, hormone levels might have been altered directly (by genetic linkage) or indirectly (by associated physiological mechanisms) between the lines. In this study, differences in growth and immune response between the two selected lines will be associated with differences in the hormonal status and the metabolic rate of these lines before and during the immune response.

In previous experiments (Henken *et al.*, 1983a, 1983b) it was demonstrated that moderate changes in the environmental temperature can provoke changes in the height of the immune response. In a recent study (Donker *et al.*, submitted-b), it was found that these changes can be of the same magnitude as those obtained by selection. Thus, the perception of the environment by the bird can be an important factor in the immune response mounted. A possible difference in thermal requirements or adaptive capacity of the birds between the high (H) and low (L) selection line could therefore be a contributing factor to the differences found in immune responsiveness between the lines. Measurement of heat production at varying temperatures, and of the associated feed intake and growth rates, and estimates of the critical temperature can give an impression of these differences.

Material and methods

Four experiments were performed. In Experiments 1 and 2 measurements of feed intake, growth rate and heat production were made at a range of environmental temperatures in order to estimate the thermoneutral zone and lower critical temperature. In Experiments 3 and 4, measurements of the protein and energy balance and hormone profiles were made during an immune response under thermoneutral conditions.

Birds and pre-experimental conditions

Male birds were used, which were genetically identical to the 7th generation of a high and low line that were selected for antibody titer to sheep erythrocytes (SRBC) (Van der Zijpp and Nieuwland, 1986). For each experiment, a separate hatch was used. The sexes and lines were separated at hatch and the birds were placed in brooder cages with water and commercial starter feed provided *ad libitum*.

Environmental temperature was gradually decreased during the first two weeks from approximately 32°C to approximately 25°C; the light regime was changed from 23 h light/ 1 h dark to 12 h light/ 12 h dark during the first three weeks.

Individual vaccinations against Marek's disease (at 1 d of age), infectious bronchitis (2 d of age), Gumboro's disease (12 d of age) and Newcastle disease (18 d of age) were given.

To exclude large variation in weight and growth rate during the experiments, a selection for body weight at 22 days of age was made. Only chicks with a bodyweight within a range of the median weight of that line ± 1 s.d. were used. The 16 selected birds per line were randomly distributed over four cages in the climate-respiration chamber. The high and low line were kept in separate chambers.

Climate-respiration chambers

Two medium size climate respiration chambers (1.8 m^3 each) at the experimental unit of the Department of Animal Husbandry of the Agricultural University were used. The technical equipment and possible applications of these chambers are described elsewhere (Verstegen *et al.*, 1987). Within each chamber a set of four brooder cages (± .5 x .4 x .4 m each; l x b x h) was placed. Measurement of feed intake and excreta could be performed for each cage individually; O_2-consumption and CO_2-production for indirect calorimetry (Verstegen *et al.*, 1987) could be measured for each chamber. Activity measurements, by ultra-sound activity detectors were made simultaneously (Wenk and van Es, 1976).

Experiment 1 and 2 (relation of environmental temperature and heat production)

The birds were placed in the chambers at an age of 25 days. Chamber temperatures were regulated at 25°C, RH at 70%. At 28 days of age all four birds of one cage per chamber were individually removed from the chamber and an immediate measurement of cloacal temperature was made. All 32 birds were weighed individually and after the chambers were closed again chamber temperatures were modified simultaneously in both chambers (Fig. 1). A day of adaptation to the new temperature was allowed before measurements of CO_2, O_2 and activity-measurements (Verstegen *et al.*, 1987) were started. These procedures were repeated every alternate day (Fig. 1). *Ad libitum* feed intake was measured daily.

Changes in chamber temperatures were made according to a "/\" shape in Experiment 1, starting at a minimum temperature of 15°C and a maximum temperature of 35°C. In Experiment 2 a "\/" shape was programmed in the temperatures, starting at a maximum temperature of 35°C and with a minimum temperature of 10°C (Fig. 1).

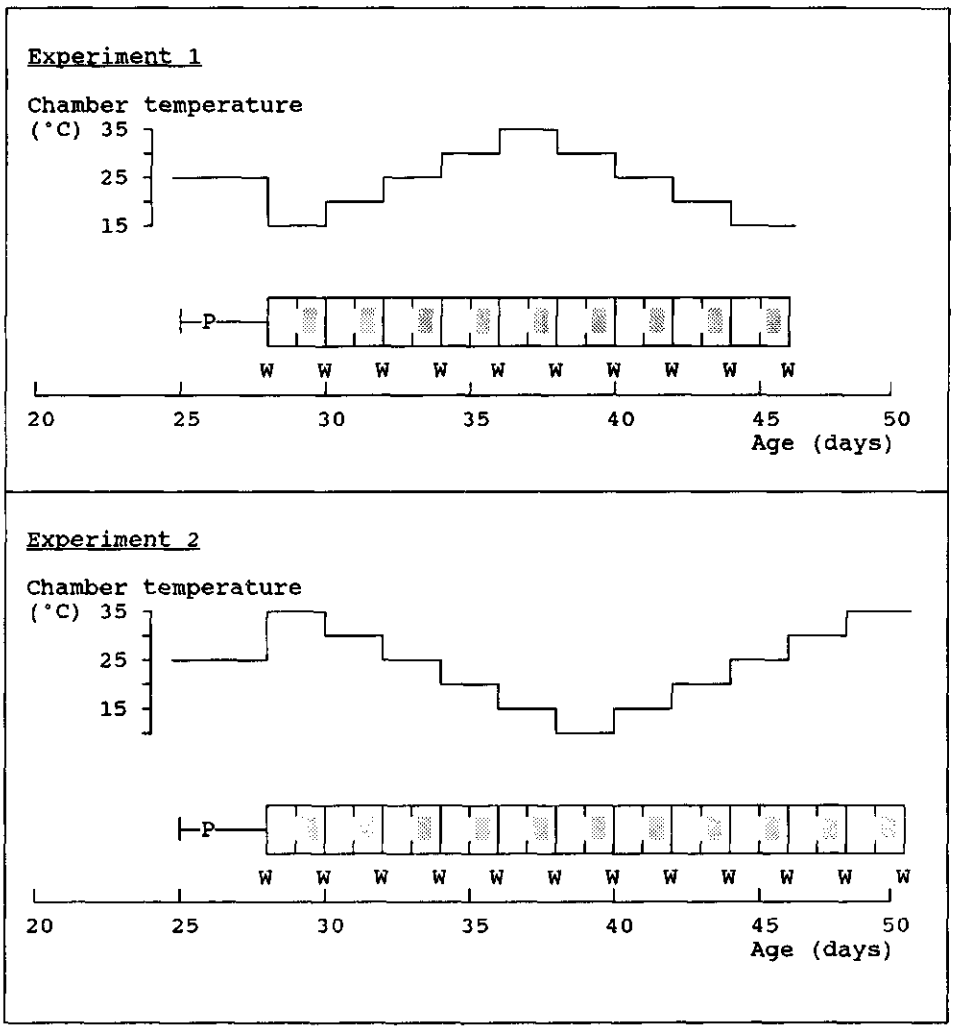
Figure 1. Experimental layout of Experiments 1 and 2.

P: Pre-experimental adaptation period

: Two day period at same temperature

: Main day (calorimetry)

W: Weighing, body temperature measurement



Experiments 3 and 4 (metabolism before and during the immune response)

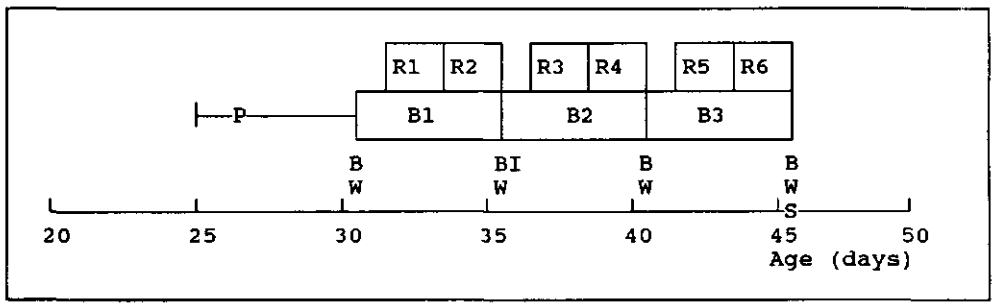
The birds were placed in the chambers at an age of 25 days. Chamber temperature was regulated at 25°C, with a RH of 70%. Light regime was 12 h light/ 12 h dark. At 31 days of age the birds were weighed and the first of three successive balance periods was started (Fig. 2). During a 5-day balance period *ad libitum* feed intake was measured daily and excreta were collected for each cage. Chambers were not opened throughout a balance period. At the second day of a balance period the first of two successive 48-hour respiration periods was started (Fig. 2). Continuous measurements of O_2-consumption and CO_2-production were made during a respiration period. At the end of each balance period, the birds were weighed again, the amount of excreta was assessed and samples of excreta and refused feed were taken.

Prior to the second balance period, a blood sample was taken from the ulnar vein (*vena cutanea ulnaris*) of all birds using a heparinized syringe. The birds were immunized intramuscularly with 1 ml 25% (v/v) packed SRBC in saline. Another blood sample was taken immediately after the end of the second and third balance period.

The chickens were killed by cervical dislocation after the third balance period and the liver, thymus, spleen and bursa were excised and weighed.

Figure 2. Experimental layout of Experiments 3 and 4.

P: Pre-experimental adaptation period in climate respiration chambers
B1, B2, B3: Balance period 1, 2, 3
R1, .., R6: 2-day respiration periods
B: Blood sampling
I: Immunization with SRBC
W: Weighing
S: Slaughter and dissection



Assays

All food and excreta samples in Experiments 3 and 4 were analyzed for energy and protein contents. In addition, energy and nitrogen loss through dust and airflow (NH_3) from the chambers were estimated.

Plasma samples were analyzed for total and 2ME-resistant antibody titer to SRBC (Van der Zijpp and Leenstra, 1982) and concentrations of triiodothyronine (T_3) (commercial kit: Dac-Cel T3; Welcome Reagents Ltd, Beckenham, Kent U.K.), thyroxine (T_4) (commercial kit: T4 RIA(PEG); Abbott Diagnostic Division, Antwerp, Belgium), growth hormone (GH) (Harvey and Scanes, 1977) and somatomedine-C (SmC) (Huybrechts *et al.*, 1985) were measured.

Statistical analysis

Heat production (H) was calculated from oxygen consumption and carbon dioxide production, using the formula of Romijn and Lokhorst (1961):

$H\ (kJ) = 16.20 \times O_2\ (l) + 5.00 \times CO_2\ (l)$.

In experiments in which metabolic traits (M) are compared, the influence of bodyweight (BW) is usually excluded by expressing $M = a\ BW^p$ (Brody, 1945) in which p is the mass exponent. For inter-species comparisons, p = .73-.75 is generally used. For intra-species comparisons, varying estimates are found. In chickens, p values varying between .3 (mature birds) and 2.1 (young chickens) were reported (van Kampen, 1987). In this study, p=1 is used since Kuenzel and Kuenzel (1977) and Henken *et al.* (1982a) demonstrated this to be the most appropriate estimate for young chickens. For this reason, all characteristics are expressed on the basis of kg^{-1}. However, calculations have also been done using p=.75, giving results which were essentially the same as presented here.

Data were statistically evaluated in analyses of variance using the SAS procedures (SAS, 1985).

In Experiment 1 and 2, individual growth, relative growth (growth in g / d / 100 g bodyweight), cloacal temperature and ME-intake per cage were analyzed in an analysis of variance with day and line as factors. Heat production was analyzed by regression on chamber temperature, including line as factor. Comparisons were made between data from the total 24 hours and data from the 12 hour light and 12 hour dark periods taken separately. Separate analyses were done for the phases with rising or decreasing temperature (Fig. 1). For estimates of critical temperature (T_c) separate regression lines were calculated using data from 20°C and lower and 30° and higher. When these lines crossed above 25°, data from 25° were added to those from 20°C and lower, and *vice versa* (Henken *et al.*, 1982b). Differences between slopes were tested in a regression analysis. Activity-free heat production was calculated

from regression of heat production on activity data. The same analyses that were performed on total heat production were also done on activity free heat production.

Individual data from Experiments 3 and 4 were analyzed, using a model containing line, day of sampling and animal number (nested within line) as factors. Line differences were tested against animal within line; differences between days and day x line interaction were tested against the residual error. Characteristics analyzed were growth, relative growth, antibody titers (total and 2ME-resistant), GH, T3, T4 and SmC. In a combined analysis of data from Experiment 3 and 4, differences between experiments were tested against animals within line, with interactions being tested against residual error.

Balance characteristics analyzed from Experiments 3 and 4 were ME-intake ($kJ \times kg^{-1} \times day^{-1}$), energy balance EB ($kJ \times kg^{-1} \times day^{-1}$), protein retention PR ($g \times kg^{-1} \times day^{-1}$) and fat deposition FD ($g \times kg^{-1} \times day^{-1}$). ME was calculated from energy in feed minus energy lost in excreta, dust and outgoing air (NH_3); PR was calculated from N in feed minus N lost in excreta, dust and outgoing air; EB was calculated as ME minus H, in which H (heat production) was calculated by dividing total heat loss per chamber among cages relative to ME intake. FD was calculated from the difference in energy retention between EB and PR. Balance data were analyzed in the same manner as individual data, testing line differences against cage within line and balance period and interactions against residual error.

Relative organ weights were analyzed in an one-way analysis with line as a factor. A two-way analysis of variance, including experiment as a factor was used on combined data of Experiment 3 and 4.

Results

Experiment 1 and 2

Feed intake

Mean relative feed intake was higher in the L line than in the H line in Experiment 1 ($P<.05$), but not in Experiment 2 (Fig. 3). Relative feed intake was reduced at temperatures higher than 25°C in both experiments ($P<.001$). However, feed intake remained at a lower level during the phase of decreasing temperature in Experiment 1.

Body weight and growth

Mean body weight and relative growth rate (g / d / 100 g body weight) per line during Experiment 1 and 2 are also shown in Figure 3. Average body weight was higher in L line than in H line in Experiment 2 ($P<.05$), but not in Experiment 1. Relative growth rates were not significantly different between lines. Growth rate was significantly depressed at 35°C in Experiment 1 ($P<.05$) and 2 ($P<.01$).

Cloacal temperature

Cloacal temperature (Fig. 3) was not different between lines. Higher cloacal temperatures ($P<.001$) were found at 35°C.

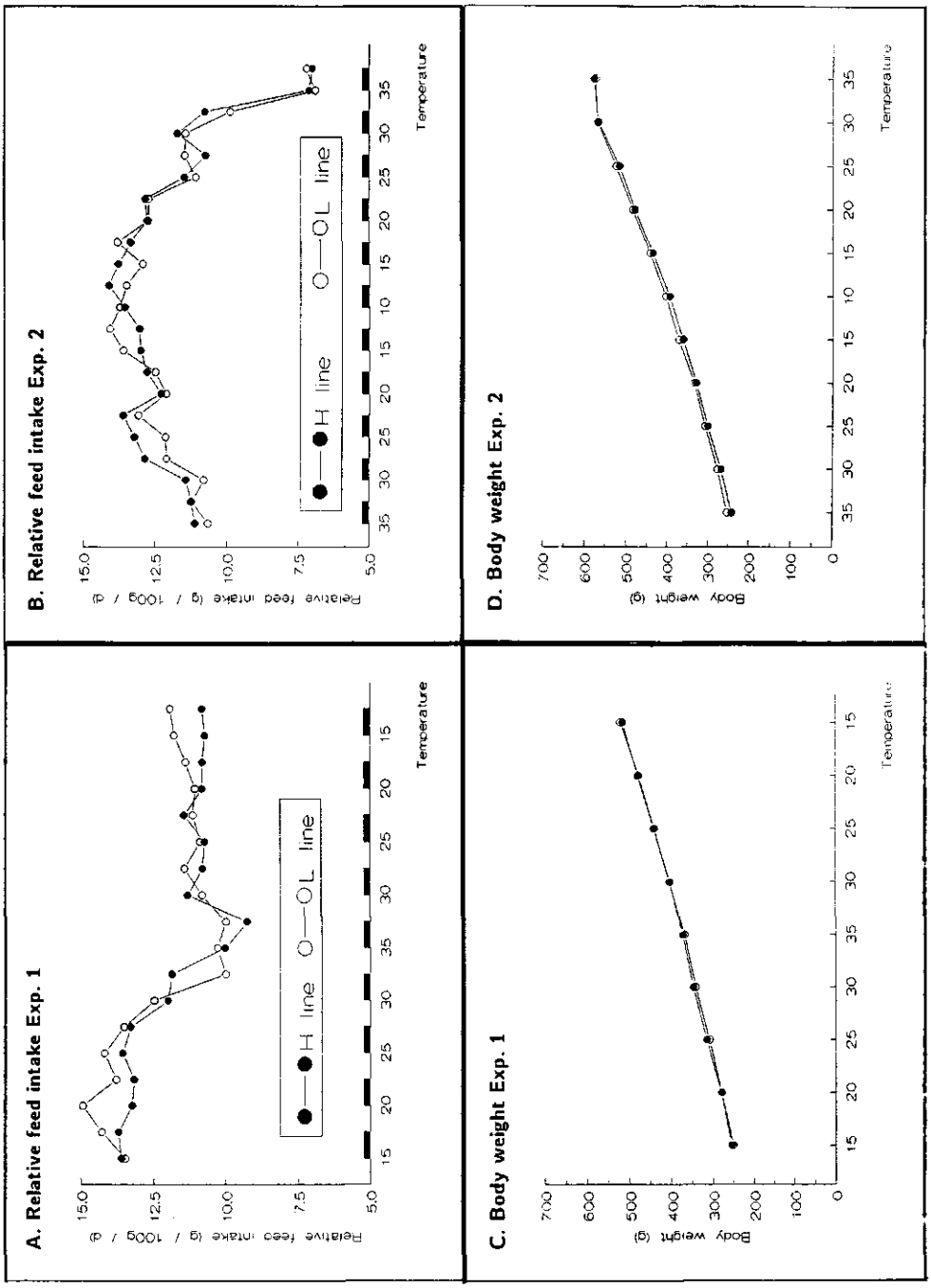
Heat production and critical temperature (T_c)

Average heat production based on 24 h data per line are presented in Figure 4. In Experiment 1 heat production during the phase of increasing temperature was higher than during the phase of decreasing temperature. In Experiment 2, the heat production during the phase of decreasing temperature was, at temperatures above 20°C, higher than during the phase of increasing temperature. The heat production in L line was higher than that of H line at temperatures below 25°C in Experiment 1, but not in Experiment 2 (Figure 4; Table 1).

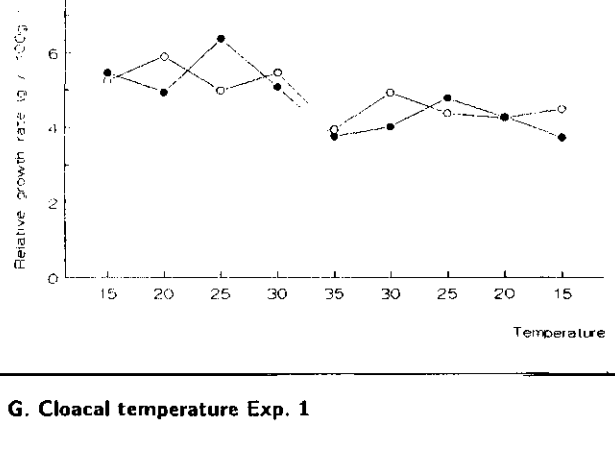
Average heat production, taken for the 12 h light period and the 12 h dark period separately is not shown, but the same differences between phases of increasing and decreasing temperatures, and lines were found as in 24 h data. However, heat production during the dark period was significantly lower ($P<.001$) than during the light period.

Regression analysis of 24 h heat production data is summarized in Table 1. The table shows significance of the factors included in the regression analysis (temperature phase, line and interaction), the significance of different slopes between lines within temperature phase and between temperature phases within lines, and the estimate of the lower critical temperature per line. In several regression analyses, differences between the two distinguished temperature sections were found. However, the differences between the estimated regression coefficients in each section usually very small, giving unrealistic estimates of the critical temperature. When the intended procedure was followed, no estimate for the lower critical temperature was found between 20° and 30°C. All regression coefficients were significantly different from 0 ($P<.001$), so no temperature zone was found in which heat production was independent from environmental temperature. Activity free heat production data are not presented, but gave similar results at a lower level.

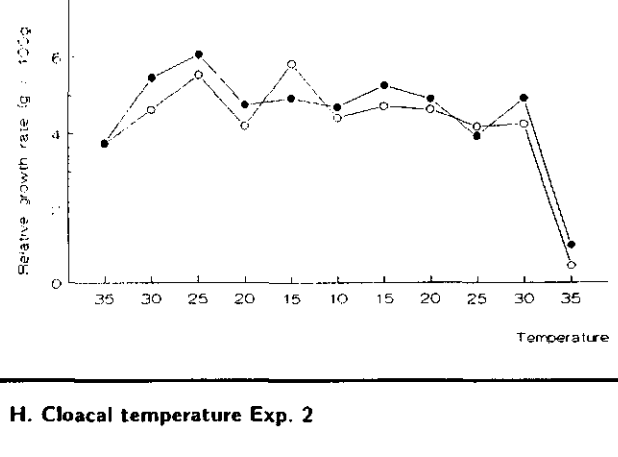
Figure 3. Average relative feed intake (A, B), average body weight (C, D), average relative growth rate (E, F) average body temperature (G, H) for H and L line in Experiment 1 and 2.



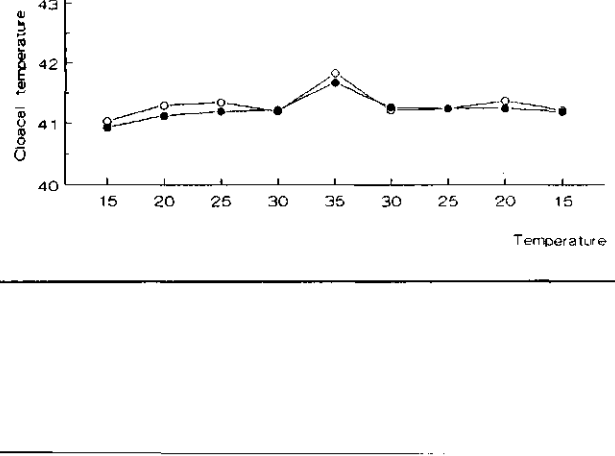
E. Relative growth rate Exp. 1



F. Relative growth rate Exp. 2



G. Cloacal temperature Exp. 1



H. Cloacal temperature Exp. 2

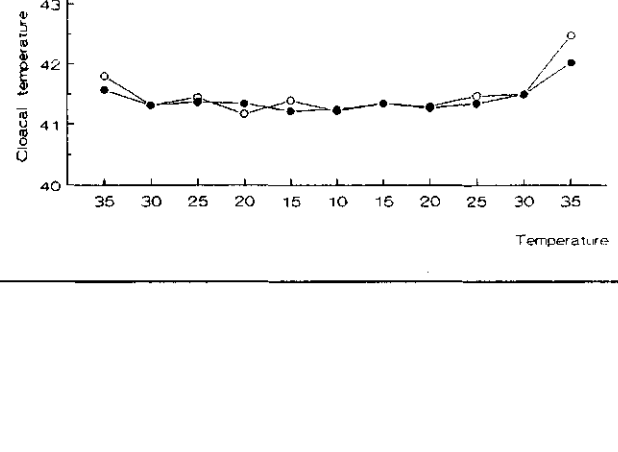


Figure 4. Average 24 h heat production vs. chamber temperature for H and L line. The arrow indicates the course of the temperature.

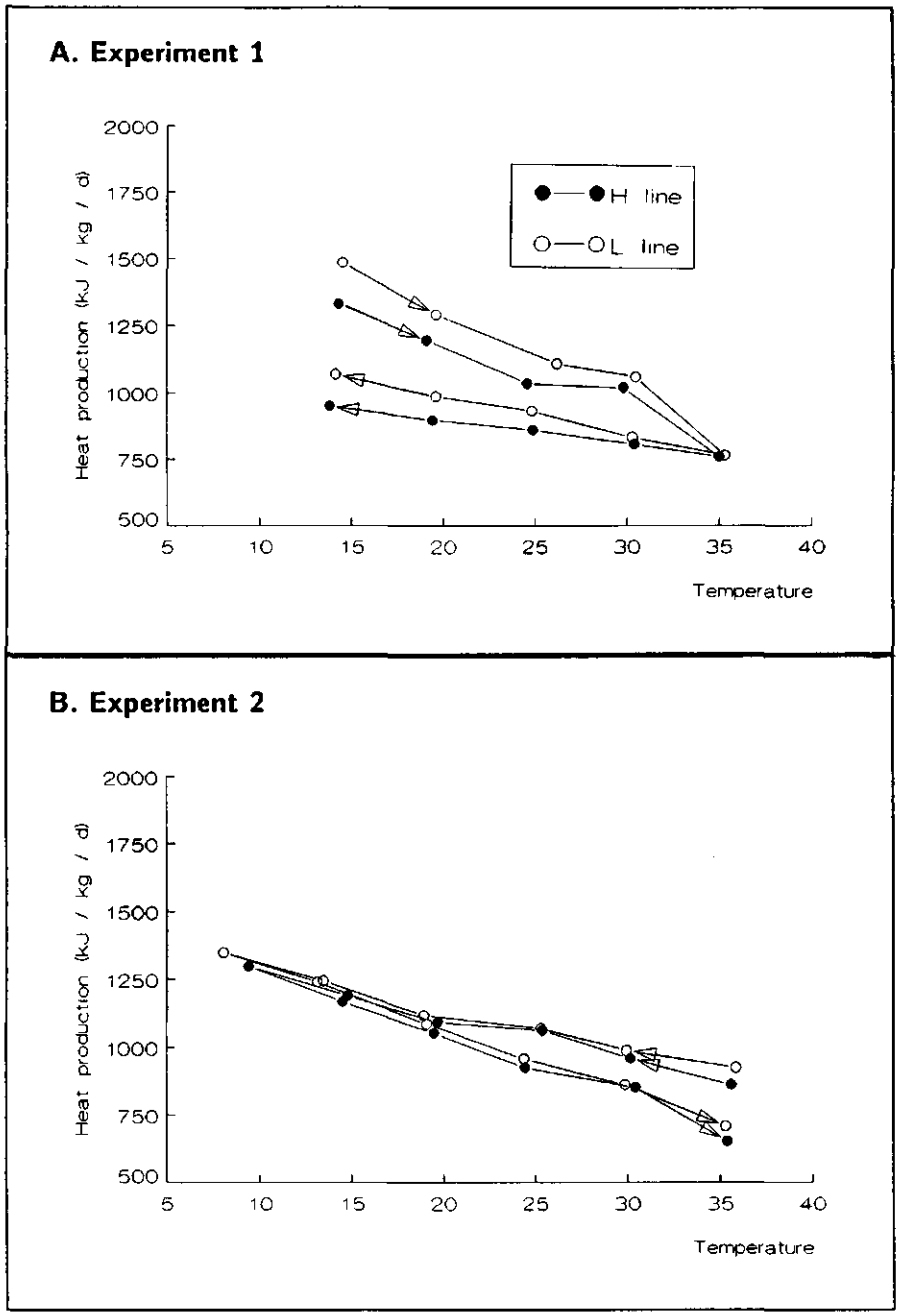


Table 1. Regression analysis of 24 h heat production on chamber temperature. Experiments 1 and 2.

		Significance[1] of effect in regression analysis:			T-test for difference between regression coëfficients:				Estimate for Tc[2] in:	
		Section[3]	Line	S x L[4]	Line in Sect. 1[5]	Line in Sect. 2	Section in H[6]	Section in L	H line	L line
Experiment 1										
T increasing phase										
15-20	30-35	***	***	***	*	**	***	***	>	>
15-25	30-35	***	***	***	ns	**	***	***	≥	≥
15-20	25-35	ns	***	***	***	***	ns	ns	21.4	<
T decreasing phase										
15-20	30-35	ns	***	***	ns	**	ns	ns	>	<
15-25	30-35	ns	***	***	*	***	ns	ns	≤	<
15-20	25-35	ns	***	***	*	***	ns	ns	26.3	≥
Experiment 2										
T increasing phase										
15-20	30-35	***	ns	**	ns	ns	***	***	>	>
15-25	30-35	***	ns	**	ns	ns	***	***	≥	≥
15-20	25-35	ns	ns	ns	ns	ns	ns	ns	<	<
T decreasing phase										
15-20	30-35	*	ns	***	ns	ns	***	***	<	<
15-25	30-35	ns	ns	ns	ns	ns	ns	ns	29.8	22.6
15-20	25-35	ns	ns	***	ns	ns	***	***	≤	≤

1) Levels of significance
ns: P>.05; *: P<.05; **: P<.01; ***:P<.001

2) Estimate of critical temperature (T_c).
>: estimate > 30°C; <: estimate < 20°C

3) Section within increasing or decreasing phase: 15-20°C, 15-25°C, etc.

4) Section x Line interaction

5) Significance of different regression coefficient between H and L line within the first section on that line (e.g. 15-20°C)

6) Significance of different regression coefficient between the two sections within H line

Experiment 3 and 4.

Antibody production

Before immunization no antibodies were detected against SRBC (Fig. 5) The antibody production after immunization, total antibody titer and 2ME-resistant titer, was higher in H line than in L line ($P<.001$; Fig. 5).

Relative organ weights

The relative weights of all measured organs were different between Experiment 3 and 4 (Table 2). No significant interaction between line and experiment was found. The weight of the spleen was significantly higher in H line birds than in those from the L line. In the combined analysis of Experiment 3 + 4 the weights of the liver, spleen and bursa were found to be significantly higher in H line.

Table 2 Relative organ weights *post mortem*. Least squares means ± s.e.m. Experiment 3 and 4.

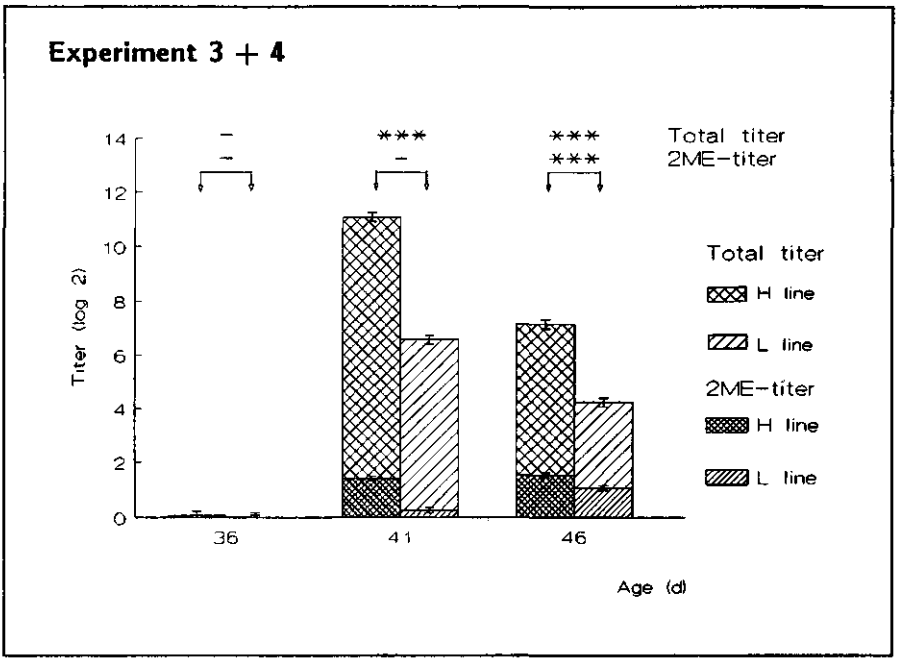
	Line		Significance	
	High	Low	H - L	Exp. 3 - 4
Experiment 3				
Liver	2.06 ± .04	2.02 ± .04	ns	
Spleen	0.22 ± .01	0.16 ± .01	**	
Bursa	0.53 ± .02	0.49 ± .02	ns	
Thymus	0.55 ± .04	0.55 ± .04	ns	
Experiment 4				
Liver	2.24 ± .06	2.08 ± .05	ns	
Spleen	0.25 ± .01	0.18 ± .01	**	
Bursa	0.58 ± .03	0.53 ± .03	ns	
Thymus	0.60 ± .03	0.57 ± .03	ns	
Experiment 3 + 4				
Liver	2.15 ± .02	2.05 ± .02	***	***
Spleen	0.23 ± .01	0.17 ± .01	***	**
Bursa	0.55 ± .01	0.51 ± .01	**	**
Thymus	0.57 ± .00	0.56 ± .00	ns	*

1) Organ weight / body weight * 100%

2) Levels of significance:
ns: $P>.05$; *: $P<.05$; **: $P<.01$; ***: $P<.001$

Figure 5. Total and 2ME-resistant antibody titer. Least squares means ± s.e.m. for H and L line from the combined results of Experiment 3 and 4.

Significance for the difference between lines within a balance period in a T-test is indicated: *** P<.001; - not tested because of skewed distribution.



Hormone levels

Growth hormone concentrations (Table 3) were significantly (P<.01) higher in H line only in Experiment 3 on day 36. In Experiment 4, significantly higher concentrations were found in L line birds on all days (P<.001). In the combined analysis of Experiment 3 and 4, no significant line effect and no significant differences between days were found.

T3 concentrations (Table 3) were overall significantly higher in H line birds than in L line (P<.01). When tested within day, however, the difference was significant only on days 36 and 41 in Experiment 3. In Experiment 3, the level was higher on day 41 than on days 36 and 46 (P<.05), but in Experiment 4, the level was highest on day 46 (n.s.). This was reflected in a significant day x experiment interaction (P<.001) in the combined analysis and no significant differences between days.

T4 concentrations (Table 3) were significantly lower in H line on day 36 in Experiment 3 and 4 (P<.01), but significantly higher in this line on day 46 in Experiment 3 and day 41 in Experiment 4 (P<.01). In the combined analysis a day x experiment x line interaction was found (P<.001).

SmC concentrations (Table 3) did not differ significantly between lines, days or experiments.

Growth and balance data

The L line birds were heavier (Fig. 6) throughout both experiments (P<.001). The difference in bodyweight remained constant as relative growth did not differ between the lines. Relative growth was also not significantly different between balance periods.

Figure 6. Body weight. Least squares means ± s.e.m. for H and L line from the combined results of Experiment 3 and 4.
Significance for the difference between lines within day in a T-test is indicated: *** P<.001

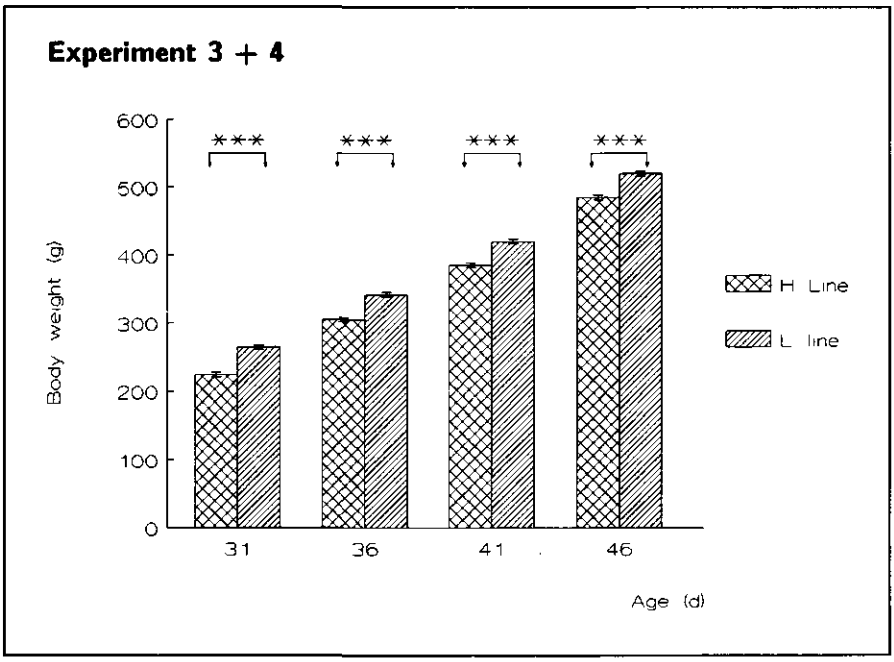


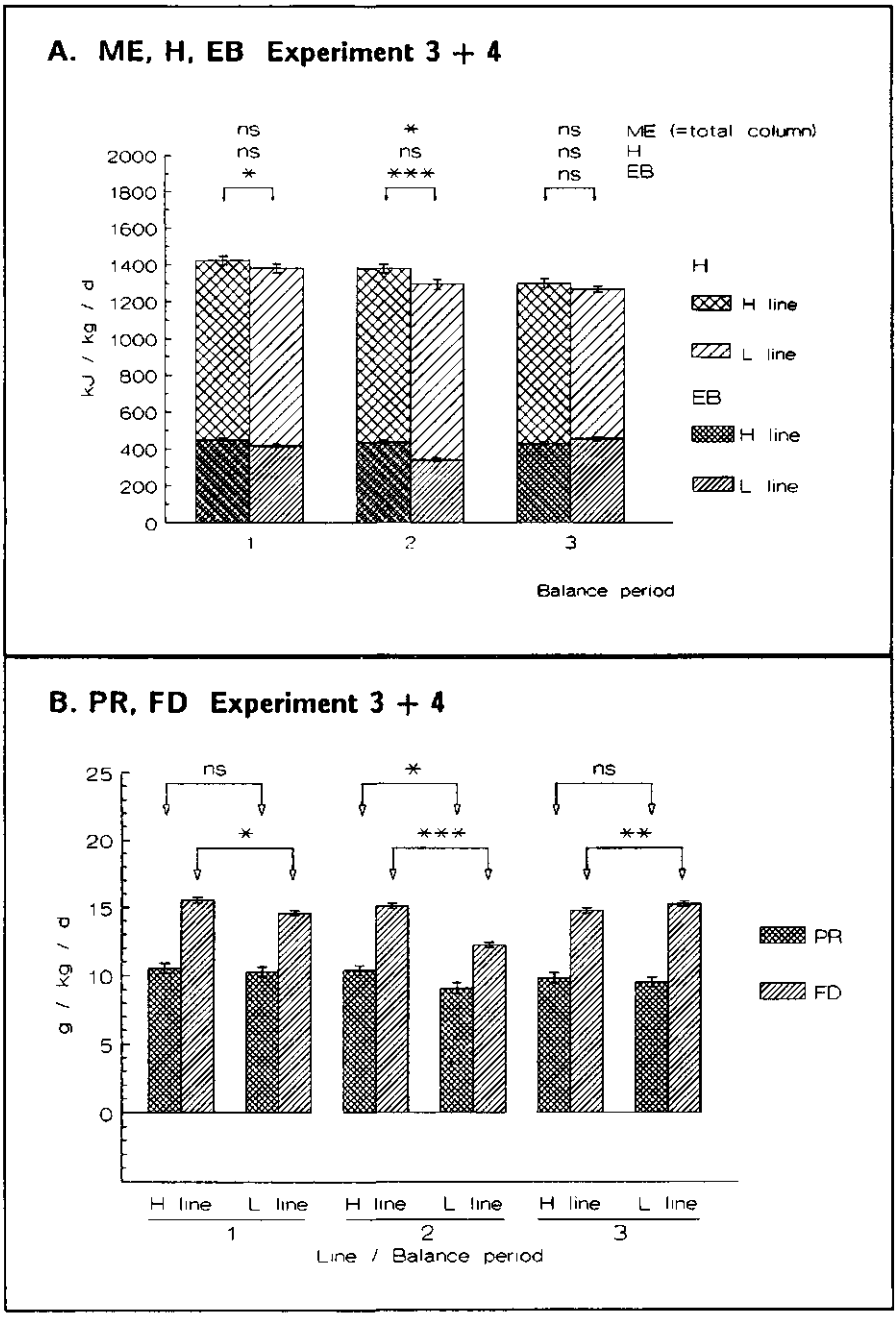
Table 3. Plasma hormone concentrations[1] per line (least squares means ± s.e.m.) and significance[2] between lines. Experiments 3 and 4

Age:	36			41			46		
Line:	H	L		H	L		H	L	
Experiment 3:									
ln GH	5.30 ± .10	4.81 ± .10	**	5.04 ± .10	5.00 ± .10	ns	4.94 ± .10	4.70 ± .10	ns
T3	1.19 ± .06	0.83 ± .06	***	1.23 ± .06	0.91 ± .06	***	0.85 ± .06	0.79 ± .06	ns
T4	11.09 ± .67	13.86 ± .67	**	11.31 ± .67	11.79 ± .67	ns	14.71 ± .67	12.63 ± .45	*
SmC	6.61 ± .45	7.00 ± .45	ns	7.71 ± .45	7.47 ± .45	ns	7.15 ± .45	7.15 ± .45	ns
Experiment 4:									
ln GH	4.64 ± .10	5.18 ± .10	***	4.51 ± .10	4.72 ± .10	*	4.63 ± .10	4.96 ± .10	**
T3	0.77 ± .09	0.82 ± .07	ns	0.82 ± .07	0.78 ± .07	ns	1.18 ± .07	1.05 ± .07	ns
T4	9.11 ± .55	11.25 ± .53	**	12.51 ± .55	10.36 ± .56	**	14.11 ± .55	15.62 ± .53	ns
SmC	7.25 ± .40	7.21 ± .39	ns	7.07 ± .40	6.95 ± .43	ns	7.45 ± .40	6.87 ± .39	ns
Combined Exp. 3 + 4:									
ln GH	4.98 ± .09	5.00 ± .09	ns	4.78 ± .09	4.86 ± .09	ns	4.79 ± .09	4.87 ± .09	ns
T3	1.05 ± .07	0.83 ± .06	*	1.03 ± .06	0.85 ± .06	*	1.01 ± .06	0.92 ± .09	ns
T4	10.13 ± .53	12.52 ± .53	**	11.88 ± .53	11.05 ± .53	ns	14.41 ± .53	13.99 ± .52	ns
SmC	6.92 ± .31	7.10 ± .31	ns	7.40 ± .31	7.22 ± .31	ns	7.29 ± .31	7.01 ± .31	ns

1) Growth hormone (GH), Triiodothyronine (T3), Thyroxine (T4): ng/ml; Somatomedine-C (SmC): I.U./ml

2) T-test between H and L line within day:
ns: P>.05; *: P<.05; **: P<.01; ***: P<.001

Figure 7. (A) Metabolized energy (ME), heat production (H) and energy balance (EB). (B) protein retention (PR) and fat deposition (FD). Least squares means ± s.e.m. for H and L line from the combined results of Experiment 3 and 4. Significance for the difference between lines within a balance period in a t-test is indicated: ns P>.05; * P<.05; ** P<.01; *** P<.001



The overall ME-intake was not significantly different between the two lines (Fig. 7A), although ME was never lower in the H than in L line. The difference between H and L line was significant ($P<.05$) within balance period 2, which caused a significant line x balance period interaction. When ME was separated into EB and H-production, no significant differences between the lines in H-production were found in any period (Fig. 7A). H-production differed ($P<.001$) between balance periods; it was highest in balance period 1 and lowest in period 3.

The EB during Experiment 3 was significantly ($P<.05$) higher in the H line than in L line, but this difference was not significant in Experiment 4. In the combined analysis of Experiment 3 and 4, several significant interactions were found between line, balance period and experiment. The EB of the H line was significantly higher than that of the L line during balance periods 1 and 2; the difference in period 2 was larger in Experiment 3 (127.7 kJ kg^{-1} d^{-1}; $P<.001$) than in Experiment 4 (56.3 kJ kg^{-1} d^{-1}; $P<.05$). For the L line, the EB was lowest in balance period 2, but no significant differences between balance periods were found in the H line.

The protein retention (PR) was not significantly influenced by balance period or line. Only in the combined analysis of Experiment 3 and 4 a significant difference was found between H and L line in balance period 2 ($P<.05$; Fig. 7B).

Fat deposition (FD) was not significantly different between lines or balance periods, although for this trait significant interactions between line, experiment and balance period were found. FD was higher in Experiment 4 than in Experiment 3, but the difference was bigger for L line than for H line. The FD in the L line was decreased in balance period 2 and increased in balance period 3 (Fig. 7B). These differences were not found in H line.

Discussion

Experiments 1 and 2

The birds were normothermic during a wide range of temperatures. They achieved this by adjusting feed intake. Feed intake was reduced, particularly at higher temperatures in order to minimize heat production and thus heat load. Reduced growth rate was a consequence. At temperatures of 30°C and lower, body temperature remained constant; 35°C caused hyperthermia equally in H and L line, as was previously found by Donker *et al.* (submitted-b). Romijn and Lokhorst (1961) reported hyperthermia at temperatures above 32°C. In Experiment 1, a marked difference between the H and L line was found in heat production at temperatures of 30°C and lower. The lower feed intake in this experiment in H line was associated with this lower heat production.

However, also an increased efficiency was evident, as growth rate was not lower than in the L line. The lower level of heat production during the phase of decreasing temperature in Experiment 1 and the phase of increasing temperature in Experiment 2 was, at least partly, related to the lower feed intake during these periods. Comparable differences between the temperature-increasing and -decreasing part were reported by Henken *et al.* (1982b), indicating an age dependent difference. Also Kuenzel and Kuenzel (1977) reported an age dependent decrease in basal metabolic rate in young broilers as well as Leghorns.

The differences between regression coefficients in the regression analysis of heat production on temperature in the different sections was often very small, even when significant. No clear thermo-neutral zone could be demonstrated in either line, during light or dark periods. Therefore, no good estimates of T_c could be made. The absence of a critical temperature in the case of *ad libitum* feeding was also reported by Henken *et al.* (1982b). In contrast to the results they obtained, better estimates could not be found during the dark than during the light period.

Because no differences between the lines existed in critical temperature or reaction in feed intake or body temperature, it is concluded that these lines can be compared for metabolic traits at any temperature. However, only temperatures between 10° and 30°C are in the thermoneutral zone for both lines. Feed intake is reduced at temperature above 25°C. Thus for comparisons under normal physiological conditions temperatures between 10° and 25°C are preferable.

Experiments 3 and 4

The differences found in antibody titers between the H and L line were as expected (Van der Zijpp *et al.*, 1988). In addition, the higher body weight in the L line is usually found in these lines (van der Zijpp and Nieuwland, unpublished data; Donker *et al.*, submitted-b).

An increased relative weight of spleen in the H line has been reported before (Donker *et al.*, submitted-a,-b). However, the increased weight of thymus and bursa in the H line were not found before. Earlier studies (Ubosi *et al.*, 1985; Donker *et al.*, submitted-a,-b) showed either no difference between selection lines or a slightly negative relation between selection for high antibody production and the weights of these organs. Those results were obtained in non-immunized birds, or in older animals after a secondary immunization. The relatively short period after the primary immunization could have accounted for increased thymus and bursa weight. The increased liver weight in H line compared to the L line in these experiments could be an indication of a somewhat increased metabolic activity, as will be discussed below.

Growth hormone, thyroid hormones and somatomedines play an important role in regulation of growth, growth rate and composition of growth (Scanes, 1987; Decuypere and Buyse, 1988). However, no consistent differences in the plasma levels of the hormones measured between H and L lines or balance periods were detected in these experiments. Only the levels of T3 showed some consistent differences across time and across experiments; these concentrations were higher in the H line. No significant correlation with either total or 2ME-resistant antibody titer was found, however. A similar increased T3 level was found in the high antibody selection line developed by Siegel and Gross (1978) (Martin *et al.*, 1988), but, also in those lines, no clear relation between antibody titers and T3 levels was found. Several other studies dealt with interactions between thyroid status and immune responses (Yam *et al.*, 1981; Scott *et al.*, 1985; Martin *et al.*, 1988). However, conflicting results were found in those experiments. In hypothyroid birds, T3 and T4 plasma levels decreased, but increased as well as decreased antibody titers were reported. Although GH and intact thyroid function are essential for the development of the immune system (Berczi, 1986; Scanes, 1987), antibody production is probably not significantly altered by thyroid hormones as long as these hormone levels are within the physiological range (Mashaly *et al.*, 1983). Possibly, thyroid hormones affect immune responses only indirectly by modifying metabolic rate (Berzci, 1986).

Hormone levels were not significantly influenced by the immunization in these experiments. Trout *et al.* (1988) reported significant changes in T3 and T4 levels after immunization with *Brucella abortus* antigen. However, significant changes were found only within 2 - 48 hours after immunization.

Like the small and inconsistent differences in the growth-related hormone levels, the differences in growth, protein retention and fat deposition between H and L line and between balance periods also were small. The difference in body weight between the lines remained constant because growth rate was the same in the two lines. Thus, although increased levels of T3 are usually related to higher growth rate (Decuypere and Buyse, 1988), this was not the case here. However, before immunization, the retained energy was higher in the H line. This was apparent as a higher deposition of fat. The ME intake was not different during this period, indicating an increased efficiency in the H line. During the second balance period, when the immune response was mounted, the difference in energy retention and fat deposition between H and L line was bigger than before. Also a higher protein retention in the H line was found in this period. Comparable differences were found by Henken and Brandsma (1982) between SRBC and sham immunized chickens. However, they reported an increased EB, PR and FD compared with the first balance

period in SRBC immunized birds, whereas we found decreased levels in the L line compared to the first balance period. Thus, a decreased efficiency in energy utilization in the L line after immunization is evident. Fat deposition is stimulated by high plasma concentrations of corticosterone (Brown *et al.*, 1958; Nagra and Meijer, 1963; Siegel, 1980; Henken and Brandsma, 1982; Kafri *et al.*, 1988). Plasma corticosterone concentration can be increased after an immunization (Siegel *et al.*, 1985; Besedovski *et al.*, 1986; Trout *et al.*, 1988). Thus a higher fat deposition in the H line could be associated with relatively higher corticosterone levels during this phase of the immune response.

During the third balance period, after the peak day in antibody titer (day 5), the differences in energy balance and protein retention disappeared again. In the L line, an increased fat deposition, which apparently was some compensation for the lower deposition in the previous period, was found.

During the experiments, ME intake and heat production decreased with age as did relative feed intake in Experiment 1 and 2. Comparable age related decreased intake was also reported by Kuenzel and Kuenzel (1977), Henken and Brandsma (1982) and Siegel *et al.* (1982b).

The response in cloacal temperature, feed intake and heat production to changing environmental temperature was the same in H and L line. No thermoneutral zone was found, thus no accurate estimates of the lower critical temperature could be made in either line. Heat production at the same temperature was higher in L line in one experiment. Some differences between the H and L selection line before and during the mounting of an immune response were evident. During the immune response, fat deposition was reduced in the L line. A slightly increased efficiency in use of metabolized energy was found in the H line. However, it is not very likely that these differences account for the different levels of the immune response found between these lines.

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References

Besedovsky H.O., E. Sorkin, M. Keller and J. Muller, 1975. Changes in blood hormone levels during the immune response. *Proc. Soc. Exp. Biol. Med.* 150: 466-470.

Besedovsky H.O., A. del Rey and E. Sorkin, 1986. Regulatory immune-neuroendocrine feedback signals. pg. 241-249 in: Berczi I. (ed.). *Pituitary function and immunity (chapter 11)*. CRC Press Inc., Boca Raton, FA.

Berczi I., 1986. Immunoregulation by pituitary hormones. pg. 227-240 in: Berczi I. (ed.). *Pituitary function and immunity (chapter 10)*. CRC Press Inc., Boca Raton, FA.

Brody S., 1945. *Bioenergetics and growth*. Reinhold Publishing Co., New York. 1023 pp.

Brown K.I., D.J. Brown and R.K. Meyer, 1958. Effect of surgical trauma, ACTH and adrenal cortical hormones on electrolytes, water balance and gluconeogenesis in male chickens. *Am. J. Physiol.* 192: 43-50.

Decuypere E. and J. Buyse, 1988. Thyroid hormones, corticosterone, growth hormone and somatomedins in avian species: general effects and possible implications in fattening. pg. 295-312 in: Leclercq and Whitehead (eds.) *Genetic, metabolic and hormonal basis of leanness in domestic birds*. Butterworth, London.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp. (*submitted-a*) Plaque-forming-cell assay and lymphoid organ development in chicken lines selected for high and low immune responsiveness.

Donker R.A., A.G.J.C. Swinkels and A.J. van der Zijpp. (*submitted-b*) The effect of environmental temperature on antibody production and some physiological parameters in chicken lines, selected for humoral immune responsiveness.

Harvey S. and C.G. Scanes, 1977. Purification and radioimmunoassay of chicken growth hormone. *J. Endocrinol.* 73: 321-330.

Henken A.M. and H.A. Brandsma, 1982. The effect of environmental temperature on immune response and metabolism of the young chicken. 2. Effect of the immune response to sheep red blood cells on energy metabolism. *Poultry Sci.* 61: 1667-1673.

Henken A.M., A.M.J. Groote Schaarsberg and M.G.B. Nieuwland, 1983a. The effect of environmental temperature on immune response and metabolism of the young chicken. 3. Effect of environmental temperature on the humoral immune response following injection of sheep red blood cells. *Poultry Sci.* 62: 51-58.

Henken A.M., G.R. Kleinhout and W. van der Hel, 1982a. The effect of environmental temperature on immune response and metabolism of the young chicken. 1. Effect of intramuscular injection on heat production. *Poultry Sci.* 61: 503-509.

Henken A.M., M.G.B. Nieuwland and G. Wensink, 1983b. The effect of environmental temperature on immune response and metabolism of the young chicken. 5. Effect of low environmental temperature on the humoral immune response to sheep red blood cells in relation to energy metabolism. *Poultry Sci.* 62: 1069-1074.

Henken A.M., M.W.A. Verstegen, W. van der Hel and E.F. Knol, 1982b. Effect of environmental temperature on heat production in pullets in relation to feeding level. In: *Proc. 9th Symp. Energy Metab. Farm. Anim.*, 1982, Lillehammer, Norway.

Huybrechts L.M., D.B. King, T.J. Lauterio, T.J. March and C.G. Scanes, 1985. Plasma concentrations of Somatomedin-C in hypophysectomized, dwarf and

intact growing domestic fowl as determined by heterologous radioimmunoassay. *J. Endocrinol.* 104: 233-239.

Kafri I., W. Rosebrough, J.P. McMurtry and C.N. Steele, 1988. Corticosterone implants and supplemental dietary ascorbic acid effects on lipid metabolism in broiler chicks. *Poultry Sci.* 67: 1356-1359.

Kampen M. van, 1987. Climatic conditions on energy metabolism of laying hens. pg. 199-216 in: M.W.A. Verstegen and A.M. Henken (eds.): *Energy metabolism in farm animals.* Martinus Nijhoff publishers. Dordrecht, Boston, Lancaster.

Kuenzel W.J. and N.T. Kuenzel, 1977. Basal metabolic rate in growing chicks *gallus domesticus. Poultry Sci.* 56: 619-627.

Martin A., F.M.A. McNabb and P.B. Siegel, 1988. Thiouracil and antibody titers of chickens lines divergently selected for antibody response to sheep erythrocytes. *Dev. Comp. Immunol.* 12: 611-619.

Mashaly M.M., S.L. Youtz and R.F. Wideman Jr., 1983. Hypothyroidism and antibody production in immature chickens. *Immunol. Commun.* 12: 551-563.

Nagra C.L. and R.K. Meijer, 1963. Influence of corticosterone on the metabolism of palmitate and glucose in cockerels. *Gen. Comp. Endocrinol.* 3: 131-138.

Romijn C. and W. Lokhorst, 1961. Climate and poultry. Heat regulation in the fowl. *Tijdschr. Diergeneesk.* 86: 153-172.

SAS, 1985. SAS User's guide: Statistics, Version 5 edition. Cary, NC: SAS Institute Inc.

Scanes C.S., 1987. The physiology of growth, growth hormone and other growth factors in poultry. *CRC Crit. Rev. Poult. Biol.* 1: 51-105.

Scott T., A. van der Zijpp and B. Glick, 1985. Effect of thiouracil-induced hypothyroidism on the humoral immunity of New Hampshire chickens. *Poultry Sci.* 64: 2211-2217.

Siegel H.S., 1980. Physiological Stress in Birds. *Bio Science* 30: 529-534.

Siegel P.B. and W.B. Gross, 1978. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Sci.* 59: 1-5.

Siegel P.B., W.B. Gross and J.A. Cherry, 1982a. Correlated responses of chickens to selection for production of antibodies to sheep erythrocytes. *Anim. Blood Gr. Biochem. Gen.* 13: 291-297.

Siegel H.S., A.M. Henken, M.W.A. Verstegen and W. van der Hel, 1982b. Heat production during the induction of an immune response to sheep red blood cells in growing pullets. *Poultry Sci.* 61: 2296-2300.

Siegel H.S., N.R. Gould & J.W. Latimer, 1985. Splenic leukocytes from chickens injected with Salmonella pullorum antigen stimulate production of corticosteroids by isolated adrenal cells. *Proc. Soc. Exp. Biol. Med.* 178:

Trout J.M., M.M. Mashaly and H.S. Siegel, 1988. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Dev. Comp. Immunol.* 12: 331-346.

Ubosi C.O., W.B. Gross and P.B. Siegel, 1985. Divergent selection of chickens for antibody production to sheep erythrocytes: Age effect in parental lines and their crosses. *Avian Dis.* 29: 150-158.

Verstegen M.W.A., W. van der Hel, H.A. Brandsma, A.M. Henken and A.M. Bransen, 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. pg. 21-62. In: M.W.A. Verstegen and A.M. Henken (eds.): *Energy metabolism in farm animals.* Martinus Nijhoff publishers. Dordrecht, Boston, Lancaster.

Wenk C. and A.J.H. van ES, 1976. Energy metabolism of growing chickens as related to their physical activity. *Proc. 7th Symp. Energy Metab. Farm Anim. (Vichy):* 189-192.

Yam D., D. Heller and N. Snapir, 1981. The effect of the thyroidal state on the immunological state of the chicken. *Dev. Comp. Immunol.* 5: 483-490.

Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterisation of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris):* 211-215.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poultry Sci.* 59: 1363-1369.

Zijpp A.J. van der, J.J. Blankert, E. Egberts and M.G.J. Tilanus, 1988. Advances in genetic disease resistance in poultry. pg. 131-138. In: S. Korver, H.A.M. van der Steen, J.A.M. van Arendonk, H. Bakker, E.W. Brascamp and J. Dommerholt (compilers). *Advances in animal breeding.* Pudoc, Wageningen, The Netherlands.

Chapter VII

Antibody production in chicken lines divergently selected for immune responsiveness, in relation to energy metabolism.

2. Energy metabolism during the immune response under hyperthermic conditions.

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Abstract

Energy metabolism with associated physiological responses was studied, during the immune response to SRBC immunization, in two chicken lines. These lines were divergently selected for the immune response to SRBC.

In each of two experiments, one group of birds (8 birds of either line) was kept in a climate respiration chamber at 25°C (70% RH) and served as a control (CC).

In the first experiment, an acute heat stress group (CH) was kept in a second, identical, climate respiration chamber. After an initial moderate climate (25°C and 70% RH) the birds were exposed to 35°C and 60% RH, immediately after an immunization with SRBC.

In the second experiment a group was kept in the second climate respiration chamber, which was continuously kept at 35°C and 60% RH (HH).

All groups consisted of 16 cockerels, 8 high and 8 low line birds, but the lines were kept in separate cages.

The energy balance, heat production, growth rate and fat deposition were decreased during the high temperature periods to the same extent in both lines. Protein retention was decreased during the 5-day balance period immediately after the temperature change in the CH treatment. Cloacal temperature was increased in the high temperature groups. Plasma concentrations of corticosterone, T3 and T4 were not different between treatments or lines. Somatomedine-C concentration was higher and growth hormone lower in the high temperature groups.

Antibody titers were consistently higher in the high selection line. In the low selection line, antibody titers were increased in the CH group 5 and 10 days after immunization and in the HH group 10 days after immunization compared to the low line birds in the CC control.

It was concluded that the differences in antibody production between the high and low selection line, and the influence of temperature on antibody production were not directly related to energy or protein metabolism.

Keywords: hyperthermia, immune response, SRBC, selection lines, metabolism,
Running title: High temperature, immune response and metabolism

Introduction

The influence of environmental temperature on antibody production can be immuno suppressive (Thaxton, 1978; Henken *et al.*, 1983a; Siegel, 1987; Donker *et al.*, submitted-d) or immuno stimulating (Henken *et al.*, 1983a; Anderson and Kühn, 1988; Donker *et al.*, submitted-d). Immunosuppressive action is usually found after acute thermal stress, in which the hypothalamo-pituitary-adrenal (HPA) axis is stimulated. Stimulation of this axis, which ends in corticosterone release, probably plays a key function in the suppression. However, results have not always been consistent (Heller *et al.*, 1979; Regnier *et al.*, 1980; Donker *et al.*, submitted-b).

Stimulatory effects on antibody production have been reported after non-acute thermal conditions, in which birds were acclimated to an environmental temperature just above the thermoneutral region (Henken *et al.*, 1983a; Donker *et al.*, submitted-d). It was postulated that in this process the hypothalamo-pituitary-thyroid (HHT) axis might be involved, with associated changes in metabolic rate (Henken *et al.*, 1983b; Donker *et al.*, submitted-d). The HHT axis takes part in the acclimation to changed environmental temperatures, and helps maintain homeostasis through influences on thermoregulation and redistribution of protein and fat metabolism. The effects of thyroid hormones on immune responsiveness recently have been the topic of intensive study (Yam *et al.*, 1981; Mashaly *et al.*, 1983; Marsh *et al.*, 1984a, 1984b; Gause and Marsh, 1985; Scott *et al.*, 1985; Bachman and Mashaly, 1986; Martin *et al.*, 1988), but the regulatory role is still not understood, due to conflicting results in hypothyroid as well as hyperthyroid birds.

By selection for antibody titers after immunization with sheep erythrocytes (SRBC), lines were obtained which differed in antibody production capacity (Van der Zijpp and Nieuwland, 1986). It is still unknown which functional changes cause these differences in antibody production capacity. However, since differences in bodyweight and growth rate are evident between the lines, it was suggested that part of the difference might be associated with changes in endocrine factors and associated metabolic changes.

The study of endocrine function and metabolic rate during the immune response in these lines is therefore relevant. Since metabolic rate is also greatly affected by environmental temperature (Henken *et al.*, 1983b), it is possible that the thermal requirements of the birds are altered in the selected birds. A combined study of endocrine function and metabolic rate during the immune response at high temperatures might give an idea of the altered thermal requirements of the selected birds, changes in endocrine function and the implications thereof for immune responsiveness.

In this study the influence of high environmental temperature was investigated in acclimated and non-acclimated chickens from the high and low selection line for immune responsiveness (Van der Zijpp and Nieuwland, 1986). The effects on plasma hormone levels (growth hormone, somatomedine, thyroid hormones, corticosterone) and energy- and protein balance were studied during the immune response after SRBC immunization.

Material and methods

Birds and pre-experimental conditions

Two experiments were carried out, using male birds which were genetically identical to the 7th generation of a high (H) and low (L) line, selected for antibody titer to sheep erythrocytes (SRBC) (Van der Zijpp and Nieuwland, 1986). For each experiment, a separate hatch was used. The birds were placed with lines separated, in brooder cages with water and commercial starter feed provided *ad libitum*.

Environmental temperature was gradually decreased during the first two weeks from approximately 32°C to approximately 25°C; the light regime was changed gradually from 23 h light/ 1 h dark to 12 h light/ 12 h dark during the first three weeks.

Individual vaccinations against Marek's disease (at 1 d of age), infectious bronchitis (2 d of age), Gumboro's disease (12 d of age) and Newcastle disease (18 d of age) were given.

To exclude large variation in weight and growth rate, a subgroup from the available birds was chosen. The selection was based on individual body weight at 22 d of age. The bodyweight of chicks used in the experiment was within a range of the median weight of that line ± 1 s.d.

Climate-respiration chambers

Two medium size climate respiration chambers (1.8 m^3 each) at the experimental unit of the Department of Animal Husbandry of the Agricultural University were used. The technical equipment and applications of these chambers is described elsewhere (Verstegen *et al.*, 1987). Within each chamber, a set of four brooder cages (± .5 x .4 x .4 m each; l x b x h) was placed. In each chamber, two cages with 4 H line birds and two cages with 4 L line birds were placed. Measurement of feed intake and excreta could be obtained for each cage separately; O_2-consumption and CO_2-production for indirect calorimetry (Verstegen *et al.*, 1987) could be measured for each chamber.

Experimental procedures

The birds were placed in the chambers at an age of 25 d (Fig. 1). Light regime was 12 h light/ 12 h dark throughout the experiments.

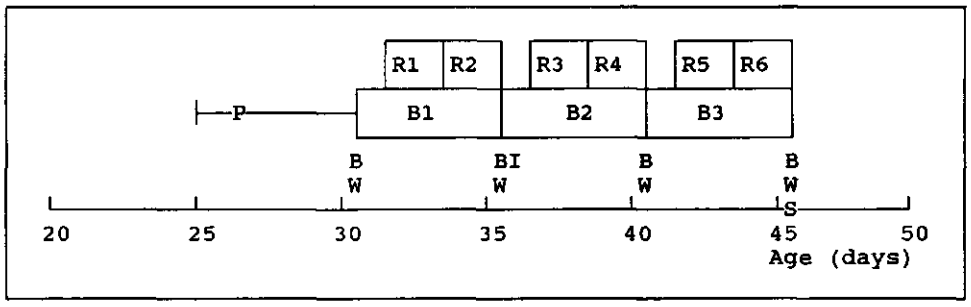
At 31 d of age all birds were removed from the chamber individually, with minimal disturbance for the other birds, in random order. A blood sample (± 1.5 ml) was taken from the ulnar vein (*vena cutanea ulnaris*) with a heparinized syringe. The entire procedure did not take longer than 45-60 seconds per bird since prolonged handling can cause increased circulating corticosterone (Beuving and Vonder, 1978). Immediately thereafter cloacal temperature was measured. The birds were weighed and replaced in the same cage as before. The first of three successive balance periods was started (Fig. 1). During a 5-day balance period, *ad libitum* feed intake was measured daily and excreta were collected. Chambers were not opened throughout a balance period. At the second day of a balance period the first of two successive 48-hour respiration periods was started (Fig. 1). Continuous measurements of O_2-consumption and CO_2-production were made during a respiration period. At the end of each balance period the same procedure for blood sampling and temperature measurement (as described above) was followed. The amount of excreta was assessed and samples of excreta and refused feed were taken.

Prior to the second balance period, the birds were immunized intramuscularly with 1 ml 25% (v/v) packed SRBC in saline.

The chickens were killed by cervical dislocation after the third balance period and the liver, thymus, spleen, bursa and adrenals were excised and weighed.

Figure 1. Experimental layout.

P: Pre-experimental adaptation period in climate respiration chambers
B1, B2, B3: Balance period 1, 2, 3
R1, .., R6: 2-day respiration periods
B: Blood sampling
I: Immunization with SRBC
W: Weighing
S: Slaughter and dissection



Experimental treatments

Three different experimental groups were distinguished based on the results of Donker *et al.* (submitted-d).

A control treatment (CC) was included in both experiments: the chamber temperature was regulated at 25°C at all time, with a relative humidity (RH) of 70%.

In Experiment 1 the other chamber provided an acute thermal stress (CH). The initial temperature was 25°C (70% RH) and this was abruptly increased to 35°C (60% RH) immediately after immunization.

In Experiment 2 the other chamber provided a continuous hot environment (HH; 35°C, 60% RH). This temperature was set before the adaptation period (Fig. 1), thus giving birds the opportunity to acclimate.

Assays

All food and excreta samples were analyzed for energy and nitrogen contents. In addition energy and nitrogen loss in dust and airflow (NH_3) from the chambers were assessed.

The blood samples were assayed for packed cell volume (PCV) and the plasma for total and 2-mercaptoethanol (2ME-) resistant antibody titer to SRBC (Van der Zijpp and Leenstra, 1982). Concentrations of corticosterone[1)] (Beuving and Vonder, 1981), triiodothyronine (T_3) (commercial kit: Dac-Cel T3; Welcome Reagents Ltd, Beckenham, Kent U.K.), thyroxine (T_4) (commercial kit: T4 RIA(PEG); Abbott Diagnostic Division, Antwerp, Belgium), growth hormone (GH) (Harvey and Scanes, 1977) and somatomedine-C (SmC) (Huybrechts *et al.*, 1985) were measured. Thyroid peroxidase activity (TPO) was estimated in the thyroid *post mortem* as an estimate of the T_4 reservoir.

Statistical analysis

All metabolic traits are expressed on the basis of kg^{-1}, as discussed by Donker *et al.* (submitted-a).

Heat production (H) was calculated from oxygen consumption and carbon dioxide production with the formula of Romijn and Lokhorst (1961):

$H\ (kJ) = 16.20 \times O_2\ (l) + 5.00 \times CO_2\ (l)$.

Balance characteristics analyzed were ME-intake ($kJ \times kg^{-1} \times day^{-1}$), energy balance EB ($kJ \times kg^{-1} \times day^{-1}$), protein retention PR ($g \times kg^{-1} \times day^{-1}$) and fat deposition FD ($g \times kg^{-1} \times day^{-1}$)). ME was calculated from energy in feed minus energy lost in excreta, dust and outgoing air; PR was calculated from N in

1) Plasma corticosterone concentrations were obtained in samples which were pooled within a day of two birds from the same cage.

feed minus N lost in excreta, dust and outgoing air (NH_3); EB was calculated as ME minus H, in which H (heat production) was calculated by dividing total heat production per chamber among cages relative to ME intake; FD was calculated from the difference in energy retention between EB and PR.

Analyses of variance were performed using the SAS procedures (SAS, 1985).

Individual data were analyzed within sampling day, using a model containing line and treatment as factors. Characteristics analyzed were growth, relative growth, antibody titer (total and 2ME-resistant), GH, T3, T4, SmC, TPO and relative organ weights. A line x treatment interaction was initially included, but excluded from the model if not significant (P>.05).

Balance data were analyzed in a comparable manner as individual data, but on the basis of cage values.

Results

Body weight and growth rate

In Experiment 1, body weight was higher at 31 d of age in the CH treatment group (Fig. 2; P<.05). Because of the higher growth rate in the CC treatment during the first balance period (P<.05) the weight was not different at 36 d of age. Lower growth during balance periods 2 and 3 (P<.01) in the CH treatment resulted in lower weights in this treatment at 41 and 46 d of age. In Experiment 2 body weight and growth rate were always lower in the HH treatment (P<.001, Fig. 2; P<.01). Body weight was higher in the L line than in the H line in Experiment 1 on all days (P<.01), but not in Experiment 2. Growth rate was not different between the lines.

Relative organ weights

The relative weights of liver, spleen, bursa and thymus were decreased by the CH treatment (Exp. 1) and the HH treatment (Exp. 2) (P<.001) (Table 1.). Higher spleen weights (P<.001) and bursa weights (P<.05) were found in the H line (Table 1). The adrenal weight was not significantly influenced by the treatments, but was higher in the H line in Experiment 1 (P<.01; Table 1).

Cloacal temperature

Cloacal temperature was increased (P<.001) when the birds were kept at 35°C in the CH treatment (Exp. 1) or HH treatment (Exp. 2). Average temperature was 41.9°C (CH and HH) vs. 41.5°C (CC). Temperatures were the same in H and L line.

Figure 2. Body weight (g) per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001

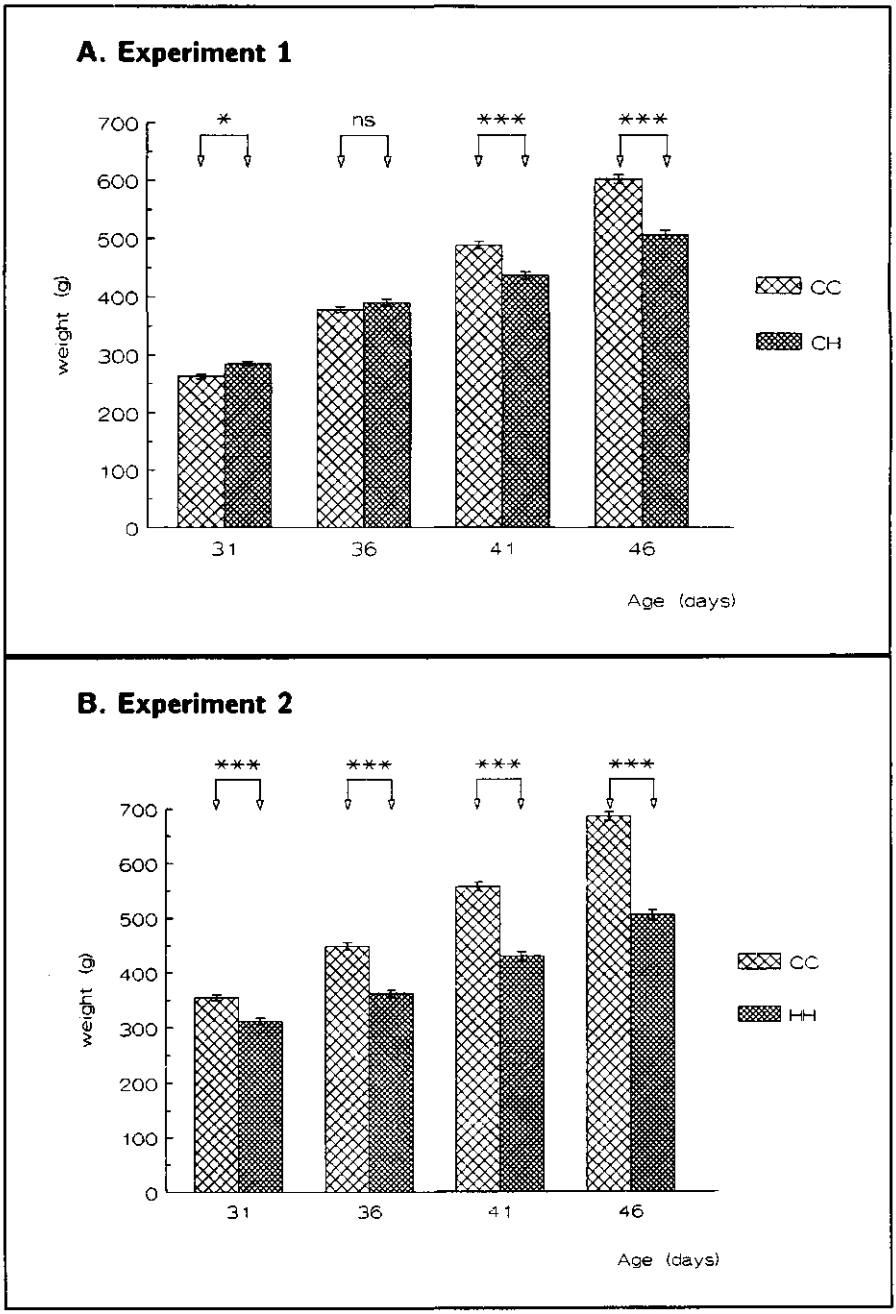


Table 1. Relative organ weight[1)] after dissection. Least squares means[2)] ± s.e.m.

Experiment 1

Treatment:	CC		HH	
Line:	High	Low	High	Low
Liver	2.49^{a} ± .06	2.40^{a} ± .06	2.02^{b} ± .06	2.07^{b} ± .06
Spleen	0.30^{a} ± .01	0.18^{bc} ± .01	0.21^{b} ± .01	0.15^{c} ± .01
Bursa	0.66^{a} ± .03	0.56^{b} ± .03	0.50^{bc} ± .03	0.44^{c} ± .03
Thymus	0.68^{a} ± .04	0.68^{a} ± .04	0.42^{b} ± .04	0.48^{b} ± .04
Adrenals	0.011^{a} ± .001	0.008^{b} ± .001	0.010^{ab} ± .001	0.009^{b} ± .001

Experiment 2

Treatment:	CC		HH	
Line:	High	Low	High	Low
Liver	2.29 ± .12	2.31 ± .12	2.03 ± .12	1.88 ± .12
Spleen	0.25^{a} ± .01	0.19^{b} ± .01	0.18^{b} ± .01	0.14^{c} ± .01
Bursa	0.57^{a} ± .04	0.50^{a} ± .04	0.48^{a} ± .04	0.34^{b} ± .04
Thymus	0.73^{a} ± .04	0.63^{a} ± .04	0.36^{b} ± .04	0.40^{b} ± .04
Adrenals	0.008 ± .001	0.009 ± .001	0.011 ± .001	0.009 ± .001

1) Organ weight / body weight x 100 %
2) Lsm within the same row bearing different superscripts differ significantly ($P<.05$)

Antibody titers

After immunization, total and 2ME-resistant antibody titers were higher in the H line than in the L line ($P<.001$; Fig. 3). Significant differences between treatments were found only in the L line. Higher total titers were found in Experiment 1 in CH treatment on day 41 ($P<.05$) and in Experiment 2 in HH treatment on day 46 ($P<.05$). Higher 2ME-resistant titers were found in CH and HH treatment on day 46 in Experiment 1 and 2 ($P<.05$).

Packed cell volume (PCV)

The PCV was lower in birds kept in the CH or HH treatments at high temperatures (Fig. 4). The PCV was higher in the H line, but the difference was significant ($P<.05$) only on days 31 and 46 in Experiment 1.

Figure 3. Total (——)and 2ME-resistant (– – –) antibody titer per line and treatment.
Least squares means ± s.e. Lsm bearing different superscripts within a day differ significantly (P<.05)

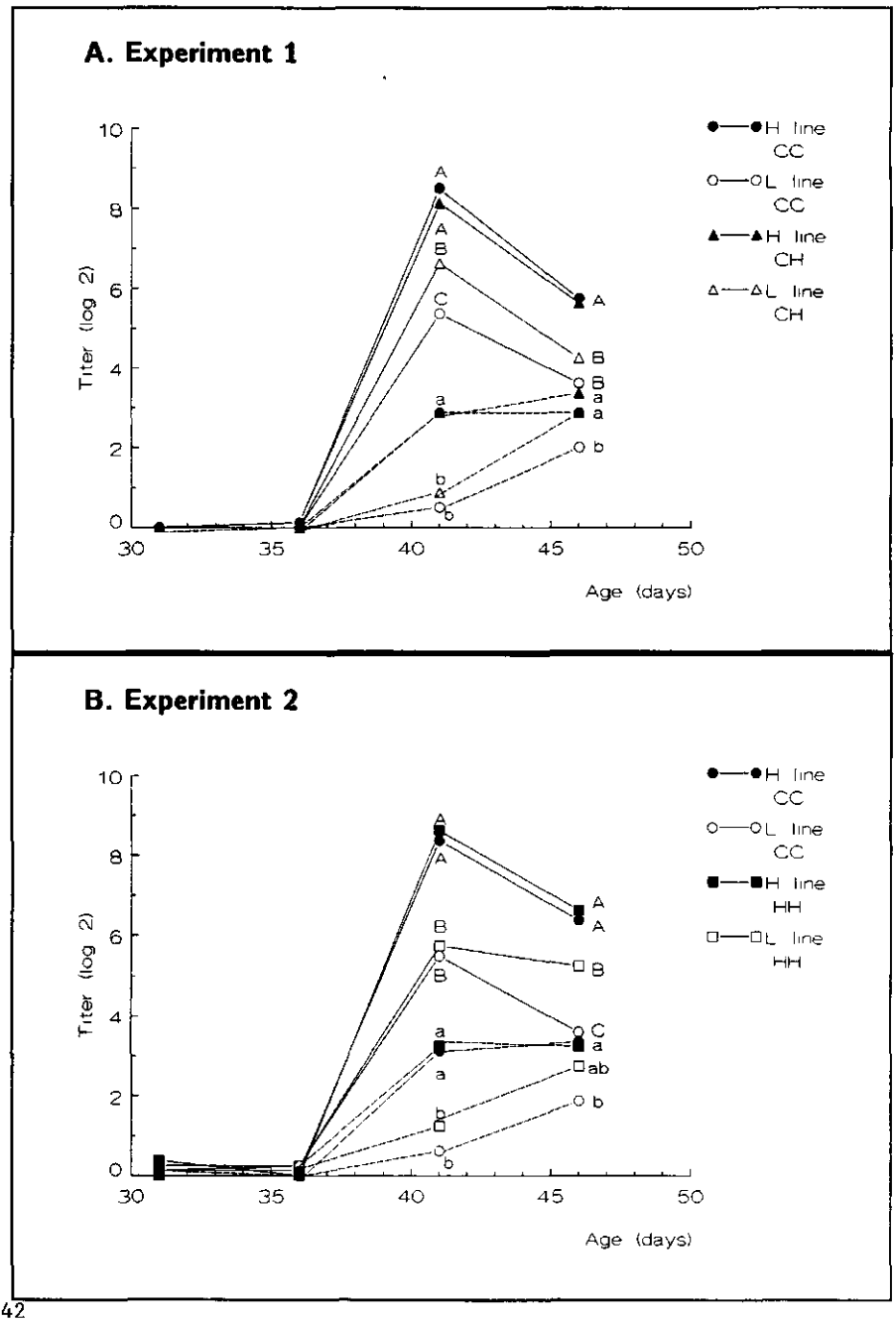
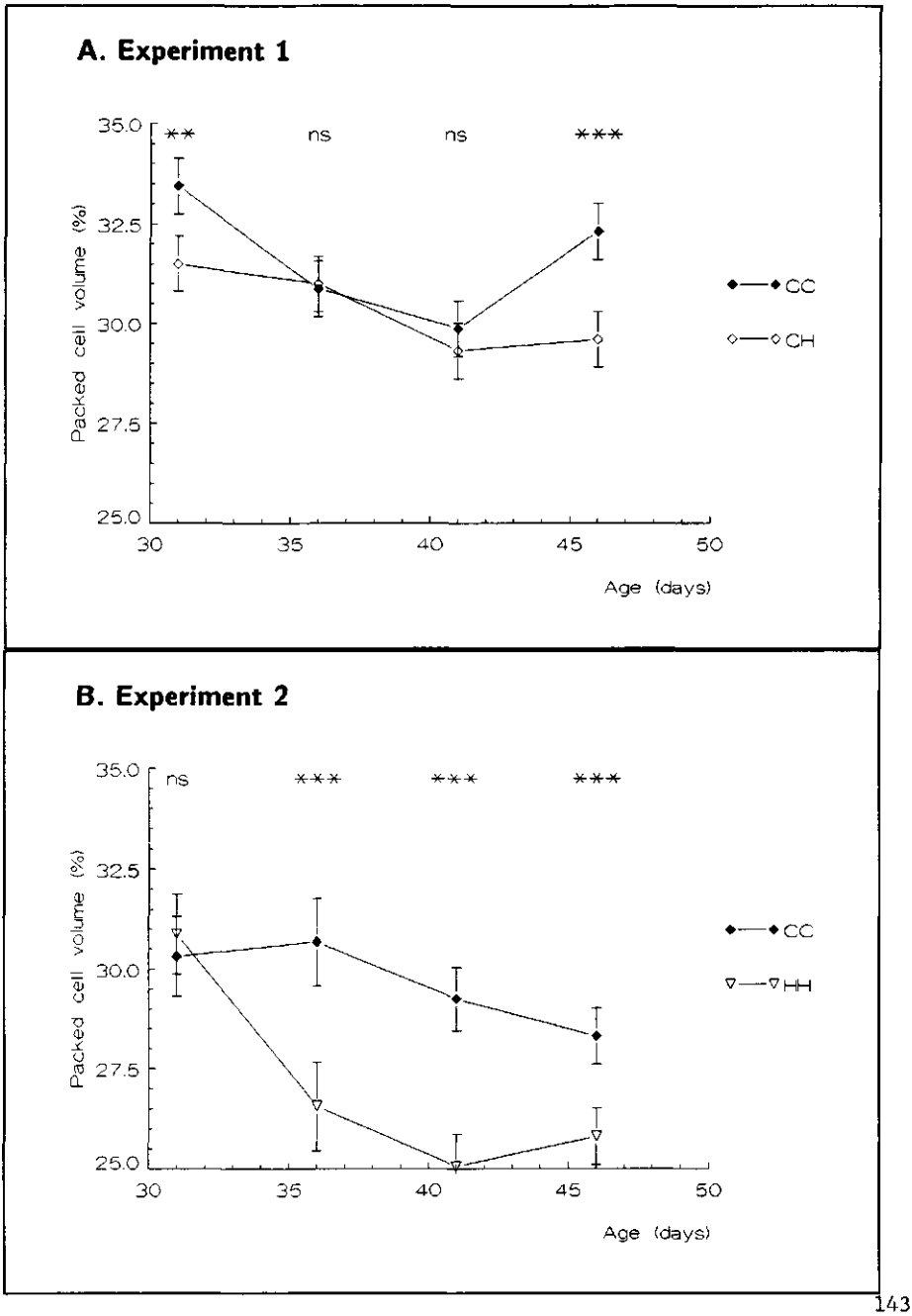


Figure 4. Packed cell volume (%) per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001



Plasma hormone concentrations

Mean plasma corticosterone concentrations varied between .8 and 5.0 ng/ml. No consistent differences between treatments or H and L selection line were found.

The average plasma concentration of the thyroid hormone T3 varied between .75 and 2.00 ng/ml, but was not significantly different between lines or treatments.

The average concentration of T4 hormone was higher in CC treatment than in CH before the immunization (13.0 vs 10.2 ng/ml; $P<.01$), but concentrations were higher in CH treatment thereafter (13.0 vs 11.9 ng/ml; $P<.01$). In Experiment 2 the concentration of T4 was significantly higher in HH treatment than in CC before immunization (12.2 vs 8.9 ng/ml; $P<.01$), but concentrations were not different thereafter (± 10 ng/ml).

Concentrations of GH were lower at high temperatures (Fig. 5A, 5B). GH-concentrations were the same in H and L line.

The concentrations of SmC were higher at high temperatures (Fig. 5C, 5D). The concentrations of SmC were lower in the H line, but only significantly in Experiment 2. In Experiment 2 the difference was on average .5 ng/ml ($P<.05$ on d 31, d 41; $P<.001$ on d 36).

Thyroid peroxidase activity

The thyroid peroxidase activity, which is a measure for the amount of T4 present in the thyroid, was .406 mμ/mg thyroid in Experiment 1 for the H line and .745 for the L line. In Experiment 2 the peroxidase activity was .103 mμ/mg thyroid in the H line and .248 in the L line. The difference was significant only in Experiment 2 ($P<.001$). No treatment influence was found on peroxidase activity.

Balance characteristics

Metabolizable energy and heat production in Experiment 1 were the same in CC and CH treatment during balance period 1 (Fig. 6A). They were higher in CC treatment in balance period 2. The ME was higher in CC than in CH treatment also during balance period 3. The energy balance was always higher in CC treatment, but the difference was bigger in balance periods 2 and 3 than in period 1 (Fig. 6A).

In Experiment 2, metabolizable energy, heat production and energy balance were higher in the CC treatment than in the HH treatment in all three balance periods (Fig. 6B).

No significant differences in metabolizable energy, heat production or energy balance were found between the H and L line in these experiments.

Figure 5. Plasma growth hormone (GH) and somatomedine-C (Sm-C) concentrations per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001

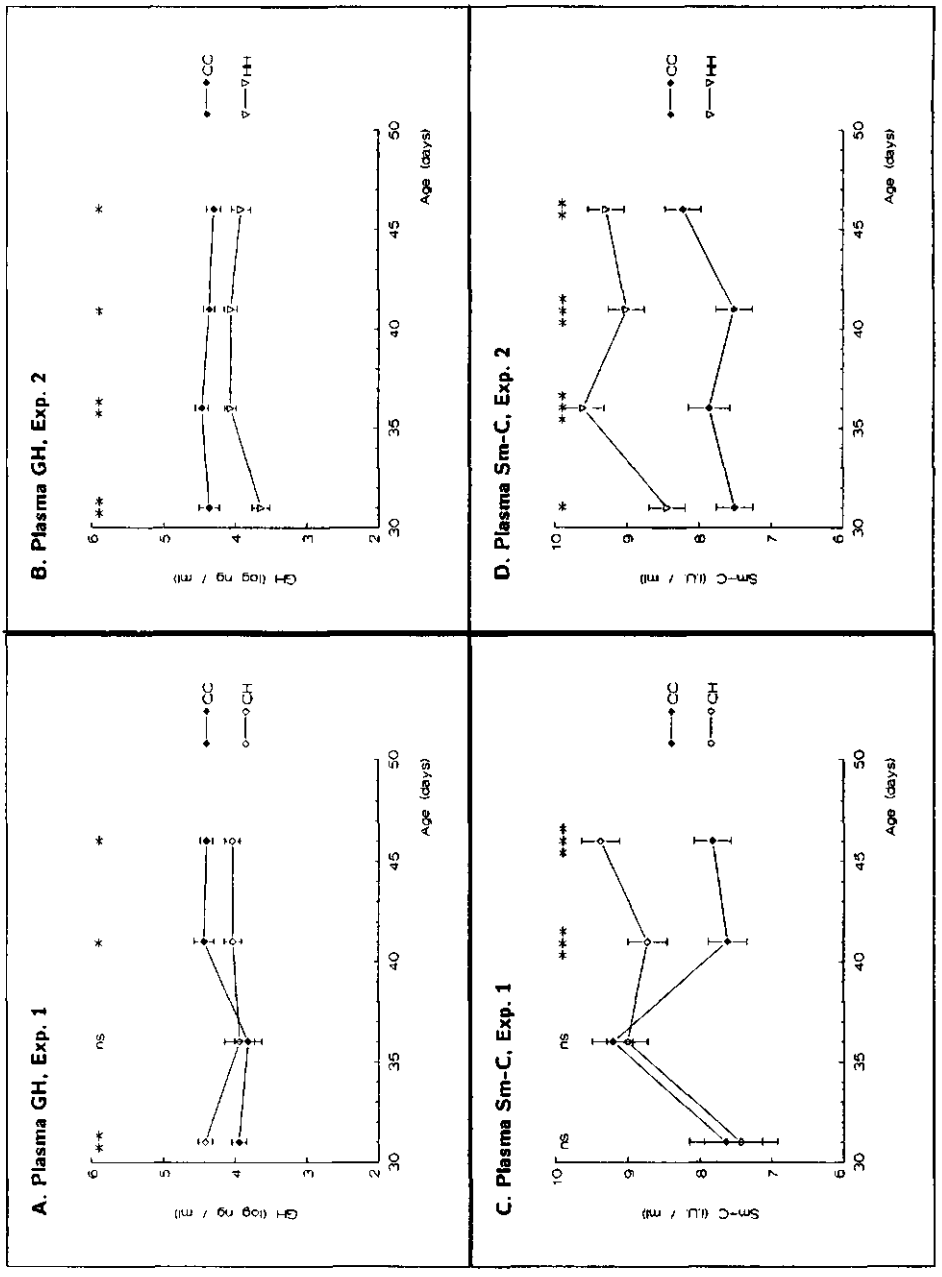


Figure 6. Metabolizable energy (ME), heat production (HP) and energy balance (EB) per balance period and treatment.
Least squares means ± s.e.m. The significance between treatments within a balance period is indicated: * P<.05, ** P<.01, *** P<.001

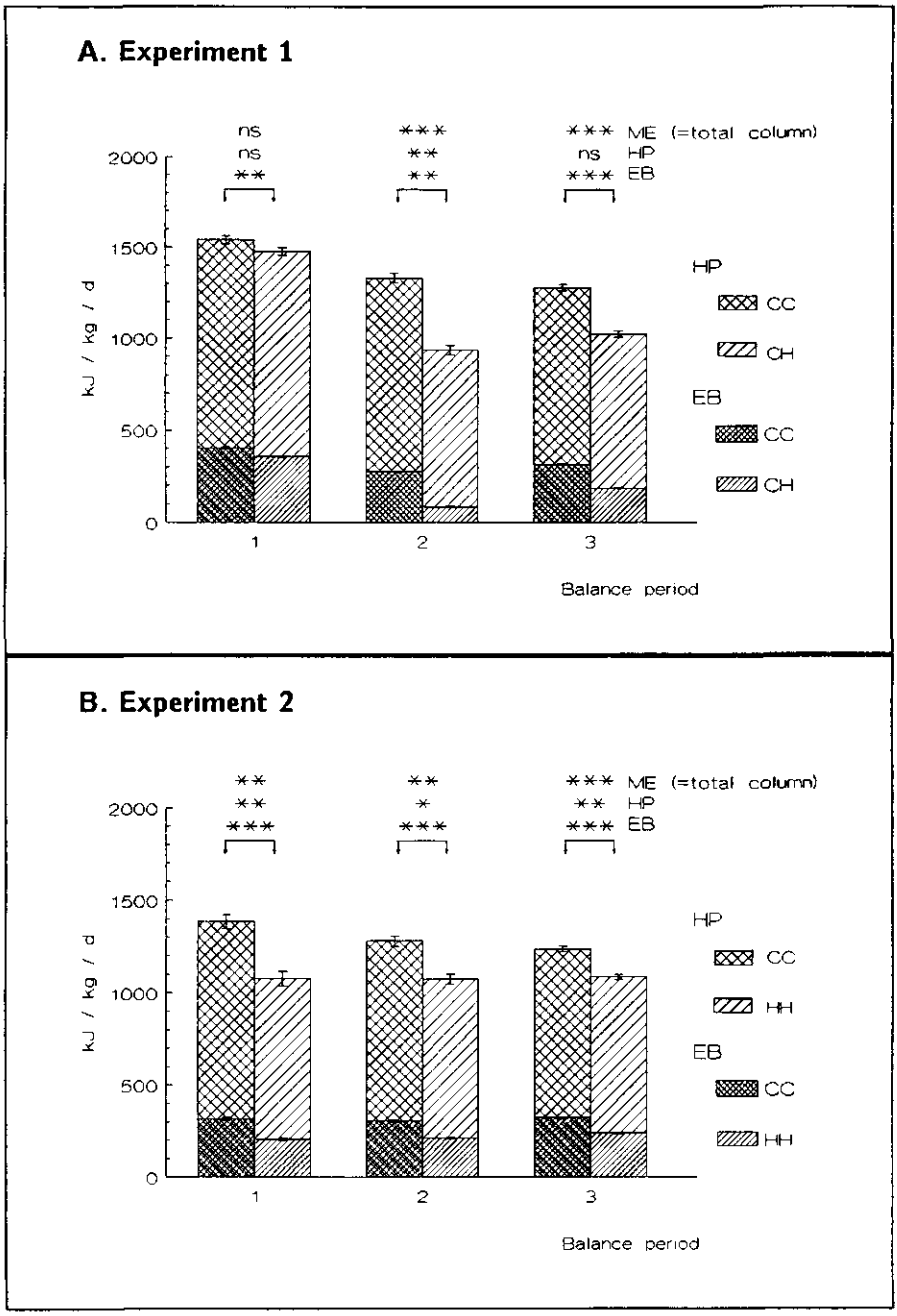
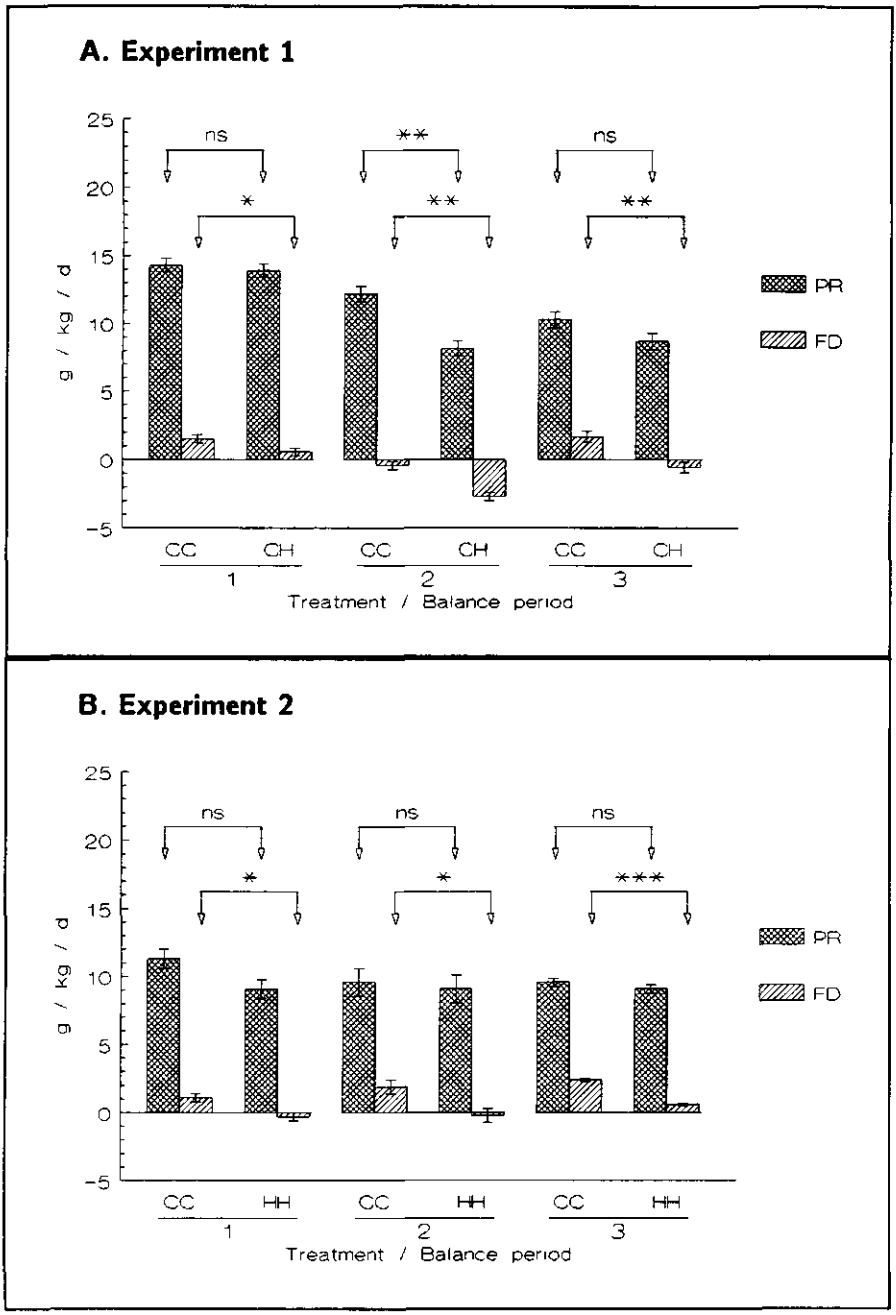


Figure 7. Relative protein retention (PR) and fat deposition (FD) per balance period and treatment.
Least squares means ± s.e.m. The significance between treatments within a balance period is indicated: * P<.05, ** P<.01, *** P<.001



A significant difference in protein retention between treatments was found in balance period 2 of Experiment 1 only (Fig. 7). The CH treatment had a lower protein retention during this period.

Significant differences in fat deposition between CC and CH treatment (Exp. 1) were found in all three balance periods. The difference was biggest in period 2, when birds in the CH treatment mobilized fat. No significant amount of fat was deposited in the CH treatment group in balance periods 1 and 3.

Fat deposition was significantly different between CC and HH treatment in Experiment 2 in all three balance periods. No significant amount of fat was deposited in birds in HH treatment during balance periods 1 and 2.

No significant differences in protein retention or fat deposition were evident between H and L line in any balance period in these experiments.

Discussion

High environmental temperature in the CH and HH treatments affected a number of physiological parameters as reported by Donker *et al.* (submitted-d). These included reduced growth rate, increased cloacal temperature, reduced weight of the liver, spleen, thymus and bursa and decreased packed cell volume. Similar observations of reductions in lymphoid organ weights and cloacal temperature were reported in chickens kept at 40° by Williamson *et al.* (1985). A decreased packed cell volume in the high temperature environment, as observed here, was reported before (Moye *et al.*, 1969; May *et al.*, 1971). This was not found, however, in birds from the same line as in the present experiments and under comparable conditions (Donker *et al.*, submitted-d).

Plasma corticosterone

The plasma concentrations of corticosterone were always in the usual physiological range, and not higher than those measured in these lines in previous experiments under standard conditions (Donker *et al.*, submitted-c). The high temperature did not increase plasma corticosterone concentrations, as was also observed by Williamson *et al.* (1985). Probably, the level did rise immediately after the temperature increased in the CH treatment, but then declined rapidly again (Donker *et al.*, submitted-c)

Thus, none of these physiological parameters or the corticosterone concentration, which could be indicative of a better acclimation to the high temperature in HH treatment than in the CH treatment, differed between these two treatments. Also none of these parameters differed between the H and L line.

Growth-associated hormones

Increased plasma concentration of somatomedine-C and decreased concentrations of growth hormone during the high temperature periods relate to decreased growth observed in CH and HH treatment. Relative high SmC and low GH levels are usually associated with low growth rate (Decuypere and Buyse, 1988).

Thyroid hormones

In the present experiment, no effects of the heat treatments on concentrations of thyroid hormones were found, in contrast to results reported by Klandorf *et al.* (1981) and Williamson *et al.* (1985). They reported decreased T3 and T4 levels during a period after moving chickens to a high

temperature (1-7 d at 32° in adult hens (Klandorf *et al.*, 1981); 1-35 d at 40°C in young chickens (Williamson *et al.*, 1985)). They found these changes were associated with decreased heat production. A decrease in heat production and feed intake were found in the present experiments at high temperatures, but this was not associated with decreased thyroid hormone levels, or recruitment of T_4 from the thyroid. Therefore, it is concluded that these changes are not necessarily initiated by thyroid hormones.

Antibody titers

Decreased antibody titers after an CH treatment and higher titers in birds in HH treatment have been reported previously (Henken *et al.*, 1983a; Donker *et al.* (submitted-d). The involvement of the HPA and HHT axes was postulated to account for these changes.

Immunosuppression was expected in the CH treatment because of the acute stress nature of this treatment (Donker *et al.*, submitted-d). However, these selection lines are relatively unsusceptible to heat stress or corticosterone, with respect to antibody production (Donker and Beuving, 1989; Donker *et al.*, submitted-b). Thus, despite physiological indications of severe thermal demand on the animals, the absence of immunosuppression is not so surprising.

Moreover, no association between thyroid hormones and the level of antibody titers was found. Increased antibody titers were found only in the low selection line in the HH treatment 5 and 10 d after immunization and in the CH treatment 10 d after immunization. The increase in the HH treatment is consistent with earlier results (Henken *et al.*, 1983a; Donker *et al.*, submitted-d), although in an earlier experiment this was found equally in the high and low line (Donker *et al.*, submitted-d). Since the antibody titers are much lower in the low line, the stimulatory effect might be detected more easily in this line.

Although the absence of immunosuppression in CH treatment is not so surprising, to find a stimulating of this treatment is unexpected. A considerable decreased protein retention and the mobilization of fat (Fig. 7A) are indicative of a severe demand on the birds of the (combination of) suddenly increased temperature and the immunization. However, analyses of continuous activity and heat production data (not shown) showed that, at least for these traits, the birds adapted within a day to the high temperature.

Since no relation was between antibody production and any measured endocrine factor or metabolic rate, it remains unclear what stimulating factor(s) increase antibody production under hyperthermic conditions. Most likely is that temperature sensitive factors which work directly on lymphocytes, e.g. lymphokines, are involved. It remains to be investigated, however, what these factors are and why these influences are so variable.

It is clear, however, that differences in circulating antibodies between high and low selection line are not regulated by corticosteroids, thyroid hormones or "general metabolic rate", since these were not different between the two lines. Moreover, profound changes in these traits caused by the temperature treatments were not associated with differences in antibody titers, either between or within lines. The differences in antibody titers between the lines remained very constant, however.

Antibody production may be considered an end-point characteristic of complex reactions in the immune system. Selection for antibody production might influence many of the underlying steps. Our understanding of the influence of temperature and acclimation thereto on antibody production can be obscured by interactions with any of the involved endocrine or cellular factors. Better understanding of the changes which occurred in the selection lines in these factors is therefore required to clarify the variability in endocrine-immunological interactions.

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References

Anderson K. and R.E. Kühn, 1988. Elevated environmental temperature overcomes immunosuppression in experimental Chagas' disease. *FASEB j.* 2: A902.

Bachman S.E. and M.M. Mashaly, 1987. Relationship between circulating thyroid hormones and cell-mediated immunity in immature male chickens. *Dev. Comp. Immunol.* 11: 203-213.

Beuving G. and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 35: 153-159.

Beuving G. and G.M.A. Vonder, 1981. The influence of ovulation and oviposition on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 44: 382-388.

Decuypere E. and J. Buyse, 1988. Thyroid hormones, corticosterone, growth hormone and somatomedins in avian species: general effects and possible implications in fattening. pg. 295-312 in: Leclercq and Whitehead (eds.) *Genetic, metabolic and hormonal basis of leanness in domestic birds.* Butterworth, London

Donker R.A and G. Beuving, 1989. The effect of corticosterone infusion on plasma corticosterone concentration, antibody production, circulating leukocytes and growth in chicken lines selected for humoral immune responsiveness. *Br. Poult. Sci.* (accepted)

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, (*submitted: b*). Heat stress in chicken lines selected for high and low immune responsiveness 1. Antibody production.

Donker R.A., S. Beers, W. v.d. Hel and E. Decuypere, (*submitted: a*). Antibody production in chicken lines divergently selected for for immune responsiveness, in relation to energy metabolism. 1. Heat production at different ambient temperatures and energy metabolism during the immune response under homeotherm conditions.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, (*submitted: c*). Heat stress in chicken lines selected for high and low immune responsiveness 2. Physiological parameters.

Donker R.A., A.G.J.C. Swinkels, A.J. v.d. Zijpp, (*submitted: d*). The effect of environmental temperature on antibody production and some physiological parameters in chicken lines, selected for humoral immune responsiveness.

Gause W.C. and J.A. Marsh, 1985. Differential effects of thyroxine on immune development and autoimmune thyroiditis in the obese strain chicken. *Dev. Comp. Immunol.* 9: 465-475.

Harvey S. and C.G. Scanes, 1977. Purification and radioimmunoassay of chicken growth hormone. *J. Endocrinol.* 73: 321-330.

Heller E.D., D. Ben-Nathan and M. Perek, 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathol.* 8:195-203.

Henken A.M., A.M.J. Groote Schaarsberg and M.G.B. Nieuwland, 1983a. The effect of environmental temperature on immune response and metabolism of the

young chicken. 3. Effect of environmental temperature on the humoral immune response following injection of sheep red blood cells. *Poultry Sci.* 62: 51-58.

Henken A.M., A.M.J. Groote Schaarsberg and W. van der Hel, 1983b. The effect of environmental temperature on immune response and metabolism of the young chicken. 4. Effect of environmental temperature on some aspects of energy and protein metabolism. *Poultry Sci.* 62: 59-67.

Huybrechts L.M., D.B. King, T.J. Lauterio, T.J. March and C.G. Scanes, 1985. Plasma concentrations of Somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. *J. Endocrinol.* 104: 233-239.

Klandorf H., P.J. Sharp and M.G. Macleod, 1981. The relationship between heat production and concentrations of plasma thyroid hormones in the domestic hen. *Gen. Comp. Endocrin.* 45: 513-520.

Marsh J.A., W.C. Gause, S. Sandhu and C.G. Scanes, 1984a. Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. *Proc. Soc. Exp. Biol. Med.* 175: 351-360.

Marsh J.A., T.J. Lauterio and C.G. Scanes, 1984b. Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. *Proc. Soc. Exp. Biol. Med.* 177: 82-91.

Martin A., F.M.A. McNabb and P.B. Siegel, 1988. Thiouracil and antibody titers of chickens lines divergently selected for antibody response to sheep erythrocytes. *Dev. Comp. Immunol.* 12: 611-619.

Mashaly M.M., S.L. Youtz and R.F. Wideman Jr., 1983. Hypothyroidism and antibody production in immature chickens. *Immunol. Commun.* 12: 551-563.

May J.D., J.W. Deaton, F.N. Reece, N. Mitlin and L.F. Kubena, 1971. The effect of environmental temperature on blood volume. *Poult. Sci.* 50: 1867-1870.

Moye R.J., K.W. Washburn and T.M. Huston, 1969. Effects of environmental temperature on erythrocyte numbers and size. *Poult. Sci.* 48: 1683-1686.

Regnier J.A., K.W. Kelley and C.T. Gaskins, 1980. Acute thermal stressors and synthesis of antibodies in chickens. *Poult. Sci.* 599: 985-990.

Romijn C. and W. Lokhorst, 1961. Climate and poultry. Heat regulation in the fowl. *Tijdschr. Diergeneesk.* 86: 153-172.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC: SAS Institute Inc.

Scott T., A. van der Zijpp and B. Glick, 1985. Effect of thiouracil-induced hypothyroidism on the humoral immunity of New Hampshire chickens. *Poult. Sci.* 64: 2211-2217.

Siegel H.S., 1987. Effects of behavioural and physical stressors on immune responses. pg. 39-70. in: Wiepkema P.R. and P.W.M. van Adrichem (eds.): *Biology of stress in farm animals: A integrative approach.* Martinus Nijhoff publ., Dordrecht, Boston, Lancaster.

Thaxton P., 1978. Influence of temperature on the immune response of birds. *Poult. Sci.* 57: 1430-1440.

Verstegen M.W.A., W. van der Hel, H.A. Brandsma, A.M. Henken and A.M. Bransen, 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. pg. 21-62 in: M.W.A. Verstegen and A.M. Henken (eds.): *Energy metabolism in farm animals. Martinus Nijhoff publishers.* Dordrecht, Boston, Lancaster.

Williamson R.A., B.H. Misson and T.F. Davison, 1985. The effect of exposure to 40° on the heat production and the serum concentrations of triiodothyronine, thyroxine and corticosterone in immature domestic fowl. *Gen. Comp. Endocrinol.* 60: 178-186.

Yam D., D. Heller and N. Snapir, 1981. The effect of the thyroidal state on the immunological state of the chicken. *Dev. Comp. Immunol.* 5: 483-490.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poult. Sci.* 59: 1363-1369.
Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterisation of lines selected for high and low antibody production. *Proc. 7th Eur.Poult.Conf. (Paris)*: 211-215.

Chapter VIII

Antibody production in chicken lines divergently selected for immune responsiveness, in relation to energy metabolism.

3. Energy metabolism during the immune response in corticosterone treated chickens, or under hyperthermic conditions.

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Abstract

The energy metabolism was studied, with associated physiological responses, before and during the immune response after an immunization with sheep red blood cells (SRBC) in two chicken lines. These lines were divergently selected for the immune response to SRBC.

In the first of two experiments one group of young cockerels, which served as a control (CC), was implanted with a latex dummy and kept in a climate respiration chamber at 25°C (70% RH). A second group was implanted with an osmotic minipump, delivering a corticosterone solution continuously, and was placed in another climate respiration chamber with the same conditions set (CS).

In Experiment 2 the group in one climate respiration chamber was assigned to the same corticosterone infusion (CS), and in the other chamber a group of birds was implanted with a dummy and climate conditions were set to 35°C and 60% RH (HH). The birds were given six days to acclimate before the experiment started.

Each group consisted of 16 young cockerels, and equal numbers of the high (8) and low (8) selection line were used, but kept in separate cages, four birds per cage.

In the CS infused group the plasma corticosterone concentration was increased, but not in the HH group. The CS infusion and HH treatment decreased growth rate and lymphoid organ development. Plasma thyroxine (T_4) concentration was lower in CS infused birds; triiodothyronine (T_3) concentration was lower in CS infused than in CC birds, but higher than in HH birds.

Metabolizable energy intake and heat production (per kg body weight) was decreased in HH birds, but not in CS infused birds. Differences in the energy balance were small, but CS infused birds had a higher energy balance during the log phase of the immune response. Fat deposition was higher in CS infused birds, particularly during the log phase of the immune response. Protein retention was lower in CS infused and HH treated birds than in CC birds.

Antibody production (total and 2ME-resistant titer) was higher in high line birds than in low line birds. CS infusion did not affect antibody titer, but HH treatment stimulated antibody production in the low line birds.

No clear relation was found between the corticosterone infusion or heat treatment with associated changes in metabolism and endocrinology and the antibody production.

Keywords: immune response, SRBC, selection lines, corticosterone, metabolism
Running title: Corticosterone, immune response and metabolism

Introduction

Increased activity of the hypothalamo-pituitary-adrenal (HPA) axis is, also in poultry, typical for a stress response. The resulting increased plasma corticosterone levels can have profound influences on the birds physiology and immunology. Immunosuppression has been reported after stimulation of the HPA axis by e.g. acute thermal stressors (Subba Rao and Glick, 1970; Thaxton and Siegel, 1970, 1973; Thaxton, 1978; Siegel, 1987). However, particularly the effects on humoral immune response have been variable (Heller *et al.*, 1979; Regnier *et al.*, 1980; Siegel, 1987; Donker *et al.*, submitted-b). Administration of corticosteroids in the feed, or by injection or infusion results in more constant elevated plasma corticosterone levels and might reduce this variability in results, and thus serve as a experimental substitute for environmental stressors.

Reduced antibody titers to sheep red blood cells (SRBC) were found if the birds were injected with cortisone acetate or corticosterone before immunization (Sato and Glick, 1970) and after feeding corticosterone (Gross *et al.*, 1980). Reduced responses to SRBC and *Brucella abortus* were reported in birds with pellets containing corticosterone implanted (Davison and Misson, 1987). Continuous infusion of corticosterone has been reported to increase the incidence of Marek's disease after challenge (Powell and Davison, 1986). However, Donker and Beuving (1989) reported no effect of infused corticosterone on antibody titers to SRBC in young chickens from lines selected for immune responsiveness, despite serious detrimental effects on growth and lymphoid development.

Placing birds of these lines (Donker *et al.*, submitted-e) or parental stock (Henken *et al.*, 1983) in a moderate or extreme hot environment, providing time to acclimate, increased the humoral response to SRBC, in spite of hyperthermia, reduced growth and other indications of severe thermal stress. The enhanced antibody response was postulated to be caused by stimulatory effects of the hypothalamo-pituitary-thyroid axis, with possible associated effects on metabolic rate (Henken *et al.*, 1983; Donker *et al.*, submitted-d). Thus the effects of extreme heat and corticosterone administration on the immune response can be quite different, despite the supposed stressful character of both.

To investigate the relations between antibody production, environmental temperature, corticosterone levels and metabolic rate more accurately a number of studies were accomplished. Energy and protein balance data were gathered during the immune response in a high and low selection line under varying conditions. In earlier reports the results at thermoneutral conditions were

presented (Donker *et al.*, submitted-a) and under hyperthermic conditions with or without previous acclimation to the high temperature (Donker *et al.*, submitted-e). In this report we present the results of endocrine and metabolic characteristics in these selection lines during the immune response, comparing those with corticosterone infusion and under hyperthermic conditions.

Material and methods

Birds and pre-experimental conditions

Two experiments were accomplished, using male birds which were genetically identical to the 7th generation of a high (H) and low (L) line, selected for antibody titer to sheep erythrocytes (SRBC) (Van der Zijpp and Nieuwland, 1986). For each experiment a separate hatch was used. After hatching, the cockerels were, lines separately, placed in brooder cages with water and commercial starter feed provided *ad libitum*.

Environmental temperature was gradually decreased during the first two weeks from approximately 32°C to approximately 25°C. The light regime was changed from 23 h light/ 1 h dark to 12 h light/ 12 h dark during the first three weeks, which remained the same throughout the experiments.

Individual vaccination against Marek's disease (at 1 d of age), infectious bronchitis (2 d of age), Gumboro's disease (12 d of age) and Newcastle disease (18 d of age) were given.

To exclude large variation in weight and growth rate a selection from the available birds was used. The selection was based on individual body weight at 22 days of age. Chicks with a body weight within a range of the median weight of that line ± 1 s.d. were used.

Climate-respiration chambers

At 25 d of age the birds were placed in one of two medium sized climate respiration chambers (1.8 m^3 each) at the experimental unit of the Department of Animal Husbandry of the Agricultural University. The technical equipment and applications of these chambers are described elsewhere (Verstegen *et al.*, 1987). Each chamber contained a set of four brooder cages (± .5 x .4 x .4 m each; l x b x h), two cages with 4 H line birds and two cages with 4 L line birds. Measurement of feed intake and excreta could be obtained for each cage separately; O_2-consumption and CO_2-production for indirect calorimetry (Verstegen *et al.*, 1987) could be measured for each chamber.

Experimental procedures

The chicks were randomly assigned to either a corticosterone infused group (CS), a control group (CC; in Exp. 1) or a heat exposed group (HH; in Exp. 2). The experimental design was full factorial with equal numbers of each line in each treatment group. Birds were anaesthetized with ether on 22 d of age (Fig. 1). In the corticosterone treatment group an infusion pump with a infusion capacity for four weeks (Alzet osmotic minipumps, model 2ML4; Alza Corp., Palo Alto, CA, USA) was implanted subcutaneously dorsal of the thorax. In the CC and HH groups a sterilized dummy of a synthetic elastic polymer (382 Medical grade elastomer, Dowcorning Co., Midland, MI, USA) with approximately the same dimensions and weight was implanted at the same location. The implanted minipumps (CS group) delivered a continuous flow of corticosterone (Sigma, St Louis, MO, USA) in polyethyleneglycol (polyethyleneglycol-400, Merck, Darmstadt, Federal republic of Germany) at a rate of 8.50 μg corticosterone/h throughout the experiment.

After examination of the incision and the implant the birds were placed in the chambers at an age of 25 days for a 6 d adaptation period (Fig. 1). Inspection of the implant was repeated after every balance period (see below).

Not all implanted minipumps and dummies stayed in location where implanted. Many (approx. 7 out of every 10) migrated subcutaneously to a site in the leg. This apparently did not hinder the birds' movements. In some birds the pressure of the implant caused necrosis of the skin and eventually pierced the skin. When this was noted the bird was taken out of the experiment. Because of this reason one bird in Experiment 1 with a dummy was taken out of the experiment, before balance period 1 was started. In Experiment 2 this was found in one bird with a dummy and one CS treated bird after balance period 1, and in one bird with a dummy after balance period 2. At examination *post mortem* it was found that several implants had caused some minor irritation (red skin with small blood spots).

Balance periods

At 31 d of age all birds were removed from the chamber individually, with minimal disturbance of the other birds, in random order. A blood sample (± 1.5 ml) was taken from the ulnar vein (*vena cutanea ulnaris*) with a heparinized syringe. The entire procedure did not take longer than 45-60 seconds since prolonged handling can cause increased circulating plasma corticosterone (Beuving and Vonder, 1978). Immediately thereafter cloacal temperature was measured. After the birds were weighed and replaced in the same cage, the first of three successive balance periods was started (Fig. 1).

During the 5-day balance periods *ad libitum* feed intake was measured daily and excreta were collected. Chambers were not opened during a balance period.

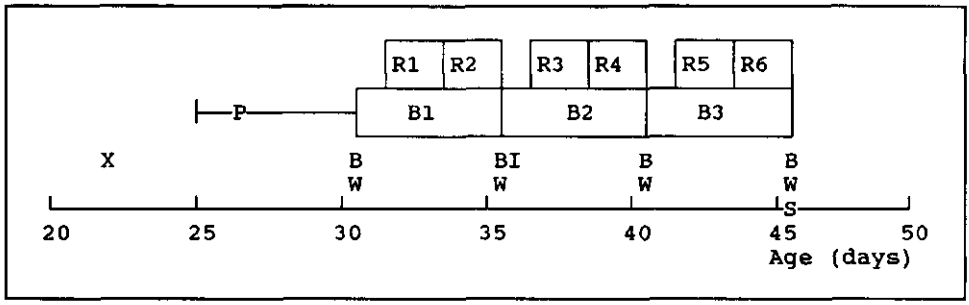
At the second day of a balance period the first of two successive 48-hour respiration periods was started (Fig. 1). Continuous measurements of O_2-consumption and CO_2-production were made during a respiration period. At the end of each balance period the same procedure for blood sampling and cloacal temperature measurement was followed, as described above; the amount of excreta was assessed and samples of excreta and refused feed were taken.

Prior to the second balance period the birds were immunized intramuscularly with 1 ml 25% (v/v) packed SRBC in saline.

The chickens were killed by cervical dislocation after the third balance period and liver, thymus, spleen, bursa and adrenals were excised and weighed.

Figure 1. Experimental layout.

X: Implantation of infusion pump or dummy
P: Pre-experimental adaptation period in climate respiration chambers
B1, B2, B3: Balance period 1, 2, 3
R1, .., R6: 2-day respiration periods
B: Blood sampling
I: Immunization with SRBC
W: Weighing
S: Slaughter and dissection



Experimental treatments

Three different experimental groups were distinguished, based on the results of Donker and Beuving (1989) and Donker *et al.* (submitted-d). Only two experimental groups could be included in each experiment.

A corticosterone infused treatment group (CS) was included in both experiments. Birds in this treatment were implanted with a minipump,

delivering corticosterone and were placed in a chamber with a temperature of 25°C, and a relative humidity (RH) of 70%.

In Experiment 1 a control group (CC) was placed in the other chamber; the birds were implanted with a dummy and placed in a chamber with a temperature of 25°C and a RH of 70%.

In Experiment 2 a high temperature treatment group (HH) was placed in the other chamber; the birds were implanted with a dummy and placed in a chamber with a temperature of 35°C with 60% RH (Donker *et al.*, submitted-a, -e). The birds were given time to acclimate to the temperature before immunization, therefore these conditions were already set during the adaptation period in the respiration chamber (Fig. 1).

Assays

All food and excreta samples were analyzed for energy and nitrogen contents. Also energy and nitrogen loss in dust and airflow (NH_3) from the chambers were assessed.

Packed cell volume (PCV) was assessed in the blood samples. Plasma samples were analyzed for total and 2-mercaptoethanol resistant (2ME-) antibody titer to SRBC (Van der Zijpp and Leenstra, 1982) and concentrations of corticosterone[1)] (Beuving and Vonder, 1981), triiodothyronine (T_3) (commercial kit: Dac-Cel T_3; Welcome Reagents Ltd, Beckenham, Kent U.K.), thyroxine (T_4) (commercial kit: T_4 RIA(PEG); Abbott Diagnostic Division, Antwerp, Belgium), growth hormone (GH) (Harvey and Scanes, 1979) and somatomedine-C (SmC) (Huybrechts *et al.*, 1985) were measured. 5'-Monodeiodinase activity (Decuypere *et al.*, 1983) was measured in liver homogenates *post mortem*, as an estimate of the T_4 to T_3 conversion capacity.

Statistical analysis

All metabolic traits were expressed on the basis of kg^{-1}, as discussed by Donker *et al.* (submitted-a).

Heat production (H) was calculated from oxygen consumption and carbon dioxide production with the formula of Romijn and Lokhorst (1961):

$H\ (kJ) = 16.20 \times O_2\ (l) + 5.00 \times CO_2\ (l)$.

Balance characteristics analyzed were ME-intake ($kJ \times kg^{-1} \times day^{-1}$), energy balance EB ($kJ \times kg^{-1} \times day^{-1}$), protein retention PR ($g \times kg^{-1} \times day^{-1}$) and fat deposition FD ($g \times kg^{-1} \times day^{-1}$)). ME was calculated from energy in feed minus energy lost in excreta, dust and outgoing air (NH_3); PR was calculated from N in feed minus N lost in excreta, dust and outgoing air; EB was calculated as ME minus H, in which H (heat production) was calculated by dividing total

1) Plasma corticosterone concentrations were obtained in samples which were pooled within a day of two birds from the same cage.

heat production per chamber among cages relative to ME intake. FD was calculated from the difference between EB and energy deposited as protein. Data were evaluated in analyses of variance using the SAS procedures (SAS, 1985).

Individual data were analyzed within sampling day, using a model containing line and treatment as factors. Characteristics analyzed were body weight, relative growth, packed cell volume, antibody titers (total and 2ME-resistant), and plasma concentrations of corticosterone, GH, T_3, T_4, SmC, monodeiodinase activity and relative organ weights. A line x treatment interaction was included in the initial model, but left from the model if not significant ($P>.05$).

Balance data were analyzed in a comparable manner as individual data, but on the basis of cage values.

Results

In the following paragraphs differences between H and L line are only mentioned if significant.

Plasma corticosterone concentrations

The plasma corticosterone concentrations were elevated on all sampling days in the CS infused birds compared to those in CC or HH treatment (Fig. 2). The concentrations in the CC treatment (Exp. 1) and the HH treatment (Exp. 2) were in the same range of values.

Body weight and growth rate

Body weight was lower in CS infused birds than birds in CC or HH treatment (Fig. 3). The difference in body weight between HH and CS (Exp. 2) was smaller than that between CC treatment and CS infusion (Exp. 1). Relative growth rate was lower in the CS infused birds than in CC treated birds ($P<.001$), but was not different between CS infusion and HH treated birds.

Relative organ weights

Relative organ weights *post mortem* are presented in Table 1.

CS infusion in Experiment 1 caused a significant decrease in the relative weight of the spleen ($P<.01$), bursa ($P<.001$) and thymus ($P<.001$) compared to CC treatment and a significant increase ($P<.001$) in liver and adrenals ($P<.05$) weights.

Figure 2. Plasma corticosterone per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: *** P<.001

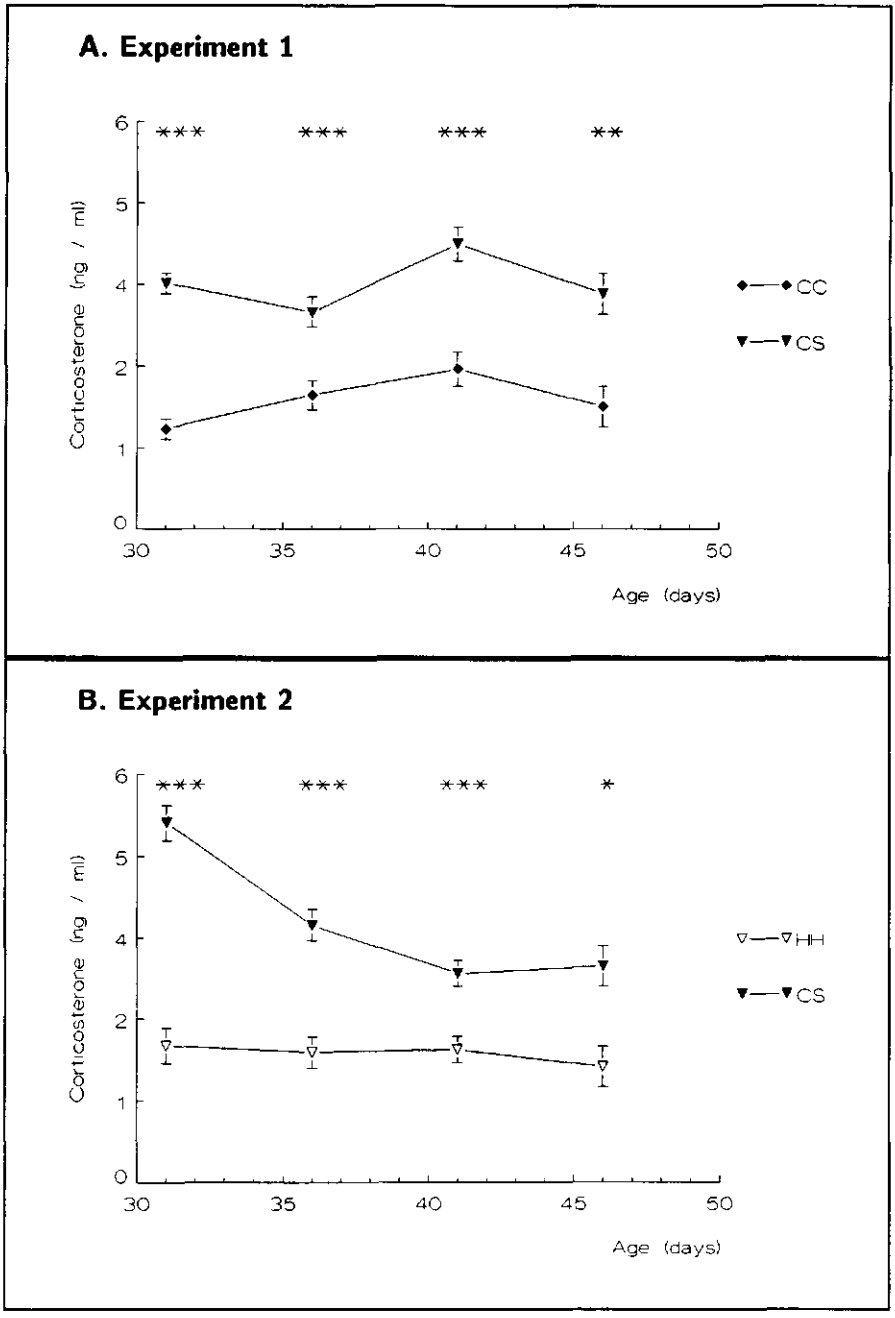


Figure 3. Body weight per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001

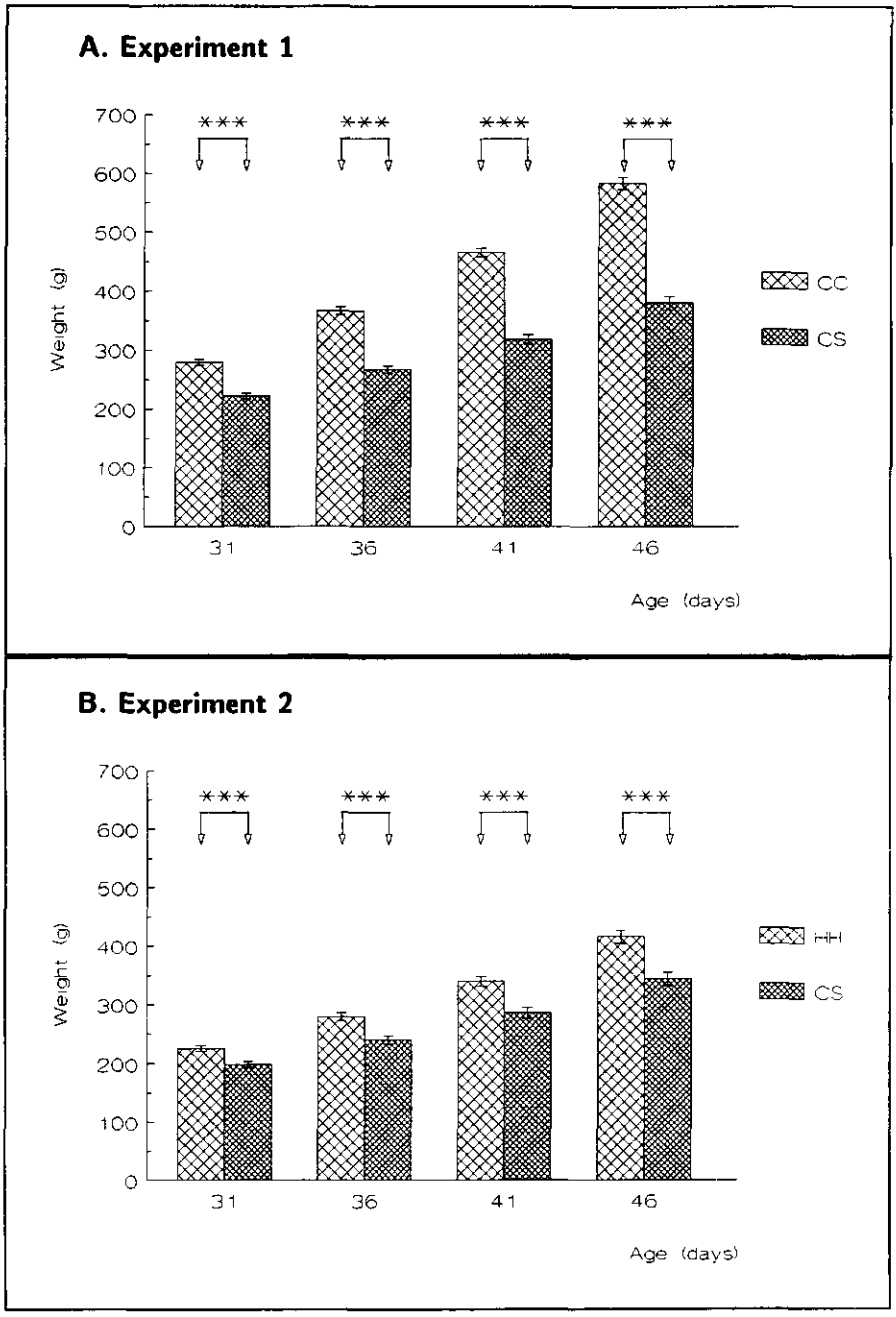


Table 1. Relative organ weights[1] and liver 5'-monodeiodinase activity[2] post mortem. Least squares means[3] ± s.e.m.

Experiment 1

Treatment:	CC		CS	
Line:	High	Low	High	Low
Liver	2.26^{c} ± .21	2.41^{bc} ± .06	3.74^{a} ± .21	3.00^{b} ± .21
Spleen	0.23^{a} ± .02	0.20^{ab} ± .02	0.17^{bc} ± .02	0.14^{c} ± .02
Bursa	0.56^{a} ± .03	0.52^{a} ± .03	0.18^{c} ± .03	0.26^{b} ± .03
Thymus	0.62^{a} ± .04	0.63^{a} ± .04	0.28^{b} ± .04	0.38^{b} ± .04
Adrenals	0.009^{b} ± .001	0.010^{ab} ± .001	0.011^{ab} ± .001	0.013^{a} ± .001
5'-monodeiodinase	0.49 ± .06	0.48 ± .06	0.48 ± .06	0.48 ± .06

Experiment 2

Treatment:	HH		CS	
Line:	High	Low	High	Low
Liver	1.97^{b} ± .25	1.98^{b} ± .21	3.08^{a} ± .25	3.16^{a} ± .21
Spleen	0.20^{a} ± .01	0.12^{c} ± .01	0.19^{ab} ± .10	0.16^{b} ± .01
Bursa	0.41^{a} ± .04	0.35^{ab} ± .04	0.24^{b} ± .04	0.26^{b} ± .04
Thymus	0.45^{a} ± .04	0.40^{a} ± .04	0.33^{ab} ± .04	0.28^{b} ± .04
Adrenals	0.010^{ab} ± .001	0.009^{b} ± .001	0.011^{ab} ± .001	0.013^{a} ± .001
5'-monodeiodinase	0.45^{a} ± 0.07	0.27^{bc} ± .06	0.29^{ab} ± .06	0.14^{c} ± .06

1) Organ weight / body weight * 100%
2) In vitro production ng T_3 / mg liver protein
3) lsm within the same row bearing different superscripts differ significantly ($P<.05$)

CS infusion in Experiment 2 caused a significant decrease in the relative weight of thymus ($P<.01$) and bursa ($P<.01$) compared to the HH treatment and a significant increase in liver ($P<.001$) and adrenal ($P<.05$) weights.

The relative weight of the spleen was higher in the H line than in the L line ($P<.01$ in Exp. 1; $P<.001$ in Exp. 2).

Cloacal temperature

Cloacal temperature was not different between CS infused or control chickens in Experiment 1. Cloacal temperature in Experiment 2 was on average .3°C higher in HH treatment than in CS treatment on all days (41.7°C vs. 41.4°C; $P<.001$).

Antibody titers

Antibody titers (total titer and 2ME-resistant titer) were significantly higher in the H line than in the L line 5 and 10 days after immunization (41 c.q. 46 d of age) in Experiment 1 and Experiment 2 ($P<.001$) (Fig. 4).

Antibody titers (total titer and 2ME-resistant titer) were not significantly influenced by CS infusion in Experiment 1. In Experiment 2 the titers were significantly higher in the HH treated birds of the L line than the CS infused birds of the same line ($P<.05$) on 5 and 10 d after immunization. The same difference in the H line was significant only for the 2ME resistant titers.

Packed cell volume (PCV)

The packed cell volume was higher in CS infused birds than in control birds in Experiment 1 (Fig. 5), although the difference was significant only on 31 ($P<.05$) and 36 ($P<.001$) d of age. In Experiment 2 packed cell volume was significantly lower in HH treated birds on all days than in those which were CS infused (Fig. 5).

Plasma hormone concentrations

Plasma triiodothyronine (T_3) concentration was lower in CS infused birds in Experiment 1 (Fig. 6), though only significantly at 31 ($P<.01$) and 41 d of age ($P<.05$). In Experiment 2 T_3 concentration was significantly lower on all days in HH treated birds than in CS infused birds ($P<.001$).

Plasma thyroxine concentration (T_4) was significantly lower in CS infused birds than in CC control birds ($P<.001$; Fig. 6). The CS infused birds had also a lower average T_4 concentration than HH treated birds in Experiment 2 ($P<.001$).

The liver 5'-monodeiodinase activity was not different between lines or treatments in Experiment 1 (Table 1). In Experiment 2 monodeiodinase activity

Figure 4. Total (——)and 2ME-resistant (– – –) antibody titer per line and treatment.
Least squares means ± s.e.m. Lsm bearing different superscripts within a day differ significantly (P<.05)

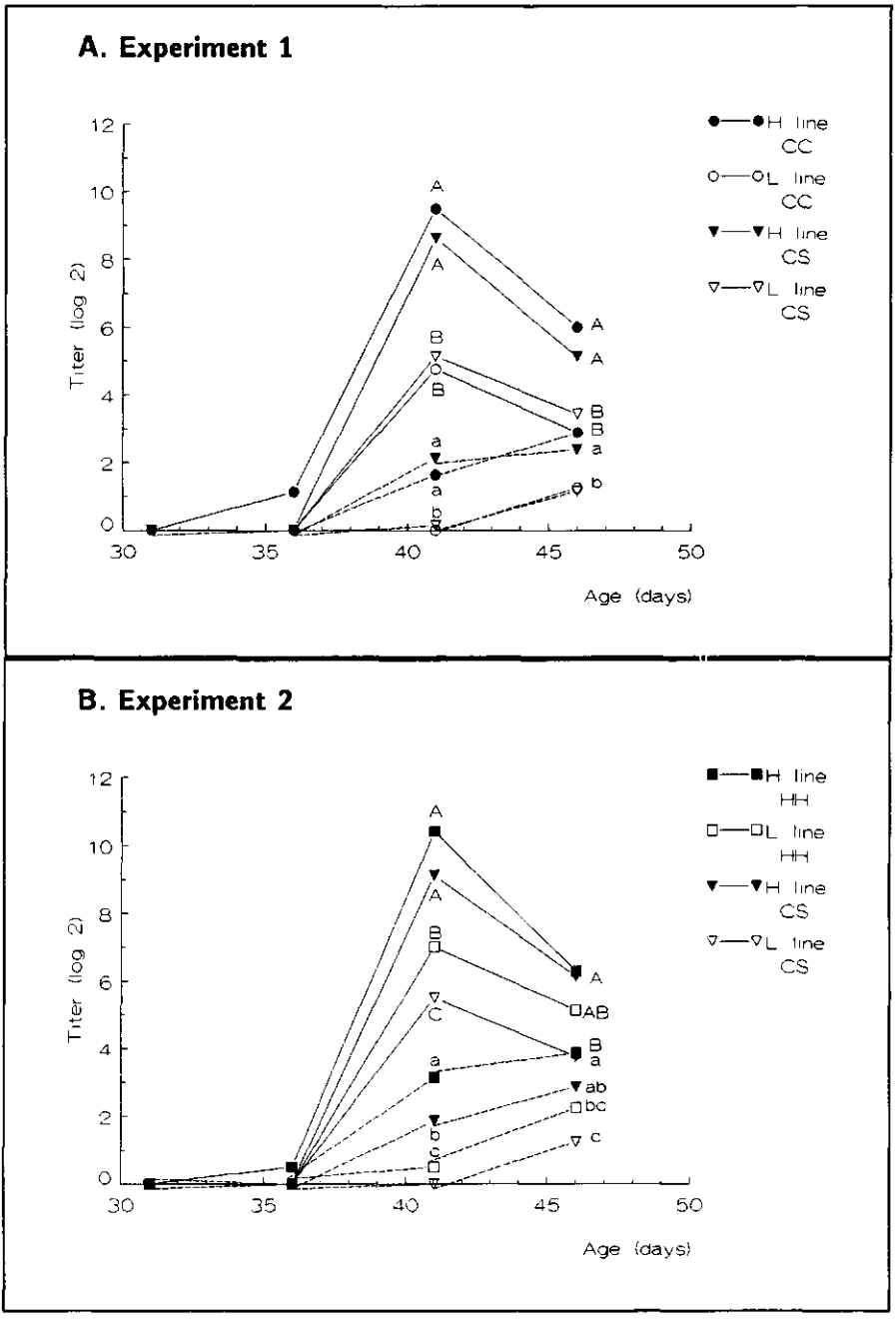


Figure 5. Packed cell volume per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001

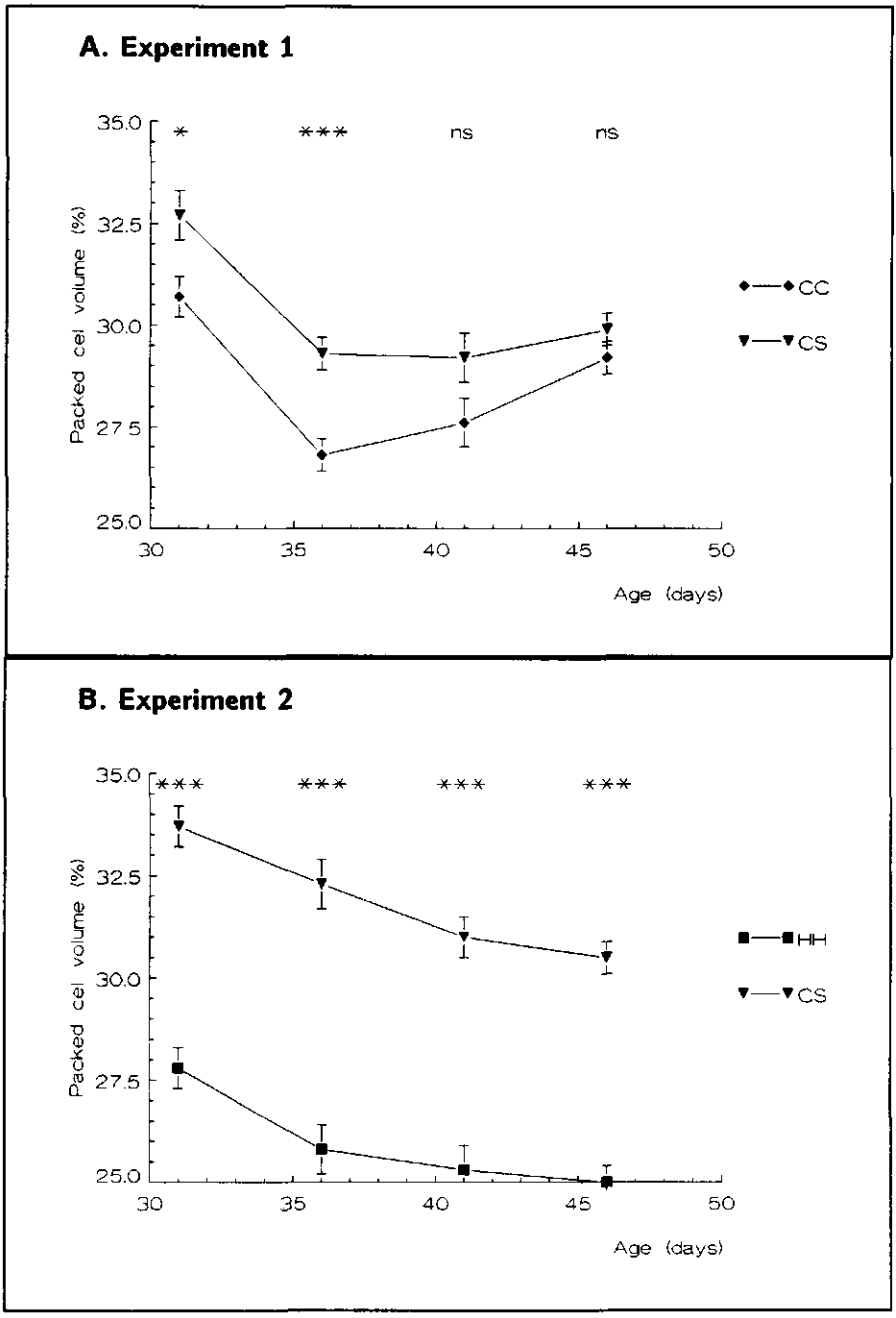
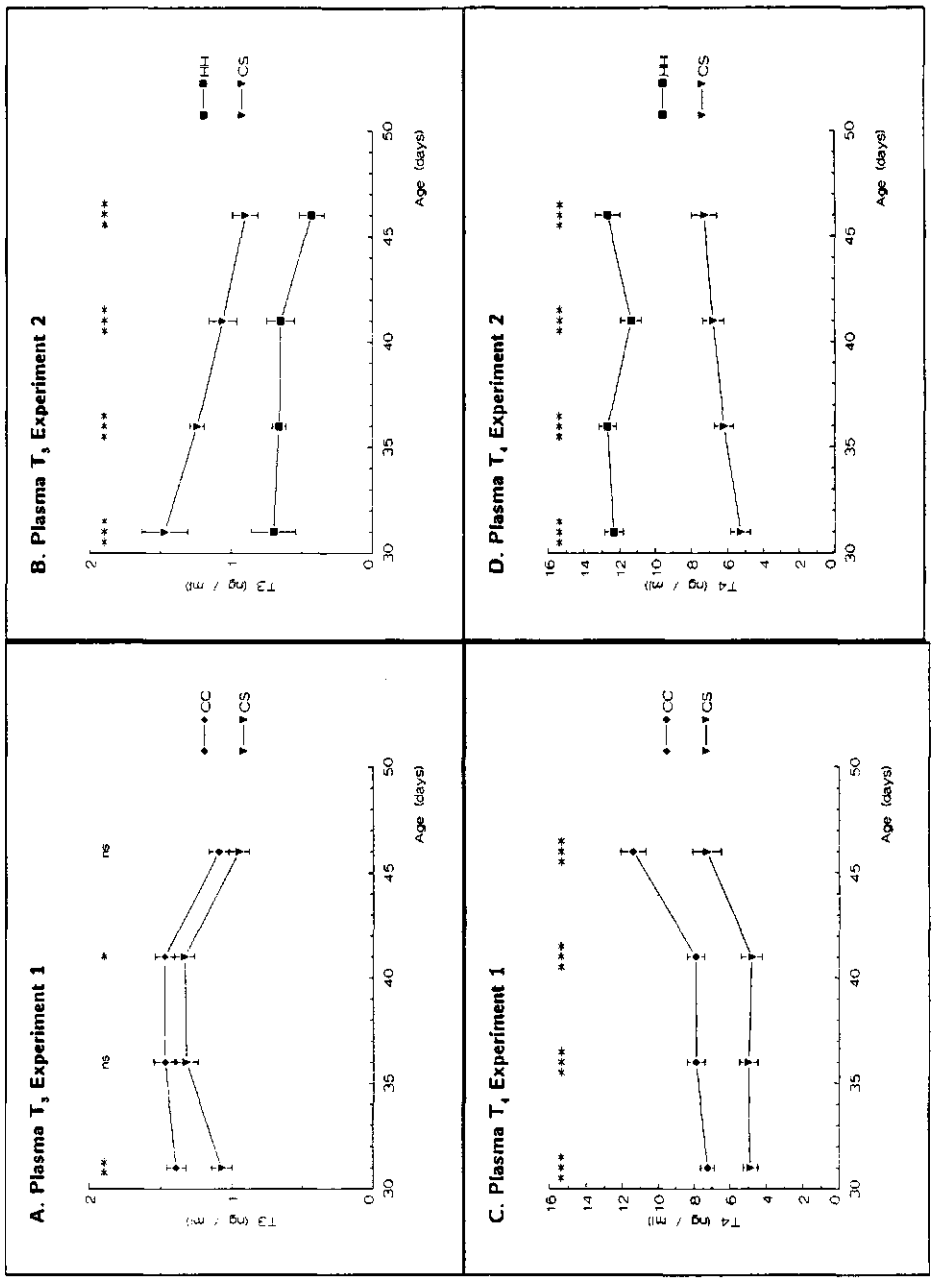


Figure 6. Plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations per treatment.
Least squares means ± s.e.m. The significance between treatments within a day is indicated: * P<.05, ** P<.01, *** P<.001



was higher in the H line than in the L line ($P<.05$), and lower in CS infused birds than in HH treated birds ($P<.05$).

Average plasma growth hormone concentration was not significantly different between control birds (4.04 ng/ml) and CS infused birds (3.89 ng/ml) in Experiment 1. HH birds in Experiment 2 had a significantly higher average growth hormone concentration (4.45 ng/ml) than CS infused birds (3.81 ng/ml; $P<.001$).

The average concentration of somatomedine-C was lower in CS infused birds (5.49 I.U./ml) than in control birds (8.35 I.U./ml; $P<.001$) in Experiment 1. Somatomedine concentrations in Experiment 2 were not significantly different between CS infused (7.43 I.U./ml) or HH treated birds (7.42 I.U./ml), except on 31 days of age, when concentrations were lower in CS infused birds (5.63 I.U./ml vs. 6.99 I.U./ml; $P<.001$).

Balance characteristics

Metabolizable energy (per kg body weight) was not significantly different between CS infused and control birds in Experiment 1 in any balance period (Fig.7). Metabolizable energy was significantly lower in HH treated birds than in CS infused birds in Experiment 2 in all balance periods.

Heat production was somewhat higher in CS infused birds than in CC-control birds during balance period 1 (Fig. 7; $P<.05$). They were not different in balance periods 2 and 3. In Experiment 2 the heat production was lower in HH treated birds than in CS infused birds.

A small difference was found in the energy balance between CS infused birds and the CC control in balance period 1, Experiment 1 (Fig. 7). A higher energy balance was found in balance period 2 in the CS infused chickens. The energy balance was not significantly different in balance period 3.

In Experiment 2 the energy balance was lower in CS infused birds than in HH treated birds in balance periods 1 and 3, but higher during balance period 2.

The protein retention and fat deposition are presented in Figure 8. In Experiment 1 the protein retention was lower in the CS infused chickens than in CC controls, which was significant in balance periods 1 and 3. Fat deposition was always higher in CS infused chickens, particularly during balance period 2. In Experiment 2 protein retention was not different between CS infused and HH treated chickens. Fat deposition was lower in CS infused chickens during balance periods 1 and 3, but higher during balance period 2.

Figure 7. Metabolizable energy (ME), heat production (HP) and energy balance (EB) per balance period and treatment.
Least squares means ± s.e.m. The significance between treatments within a balance period is indicated: * P<.05, ** P<.01, *** P<.001

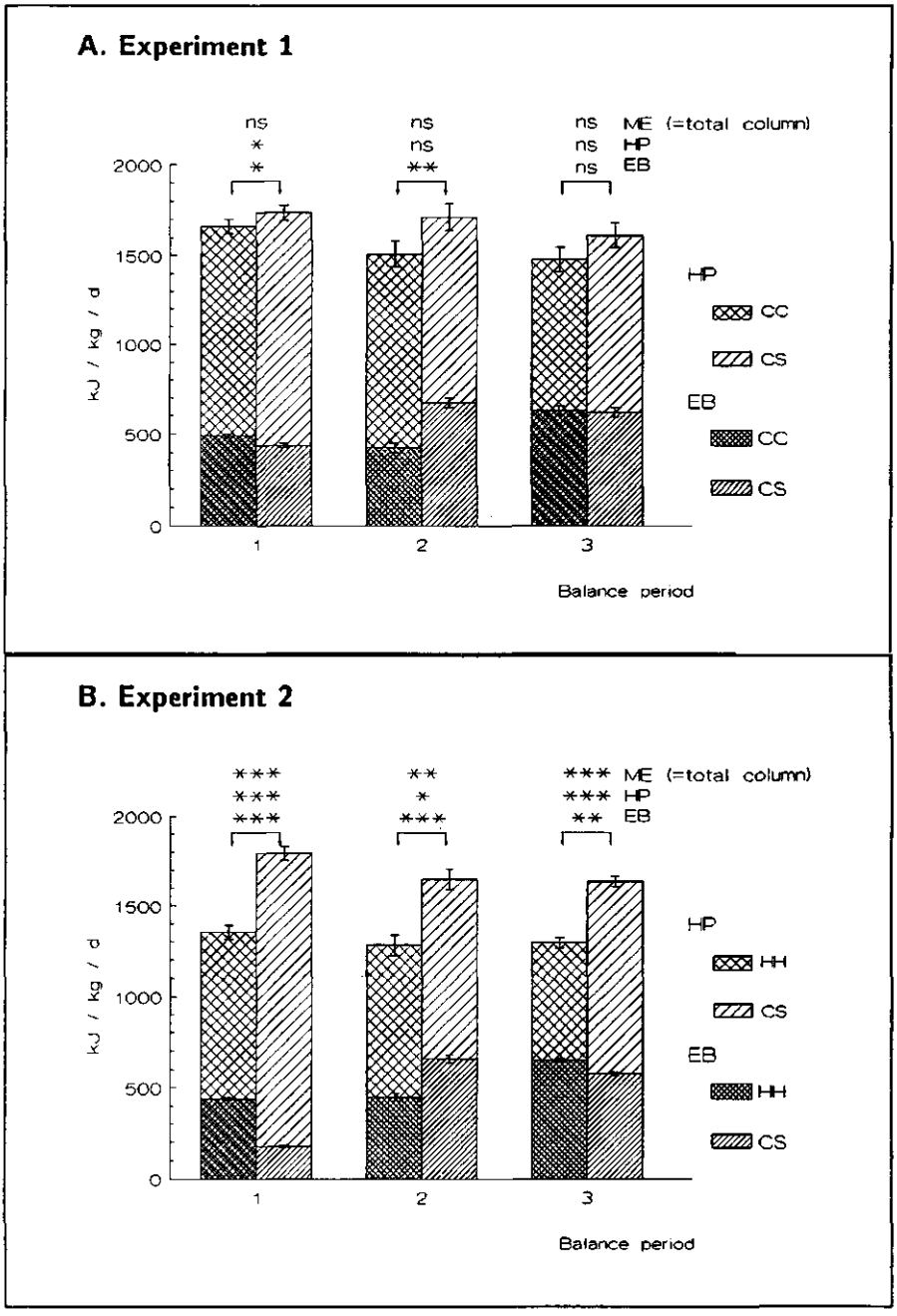
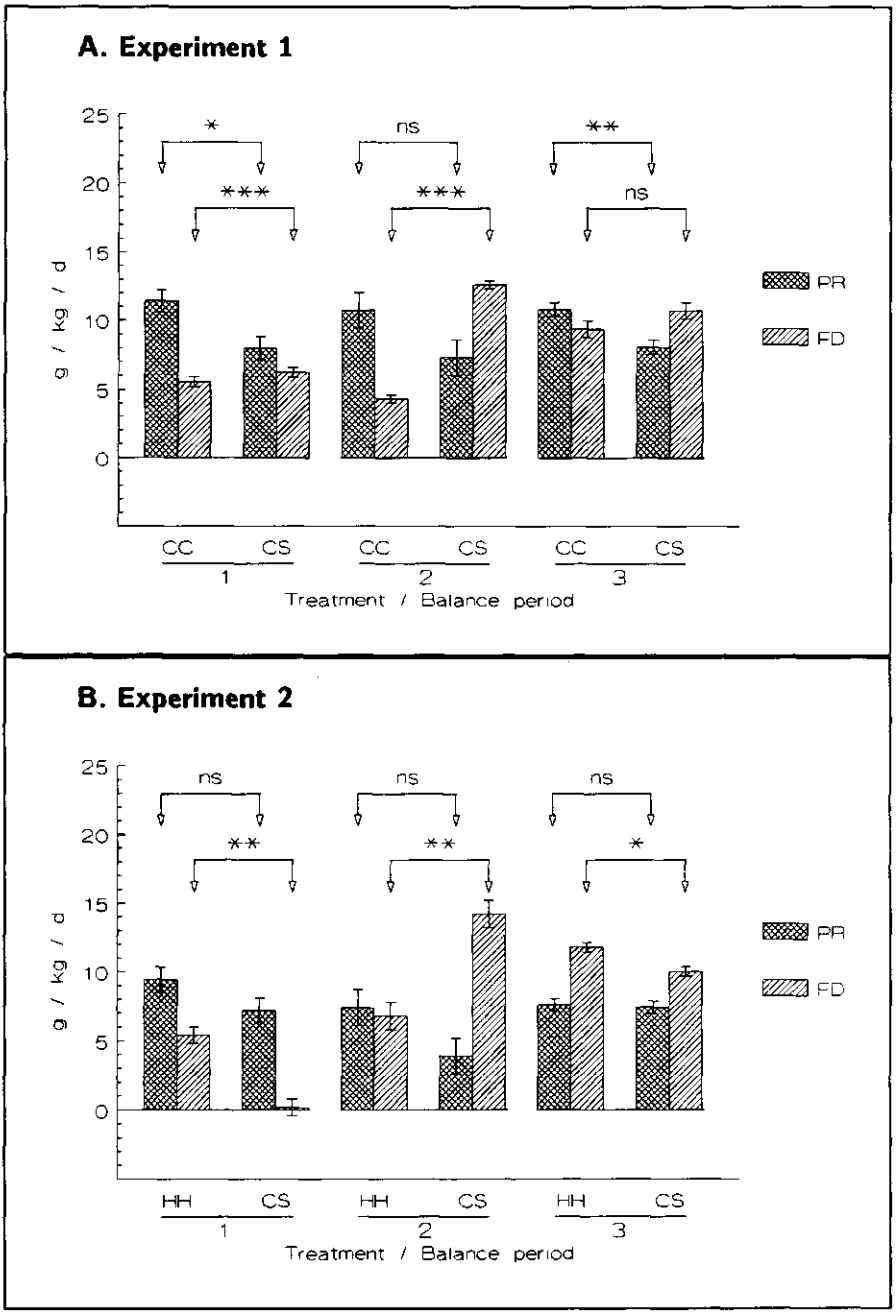


Figure 8. Protein retention (PR) and fat deposition (FD) per balance period and treatment.
Least squares means ± s.e.m. The significance between treatments within a balance period is indicated: * P<.05, ** P<.01, *** P<.001



Discussion

The infused corticosterone increased plasma corticosterone levels throughout the experiments, but the concentrations remained within a range as frequently observed under physiological conditions (Davison *et al.*, 1985; Beuving and Vonder, 1986; Donker and Beuving, 1989). The plasma corticosterone concentrations in the CC and HH treated birds were at the same level as reported before in these birds as normal base levels (Donker *et al.*, submitted-e) or after minipumps had been implanted releasing vehicle only (Donker and Beuving, 1989). Thus the implanted dummies did not result in increased levels of corticosterone, like in adult hens (Beuving, personal communication). Also the high temperature did not result in elevated plasma corticosterone concentrations, as previously reported by Williamson *et al.* (1985) and Donker *et al.* (submitted-e). However, plasma corticosterone was probably increased initially after the birds were placed in the hot environment (Donker *et al.*, submitted-b), but decreased again soon thereafter. Beuving and Vonder (1978) reported that, in adult hens, plasma corticosterone concentrations were back to the base level four days after placing them at 37°C. Williamson *et al.* (1985), however, did not find increased plasma corticosterone concentrations already 24 h after placing young birds in a 40°C environment.

Decreased growth and detrimental effects on lymphoid organs after artificial corticosterone administration are well described (Siegel and van Kampen, 1984, Davison *et al.*, 1985; Kafri *et al.*, 1988; Donker and Beuving, 1989). Comparable effects on growth and lymphoid organs of hot environments were also reported (Williamson *et al.*, 1985; Donker *et al.*, submitted-d, -e). The lower growth in corticosterone infused or heat treated birds was not reflected in altered plasma concentrations of growth hormone. Somatomedine levels were decreased in corticosterone infused birds and on the same level in those in the high temperature environment. Comparable observations were made by Buyse *et al.* (1987) in corticosterone injected birds.

Increased liver weight after corticosterone administration is related to the increased lipogenesis and agrees with the increased fat deposition (Siegel and van Kampen, 1983; Davison *et al.*, 1985; Buyse *et al.*, 1987; Kafri *et al.*, 1988). Increased adrenal weights in the CS infused group was not expected and conflicting with other reports (Davison *et al.*, 1985; Kafri *et al.*, 1988).

The decreased packed cell volume in the HH treatment may have been caused by either a decrease in the number of erythrocytes or a decrease in the volume per cell (May *et al.*, 1971). In either case haemoglobin capacity is probably decreased. This might reflect the lower oxygen demand because of the decreased growth rate in this treatment. The increased packed cell volume in CS infused

birds could on the contrary reflect the higher oxygen demand, related to deposition of more fat.

Decreased T_3 plasma concentrations as a result of the corticosterone infusion and high environmental temperature are in line with other recent observations (Davison *et al.*, 1985; Williamson *et al.*, 1985; Buyse *et al.*, 1987; Williamson and Davison, 1987). Triiodothyronine is probably an important factor in regulating feed intake and metabolic rate (Bobek *et al.*, 1977; Klandorf *et al.*, 1981; Williamson *et al.*, 1985).

After corticosterone infusion often an increased level of T_4 is found. However, decreased T_4 concentrations after corticosterone administration, as found in the present experiments, were reported also by Decuypere *et al.* (1983). Davison *et al.* (1985) and Williamson and Davison (1987) reported an increase in T_4 levels was found only above some threshold dose of corticosterone. An explanation for the increased T_4 concentration could also be the corticosterone induced decrease in 5'-monodeiodinase (Decuypere *et al.*, 1983; Williamson and Davison, 1987). However, also thyroidal release of thyroxine can be influenced by corticosterone (Decuypere *et al.*, 1983; Davison *et al.*, 1985), resulting in increased T_4 concentrations. Both factors could explain higher thyroxine concentrations in HH treatment, although these differences were not observed in earlier experiments (Donker *et al.*, submitted-e).

As expected the high environmental temperature decreased total metabolizable energy and heat production in the birds (Henken *et al.*, 1983; Donker *et al.*, submitted-e); which is probably related to lower T_3 levels (Klandorf *et al.*, 1981; Williamson *et al.*, 1985). Remarkable and unexplained is the fat deposition measured in HH treatment in Experiment 2. This was considerably higher than results in the same treatment already reported by Donker *et al.* (submitted-e). Fat deposition in CS-infused birds was controversially quite low. In balance periods 1 and 3 lower even than in HH treatment birds. Although no measurements were made, it was very obvious at examination *post mortem* that fat depots in CS infused birds were considerable bigger than in CC or HH treated birds. Also a higher ME intake in the CS-infused group combined with a growth rate that was lower than CC treatment (Exp. 1) or equal to HH treatment (Exp. 2) indicates a higher fat deposition in CS-infused birds.

Thus the effects of corticosterone infusion on balance characteristics were not so clear. But an obviously higher energy balance was found during balance period 2, in which the immune response was mounted. This increase was associated with considerably increased fat deposition during the same period. These changes are somehow induced by the immune response, as a comparable difference was found in the same balance period when SRBC and PBS injected birds were compared (Henken and Brandsma, 1982) or when the H and L response

lines were compared (Donker *et al.*, submitted-a). In those experiments the experimental groups which mounted the highest immune response had higher energy retention and higher fat deposition.

No suppressive effects of corticosterone infusion were found on antibody titers in either the H or the L selection line. The selection difference in antibody titer between the lines was consistent. This was also previously reported by Donker and Beuving (1989). It has been suggested that exogenous corticosterone might be a relatively weak immuno suppressant (Sato and Glick, 1970). Davison and Misson (1987), however, reported dose dependent depressions of implanted pellets containing corticosterone on antibody titers to a T-cell dependent (SRBC) as well as a T-cell independent (*Brucella Abortus*) antigen. Although implantation of pellets containing corticosterone might affect immune function differently than infusion, it seems likely that the chickens used in the present experiments are somehow rather unsusceptible to the effects of corticosterone on lymphocyte action, with respect of antibody production, despite the detrimental effects on lymphoid tissue.

Increased titers were found on day 5 after immunization in HH treated compared to CS infused chickens in Experiment 2. These were rather caused by stimulatory factors in the HH treatment as postulated by Donker *et al.* (submitted-d, -e), than suppressive factors in CS infused birds (see Fig. 4a and 4b). But it remains unclear what these factors are. Decreased T_3 and increased T_4 concentrations could be of importance, since thyroid hormone are associated with immune function. However, controversial results were reported in both hypo- and hyperthyroid birds (Gause and Marsh, 1985; Kai *et al.*, 1987, 1988; Marsh *et al.*, 1984a, 1984b; Mashaly *et al.*, 1983; Scott *et al.*, 1985; Yam *et al.*, 1981). Donker *et al.* (submitted-e) reported no influence of the high temperature on thyroid hormone concentrations and thus no relation with the observed immuno stimulatory effect of high temperature. Mashaly *et al.* (1983) postulated that thyroid hormones probably do not regulate immune function as long as levels remain in the normal physiological range.

Although the effects of CS infusion and the hot environment were comparable in some characteristics (depressed growth, lymphoid involution), many other effects were very different. Continuously high plasma concentrations were not typical of the hot environment, as also reported by Williamson *et al.* (1985). The effects on thyroid hormones, packed cell volume, energy balance and composition of gain were quite different between CS infusion and high temperature. Also the effects on antibody production were, although small, different. The validity of continuous CS infusion as a experimental substitute for chronic stress is therefore questionable.

Thus some very distinct differences in metabolic characteristics and stress associated parameters, caused by either corticosterone infusion or high environmental temperature were found. These were not associated, however, with

differences in antibody titers. Differences in antibody production between the high and low selection line on the other hand were unaffected by experimental treatments and also not related to endocrine levels or general metabolic characteristics. Therefore these results are proof of the small impact of environmental influences or corticosterone on the height of the immune response in these selection lines.

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References

Beuving G. and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 35: 153-159.

Beuving G. and G.M.A. Vonder, 1981. The influence of ovulation and oviposition on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 44: 382-388.

Beuving G. and G.M.A. Vonder, 1986. Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and plasma baseline levels of corticosterone. *Gen. Comp. Endocrinol.* 62: 353-358.

Bobek S., M. Jastrzebski and M. Pietras, 1977. Age-related changes in oxygen consumption and plasma thyroid hormone concentration in the young chicken. *Gen. Comp. Endocrinol.* 31: 169-174.

Buyse J., E. Decuypere, P.J. Sharp, L.M. Huybrechts, E.R. Kühn and C. Whitehead, 1987. Effect of corticosterone on circulating concentrations of corticosterone, prolactin, thyroid hormones and somatomedin C and on fattening in broilers selected for high or low fat content. *J. Endocrinol.* 112: 229-237.

Davison T.F., B.M. Freeman and J. Rea, 1985a. Effects of continuous treatment with synthetic ACTH or corticosterone on immature gallus domesticus. *Gen. Comp. Endocrinol.* 59: 416-423.

Davison T.F. and B.H. Misson, 1987. Effect of corticosterone on antibody responses to a T-dependent and T-independent antigen in domestic fowl. *Med. Sci. Res.* 15: 967-968.

Davison T.F., B.H. Misson, R.A. Williamson and J. Rea, 1988. Effect of increased circulating corticosterone in the immature fowl on the blastogenic responses of peripheral blood lymphocytes. *Dev. Comp. Immunol.* 12: 131-144.

Davison T.F., J. Rea and B.M. Freeman, 1985. Effect of increased circulating corticosterone on the plasma concentrations of thyroxine and triiodothyronine in chickens. *IRCS Med. Sci.* 13: 692-693.

Davison T.F., M. Rennie and J. Rea, 1986. Effect of increased circulating corticosterone on the subpopulations of B and T lymphocytes in blood of immature domestic fowl. *IRCS Med. Sci.* 14: 563-564.

Decuypere E., C.G. Scanes and E.R. Kühn, 1983. Effects of glucocorticoids on circulating concentrations of thyroxine (T_4) and triiodothyronine (T_3) and on peripheral monodeiodination in pre- and post-hatching chickens. *Horm. Metabol. Res.* 15: 233-236.

Donker R.A. and G. Beuving, 1989. The influence of corticosterone infusion on antibody production, growth and some blood parameters in antibody-selected chicken lines. *Br.Poult.Sci.* (in press)

Donker R.A., S. Beers, W. v.d. Hel and E. Decuypere. *(submitted-a)* Energy metabolism characteristics in chicken lines divergently selected for humoral immune responsiveness. 1. Heat production at different ambient temperatures and energy metabolism during the immune response under normothermic conditions.

Donker R.A., M.G.B. Nieuwland and A.J. van der Zijpp, *(submitted-b)*. Heat stress in chicken lines selected for high and low immuneresponsiveness 1. Antibody production.

Donker R.A., M.G.B. Nieuwland, A.J. van der Zijpp and C.A. Makkink *(submitted-c)*. Heat stress in chicken lines selected for high and low immuneresponsiveness 2. Physiological parameters.

Donker R.A., A.G.J.C. Swinkels, A.J. v.d. Zijpp *(submitted-d)* The effect of environmental temperature on antibody production and some physiological parameters in chicken lines selected for humoral immune responsiveness.

Donker R.A., R. v.d. Wal, W. v.d. Hel and E. Decuypere. *(submitted-e)* Energy metabolism characteristics in chicken lines divergently selected for humoral immune responsiveness. 2. Energy metabolism during the immune response under hyperthermic conditions in acclimated and non-acclimated chickens.

Gause W.C. and J.A. Marsh, 1985. Differential effects of thyroxine on immune development and autoimmune thyroiditis in the obese strain chicken. *Dev. Comp. Immunol.* 9: 465-475.

Gross W.B., P.B. Siegel and R.T. DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poult. Sci.* 59: 516-522.

Harvey S. and C.G. Scanes, 1977. Purification and radioimmunoassay of chicken growth hormone. *J. Endocrinol.* 73: 321-330.

Heller E.D., D. Ben-Nathan and M. Perek, 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathol.* 8:195-203.

Henken A.M. and H.A. Brandsma, 1982. The effect of environmental temperature on immune response and metabolism of the young chicken. 2. Effect of the immune response to sheep red blood cells on energy metabolism. *Poult. Sci.* 61: 1667-1673.

Henken A.M., A.M.J. Groote Schaarsberg and W. van der Hel, 1983. The effect of environmental temperature on immune response and metabolism of the young chicken. 4. Effect of environmental temperature on some aspects of energy and protein metabolism. *Poult. Sci.* 62: 59-67.

Huybrechts L.M., D.B. King, T.J. Lauterio, T.J. March and C.G. Scanes, 1985. Plasma concentrations of Somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. *J. Endocrinol.* 104: 233-239.

Kafri I., R.W. Rosebrough, J.P. McMurtry and N.C. Steele, 1988. Corticosterone implants and supplemental dietary ascorbic acid effects on lipid metabolism in broiler chicks. *Poult. Sci.* 67: 1356-1359.

Kai O., M. Imada, Y. Imada and K. Sato, 1987. Effects of the thyroid status on the immune system of chickens. *Jap. Poult. Sci.* 24: 150-159.

Kai O., H. Nagase, N. Ishikawa, M. Suzuki, T. Kakegawa and K. Sato, 1988. Effects of propylthiouracil (PTU) on the immunological status of the chicken. *Dev. Comp. Immunol.* 12: 145-156.

Klandorf H., P.J. Sharp and M.G. Macleod, 1981. The relationship between heat production and concentrations of plasma thyroid hormones in the domestic hen. *Gen. Comp. Endocrin.* 45: 513-520.

Marsh J.A., W.C. Gause, S. Sandhu and C.G. Scanes, 1984a. Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. *Proc. Soc. Exp. Biol. Med.* 175: 351-360.

Marsh J.A., T.J. Lauterio and C.G. Scanes, 1984b. Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. *Proc. Soc. Exp. Biol. Med.* 177: 82-91.

Mashaly M.M., S.L. Youtz and R.F. Wideman Jr., 1983. Hypothyroidism and antibody production in immature chickens. *Immunol. Commun.* 12: 551-563.

May J.D., J.W. Deaton, F.N. Reece, N. Mitlin and L.F. Kubena, 1971. The effect of environmental temperature on blood volume. *Poult. Sci.* 50:1867-1870.

Powell P.C. and T.F. Davison, 1986. Induction of Marek's disease in vaccinated chickens by treatment with betamethasone or corticosterone. *Isr. J. Vet. Med.* 42: 73-78.

Regnier J.A., K.W. Kelley and C.T. Gaskins, 1980. Acute thermal stressors and synthesis of antibodies in chickens. *Poult. Sci.* 599: 985-990.

Romijn C. and W. Lokhorst, 1961. Climate and poultry. Heat regulation in the fowl. *Tijdschr. Diergeneesk.* 86: 153-172.

SAS, 1985. *SAS User's guide: Statistics*, Version 5 edition. Cary, NC: SAS Institute Inc.

Sato K. and B. Glick, 1970. Antibody and cell mediated immunity in corticosteroid-treated chicks. *Poult. Sci.* 49: 982-986.

Scott T., A. van der Zijpp and B. Glick, 1985. Effect of thiouracil-induced hypothyroidism on the humoral immunity of New Hampshire chickens. *Poult. Sci.* 64: 2211-2217.

Siegel H.S. and M. v. Kampen, 1984. Energy relationship in growing chickens given daily injections of corticosterone. *Br. Poultry Sc.* 25: 477-485.

Subba Rao D.S.V. and B. Glick, 1970. Immunosuppressive action of heat in chickens. *Proc. Soc. Exp. Biol. Med.* 133: 445-448.

Thaxton P., 1978. influence of temperature on the immune response of birds. *Poult. Sci.* 57: 1430-1440.

Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. *Poult. Sci.* 49: 202-205.

Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poult. Sci.* 52: 618-624.

Verstegen M.W.A., W. van der Hel, H.A. Brandsma, A.M. Henken and A.M. Bransen, 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. pg. 21-62 in: M.W.A. Verstegen and A.M. Henken (eds.): *Energy metabolism in farm animals*. Martinus Nijhoff publishers. Dordrecht, Boston, Lancaster.

Williamson R.A. and T.F. Davison, 1987. Effect of increased circulating corticosterone on serum and thyroidal concentrations of iodothyronines and the responses to thyrotrophin in the immature fowl (*Gallus domesticus*). *Gen. Comp. Endocrinol.* 65: 65-72.

Williamson R.A., B.H. Misson and T.F. Davison, 1985. The effect of exposure to 40° on the heat production and the serum concentrations of triiodothyronine, thyroxine and corticosterone in immature domestic fowl. *Gen. Comp. Endocrinol.* 60: 178-186.

Yam D., D. Heller and N. Snapir, 1981. The effect of the thyroidal state on the immunological state of the chicken. *Dev. Comp. Immunol.* 5: 483-490.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poult. Sci.* 59: 1363-1369.
Zijpp A.J. van der and M.G.B. Nieuwland, 1986. Immunological characterisation of lines selected for high and low antibody production. *Proc. 7th Eur. Poult. Conf. (Paris):* 211-215.

Thermal influences on antibody production and metabolism in chicken lines divergently selected for immune responsiveness.

General Discussion.

Introduction

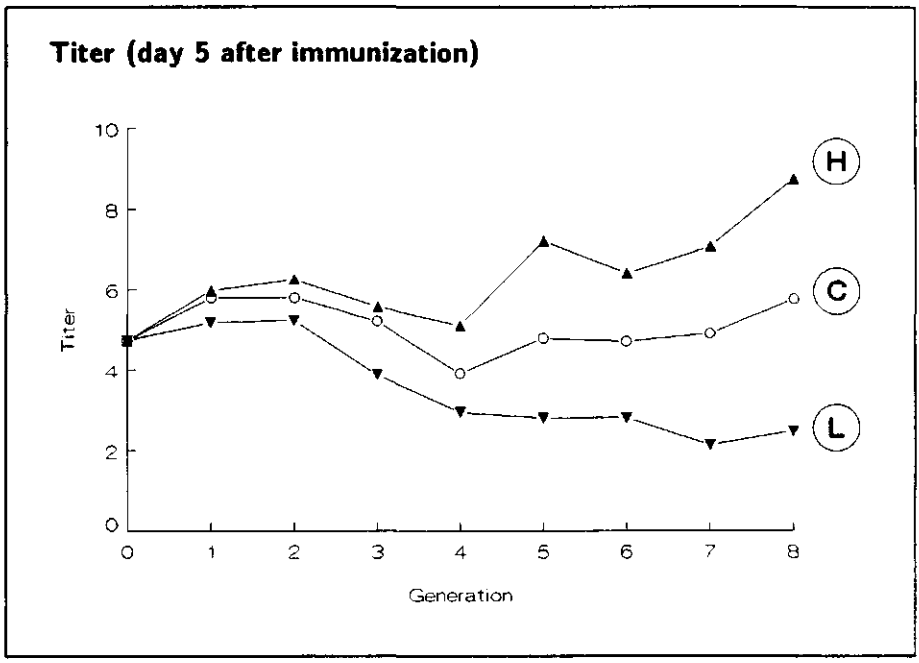
Genetic improvement of disease resistance is an attractive approach to minimize risk of disease outbreak and reduce the cost of medication and vaccination programs. The chosen approach of selection for responsiveness to a complex, though non-pathogenic antigen (SRBC) proved to be worthwhile. The lines diverged steadily from the first generation on. The justification of this approach is found in the results of experiments in which other antigens and infectious agents were used (Van der Zijpp *et al.*, 1988). In these experiments the high (H) line performed better (higher titers, lower mortality) than the low (L) line.

However, the response to a given antigen is not solely genetically determined. Heritability estimates of peak titers (5 days after immunization) were about .20 - .25 (Van der Zijpp *et al.*, 1988). Thus, environmental influences modulate the response to the SRBC immunization, even under standardized conditions. Although the repeatability of the assay itself is high (about .95; Van der Zijpp and Leenstra, 1980), variation from experiment to experiment and from year to year is evident. This can be illustrated by the antibody titers on day 5 after the SRBC immunization over the selection generations (Fig. 1; compare to Fig. 1 in the General Introduction). Although the differences between lines diverged consistently, the levels vary considerably.

These environmental influences on the responses, although the precise nature of the source is unknown, were the source of concern about possible genotype x environment interactions, and thus provided the motive for the present experiments. If genotype x environmental temperature interactions were evident, the selection could be impaired.

From the present experiments it was concluded that this is not the case. No interactions were found on either physiological parameters, metabolic rate or antibody production. This general conclusion will be discussed in more detail.

Figure 1. Selection result in antibody titers 5 days after immunization. H: High line; C: random bred Control line; L: low line



Stress parameters

During the experiments a number of indicators of the impact of treatments on the birds were measured.

The acute heat stress (in which the chickens were kept for ½ h periods at 42°C, with ½ h intervals), as described in Chapters I and II, did affect cloacal temperature and stimulated the hypothalamo-pituitary-adrenal (HPA) axis in the chickens. These responses, measured by plasma corticosterone concentrations and cloacal temperature, were in accordance with those reported elsewhere (Ben Nathan *et al.*, 1976; Edens and Siegel, 1975, 1976; Siegel and Gould, 1982; Williamson *et al.*, 1985), but more importantly, they were not different between the H and L line. Also, when the adrenals were stimulated directly by injecting ACTH, the increase in plasma corticosteroids did not differ between H and L line (unpublished results). The effects on both cloacal temperature and plasma corticosteroids were temporary, as can be concluded from the return to initial levels in the intervening ½ hour periods between the high temperature periods (Chapter II) and the absence of elevated corticosterone levels when temperature was increased from 25°C to 35°C (Chapter VII).

When the birds were placed in high temperature environments (35°C) during longer periods, either with or without the opportunity for acclimation (Chapters III, VII, VIII), they were hyperthermic continuously. No effects were found on plasma corticosterone levels. Thus, in these experiments, the HPA axis was not stimulated or, more probably, during acclimation the corticosteroid levels were lowered to their base levels again. This requires clearing of the corticosterone from the blood stream during the ½ h intervals (Chapter I and II) and soon after the temperature increase from 25° to 35°C (Chapters III, VII). This implies endocrine feedback, and binding in lymphocytes (Gould and Siegel, 1974, 1978, 1980, 1984, 1985; Siegel and Gould, 1982).

Also depression in feed intake, growth rate, reduced protein retention and fat deposition and the detrimental effects on lymphoid tissue were clear testimonies of the impact of the hot environment. But, here again, the effects on the birds were equal in the H and L line.

Some of the other measured parameters were either not affected at all or results were inconsistent.

Changes in heterophils and lymphocytes, or more particularly the ratio between these two, was reported to be a reliable estimate of stress responses (Chancellor and Glick, 1960; Ben Nathan *et al.*, 1976; Heller *et al.*, 1979; Gross *et al.*, 1980; Gross and Siegel, 1983). We could not confirm these observations in the present experiments. No consistent changes were found that reflected the impact of heat stress, either after short during acute stress (Chapter II), or during the prolonged heat periods (Chapter III, unpublished results). Some interesting relations were manifest, however, in the response to immunization (Chapter II). These were proof of the involvement of the heterophils (the chickens equivalent of the mammals' "polymorphic neutrophil") in the immune response. Comparable results were reported by Trout *et al.* (1988a). Recent observations (Trout *et al.*, 1988b) revealed morphological changes in these cells during the initiation of the immune response. Despite this proven role in immune function, no difference in number of these cells was found between H and L line. However, more precise study of these cells during the early phases of an immune response, for example using FACS-equipment, might be worthwhile.

Two of the "acute phase proteins", fibrinogen and albumin, did not prove useful as indicators of stress. Acute phase proteins are affected by infection or injury and show rapid changes in concentration, usually in relation to body temperature (fever). Although they do change together with cloacal temperature during a progressing infection in poultry (Sijtsma, personal communication), no relation with increased cloacal temperature was found in the present experiments (Chapter II; Chapter III, unpublished results). Unexpected, but remarkable changes during the immune response were found, on the day peak

titer is reached, dependent on immunization route (intramuscular or intravenous). This indicates therefore some relation with antibody production. The implications of this finding are unclear, but deserve further study.

During the experiments which were performed in the climate-respiration chambers (Chapters VI, VII, VIII) also endocrine data were gathered.

Thyroid hormones are frequently associated with immune function (Gause and Marsh, 1985; Kai *et al.*, 1987, 1988; Marsh *et al.*, 1984a, 1984b; Mashaly *et al.*, 1983; Scott *et al.*, 1985; Yam *et al.*, 1981). Very variable results were obtained, however, also in the present studies. Only when the H and L line were compared under "standard" 25°C conditions (Chapter VI) was a small, but consistent, difference in T_3 concentration between the lines found. This higher level in the H line was similar to a recently reported difference between lines bred to immune response after intravenous immunization with SRBC (Martin *et al.*, 1988). In the other experiments (Chapters VII, VIII), no reliable relations between thyroid hormones and immune function, selection line or treatment (high temperature or corticosterone infusion) were evident.

Growth hormone concentration was lower during the periods at high temperatures (Chapter VII) than at control temperatures, either with or without previous acclimation. Somatomedin-C, an inhibitor of growth hormone was higher at the same time. These findings match with the decreased growth during these periods. No relations were observed however, that would reveal an explanation for differences in antibody production between the H and L line, or between temperature treatments.

Also no apparent differences were found between H and L line in metabolic rate. Heat production was dependent on environmental temperature during a wide range of temperatures (Chapter VI), equally in the two lines. Thermoneutrality was maintained by adjustment of the *ad libitum* feed intake. Only a small difference in energy balance, heat production and fat deposition was evident between the lines (all higher in H line), during the balance period immediately after immunization under 25°C conditions (Chapter VI). These results were analogous to those found by Henken *et al.* (1983) when they compared SRBC immunized chickens with a group that received a sham injection.

The high temperature treatments (continuously hot, or sudden change from 25° to 35°C; Chapters VII, VIII) and the corticosterone infusion (Chapter VIII) had a major impact on metabolic rate. Metabolizable energy, energy balance, heat production and fat deposition were considerably decreased, but protein retention was rather constant. Only during the balance period immediately following the temperature change from 25° to 35°C (Chapter VII) a decreased protein retention was found, an indication of severe stress during this period.

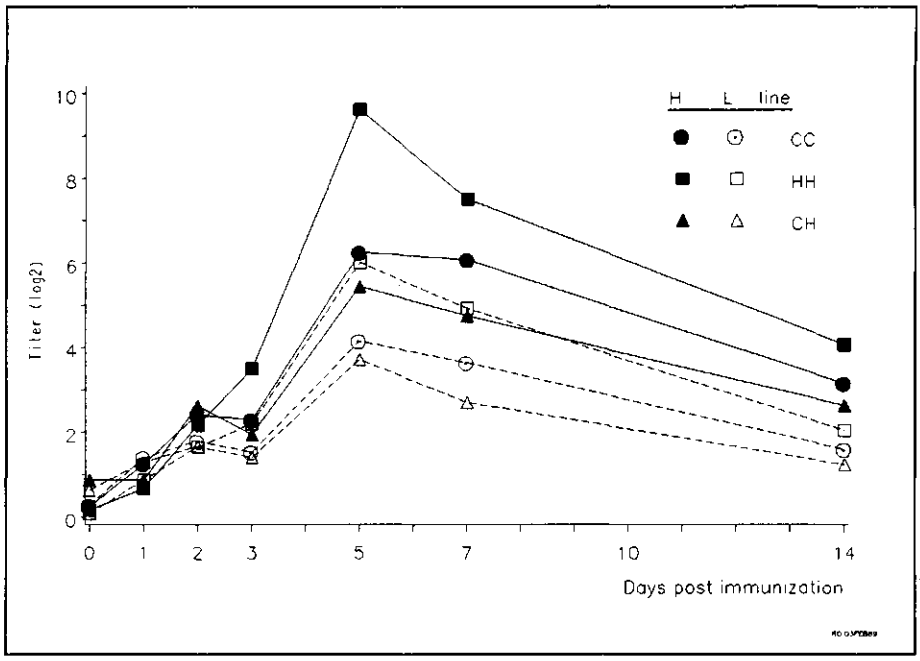
Interactions with antibody production

Acute stressors

Although a number of the physiological parameters were indicative of major influences of the thermal treatments on the birds, effects on the antibody titers were small.

A major influence of acute stressors on immune responses is generally accepted to be mediated through the HPA axis (see General Introduction). In the case of antibody production, the period most sensitive to heat stress treatment was reported to be between 24 h before and 12 h after the immunization (Subba Rao and Glick, 1970). Despite the fact that the acute short thermal stressor (four ½ h periods at 42°C; Chapter I) or the acute change in temperature (from 25° to 35°C; Chapters III, VII) was given within this period, and it was clear that the HPA axis was stimulated in both lines, only incidently was an immunosuppressive action found. After the short period heat stress a small suppression was found in only one experiment (Chapter I), and in the H line only. But the effect of suppression was considerably smaller than the obtained selection difference in this (6th) generation. The most pronounced immunosuppression was found in the experiment described in Chapter III, after transfer from a 25°C environment to a 35°C environment (Fig. 2). It is important to notice that this suppression was similar in the H and L line.

Figure 2. Antibody production in CC, HH and CH treatment after primary immunization with SRBC (Chapter III).
CC: 25°C environment; HH: 35°C environment; CH: moved on day of immunization from 25° to 35°C environment.



Also in literature considerable variation in the suppressive effects of heat stress on antibody production has been reported (Heller *et al.*, 1979; Regnier *et al.*, 1980). A number of factors may interfere with the occurrence of immunosuppression, as discussed in Chapter I. It is noteworthy that the most significant heat stress mediated immunosuppression on humoral immune responses reported in literature was found either in a New Hampshire line, selected for high bursal weight (Subba Rao and Glick, 1970; 1977) or in Athens Randombred that were selected for high stress responsiveness (Thaxton and Siegel, 1970; 1972; 1973; personal communication), although this was not always clearly stated. Regnier *et al.* (1980) used birds from the same breeds, which were not selected for these traits in comparable experiments. They did not find suppressive effects on antibody titers. Also Davison *et al.* did not find suppressive effects of a similar heat stress treatment in their birds (personal communication). Heller *et al.* (1979) even reported enhancing effects of the same acute heat stress treatment, in the chicken lines they used.

Therefore, it is even more notable that in the present experiments, if suppressive effects were found, the H and L line reacted in such similar manner. This demonstrates the absence of an increased stress susceptibility in either line. It emphasizes, however, in combination with the literature cited, that genetic differences can exist between poultry stock in susceptibility to a stressor with varying outcome in antibody response.

Only one other study of environment x genotype interactions in antibody production, using birds from the same genetic origin, has been reported so far. Gross (1986) studied the immune responses in different social environments of birds which were selectively bred for high or low antibody production after intravenous immunization with SRBC (Siegel and Gross, 1980). In that study more pronounced and consistent results in responses to SRBC and bacterial antigen between the H and L line were found in 'socialized' birds. Comparable differences were found however in 'normal' treated or 'harassed' birds. So in that study no clear line x social environment interaction was found on the immune response.

More surprising than the absence of suppressive action of acute stress in the present studies, was the absence of immunosuppressive action of direct injection of ACTH (unpublished results) and the corticosterone infusion in the experiments described in Chapters IV and VIII. Direct administration of ACTH or corticosteroids supplied in the feed or by means of infusion, pellet implantation or injection usually cause a significant decrease in antibody production (Sato and Glick, 1970; Gross *et al.*, 1980; Davison and Misson, 1987), or cause an increase in the incidence of Mareks' disease (Powell and Davison, 1985; 1986). All other effects of the infused corticosterone in the present experiments, (growth depression, lymphoid regression, leukocyte

changes, fat deposition) were very typical for chronic stress, or at least for a chronic high plasma corticosterone level.

From these observations it is therefore concluded that the H as well as the L selection line is particularly tolerant to heat stress or corticosteroid effects on antibody production.

Metabolic rate

Stimulatory influences on antibody production were found if birds were kept for long periods in high temperature environments (Chapters III, VII, VIII). In Chapter III an increase of the antibody production in both lines was found if the birds were given time to acclimate (Fig. 2). This is similar to results reported by Henken *et al.* (1983). These effects could have been caused by stimulation of the hypothalamo-pituitary-thyroid (HHT) axis, and thus increased metabolic rate (Henken, 1983), although these were not measured in this experiment. The same treatment repeated in the climate respiration chambers, however, did not consistently result in an increase in antibody production (Chapters VII, VIII), and the involvement of thyroid regulation could not be confirmed. During these experiments in the climate respiration chambers considerable changes in metabolic rate caused by temperature treatments or corticosterone infusion were evident, but could not be clearly related to changes in the measured hormones or the height of the immune response. *Vice versa*, considerable differences in antibody production between the lines were not reflected in differences in metabolic rate or hormone levels (Chapter VI, VII, VIII).

Thus, from these experiments in the climate respiration chambers it is clear that the differences in antibody production between high and low line are not based on aspecific differences in metabolic rate.

Ontogeny

The experiments described in Chapter IV, revealed a significant difference between the high and low line in the weight of one of the immunologic most important organs, the spleen. In all other experiments higher spleen weight were found in the high line too, but in this experiment it was found to be so during the whole ontogeny of the bird, even before immunization. Moreover, it was demonstrated that after immunization higher numbers of plaque-forming-cells were present in the spleen of high line birds. This certainly contributes to the immunologic difference between the lines. These findings imply that functional differences in immune responsiveness are probably based on cellular activity and cooperation.

Another important finding, which justifies more basic research into cell-cell and cell-antigen interactions, is that the frequencies of some haplotypes of the major histocompatibility complex (B-complex) are different in the

successive lines (Van der Zijpp and Nieuwland, unpublished results). This probably has implications on a number of important immunologic cell-interactions, with consequences for disease resistance.

Consistency of results

As outlined in the first paragraph of this discussion, considerable variation between experiments can be found in antibody production levels. This was also found in the present series of experiments. Sometimes immunosuppression was found; the same treatment repeated in a second experiment did not give any immunosuppression. Even under these highly controlled experimental conditions there were apparently a number of factors which influenced the occurrence of stimulation or suppression. It is therefore comforting to find that, even if a environmental factor influenced the response, the impact was always smaller than the selection difference obtained in the six or seven generations. Moreover, the difference between selection lines was consistent under all conditions studied in these experiments. It is questionable whether high temperature influences under practical conditions, unless being lethal in itself, would have greater impact on the immunology of the birds than in the experiments presented here. The importance of high temperature interactions in these lines can therefore be neglected, also under practical conditions.

Conclusions:

From these experiments it is concluded that

- The selection on antibody production did not alter the physiological responses to an acute thermal stressor, or to high environmental temperature.

- Both the high and low selection line are particularly resistant to thermal stress or the effects of corticosterone, with respect to antibody production.

- Environmentally induced changes in endocrine profiles and metabolic rate, before or during the immune response, do not have a regulatory role in antibody production.

- The obtained difference in immune responsiveness between the high and low selection line is not physiologic functionally related to differences in body weight or metabolic rate.

- The size of the spleen and the total number of (potential) immunocompetent cells therein is a deterministic factor for the difference in immune responsiveness between the high and low selection line.

References

Ben-Nathan D., E.D. Heller and M. Perek, 1976. The effect of short heat stress upon leucocyte count, plasma corticosterone level, plasma and leucocyte ascorbic acid content. *Br. Poult. Sci.* 17: 481-485.

Chancellor L. and B. Glick, 1960. Effect of temperature as a stressor on white blood cells, adrenals and bursae of Fabricius of chicks. *Am. J. Physiol.* 198: 1346-1348.

Davison T.F. and B.H. Misson, 1987. Effect of corticosterone on antibody responses to a T-dependent and T-independent antigen in domestic fowl. *Med. Sci. Res.* 15: 967-968.

Edens F.W. and H.S. Siegel, 1975. Adrenal responses in high and low ACTH-response lines of chickens during acute heat stress. *Gen. Comp. Endocrinol.* 25: 64-73.

Edens F.W. and H.S. Siegel, 1976. Modification of corticosterone and glucose responses by sympatholitic agents in young chickens during acute heat exposure. *Poultry Sci.* 55: 1704-1712.

Gause W.C. and J.A. Marsh, 1985. Differential effects of thyroxine on immune development and autoimmune thyroiditis in the obese strain chicken. *Dev. Comp. Immunol.* 9: 465-475.

Gross W.B. and H.S. Siegel, 1983. Evaluation of the heterophil/lymphocite ratio as a measure of stress in chickens. *Avian Dis.* 27: 972-979.

Gould N.R. and H.S. Siegel, 1974. Age variation in corticosteroid binding by serum proteins of growing chickens. *Gen. Comp. Endocrinol.* 24: 177-182.

Gould N.R. and H.S. Siegel, 1978. Effect of age and sex on the association constant and binding capacity of chicken serum for corticosteroid. *Poultry Sci.* 57: 778-784.

Gould N.R. and H.S. Siegel, 1980. Effect of adrenocorticotropin on binding of endogeneous corticosteroid by chicken bursal cells. *Poultry Sci.* 59: 1935-1940.

Gould N.R. and H.S. Siegel, 1984. Effect of adrenocorticotropin hormone injections on glucocorticoid receptors in chicken thymocytes. *Poultry Sci.* 63: 373-377.

Gould N.R. and H.S. Siegel, 1985. Effect of corticotropin and heat on corticosteroid-binding capacity and serum corticosteroid in white rock chickens. *Poultry Sci.* 64: 144-148.

Gross W.B., P.B. Siegel and R.T. DuBose, 1980. Some effects of feeding corticosterone to chickens. *Poultry Sci.* 59: 516-522.

Heller E.D., D. Ben-Nathan and M. Perek, 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathol.* 8: 195-203.

Henken A.M., A.M.J. Groote Schaarsberg and M.G.B. Nieuwland, 1983. The effect of environmental temperature on immune response and metabolism of the young chicken. 3. Effect of environmental temperature on the humoral immune response following injection of sheep red blood cells. *Poultry Sci.* 62: 51-58.

Kai O., M. Imada, Y. Imada and K. Sato, 1987. Effects of the thyroid status on the immune system of chickens. *JPN. Poult. Sci.* 24: 150-159.

Kai O., H. Nagase, N. Ishikawa, M. Suzuki, T. Kakegawa and K. Sato, 1988. Effects of propylthiooouracil (PTU) on the immunological status of the chicken. *Dev. Comp. Immunol.* 12: 145-156.

Marsh J.A., W.C. Gause, S. Sandhu and C.G. Scanes, 1984a. Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. *Proc. Soc. Exp. Biol. Med.* 175: 351-360.

Marsh J.A., T.J. Lauterio and C.G. Scanes, 1984b. Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. *Proc. Soc. Exp. Biol. Med.* 177: 82-91.

Martin A., F.M.A. McNabb and P.B. Siegel, 1988. Thiouracil and antibody titers of chickens lines divergently selected for antibody response to sheep erythrocytes. *Dev. Comp. Immunol.* 12: 611-619.

Mashaly M.M., S.L. Youtz and R.F. Wideman Jr., 1983. Hypothyroidism and antibody production in immature chickens. Immunol. Commun. 12: 551-563.

Powell P.C. and T.F. Davison, 1985. The interaction between stress and Marek's disease. (?)

Powell P.C. and T.F. Davison, 1986. induction of Marek's disease in vaccinated chickens by treatment with betamethasone or corticosterone. *Isr. J. Vet. Med.* 42: 73-78.

Regnier J.A., K.W. Kelley and C.T. Gaskins, 1980. Acute thermal stressors and synthesis of antibodies in chickens. *Poultry Sci.* 59: 985-990.

Scott T., A. van der Zijpp and B. Glick, 1985. Effect of thiouracil-induced hypothyroidism on the humoral immunity of New Hampshire chickens. *Poultry Sci.* 64: 2211-2217.

Siegel H.S. and N.R. Gould, 1982. High temperature and corticosteroid in the lymphocytes of domestic fowl (*Gallus domesticus*). *Gen. Comp. Endocrinol.* 48: 348-354.

Subba Rao D.S.V. and B. Glick, 1977. Effect of cold exposure on the immune response of chickens. *Poultry Sci.* 56: 992-996.

Thaxton P. and H.S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. *Poultry Sci.* 49: 202-205.

Thaxton P. and H.S. Siegel, 1972. Depression of secundary immunity by high environmental temperature. *Poultry Sci.* 51: 1519-1526.

Thaxton P. and H.S. Siegel, 1973. Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Sci.* 52: 618-624.

Trout J.M., M.M. Mashaly, H.S. Siegel, 1988a. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Dev. Comp. Immunol.* 12: 331-346.

Trout J.M., M.M. Mashaly, H.S. Siegel, 1988b. Changes in profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity male chickens. *Dev. Comp. Immunol.* 12: 331-346.

Williamson R.A., B.H. Misson and T.F. Davison, 1985. The effect of exposure to 40° on the heat production and the serum concentrations of triiodothyronine, thyroxine and corticosterone in immature domestic fowl. *Gen. Comp. Endocrinol.* 60: 178-186.

Yam D., D. Heller and N. Snapir, 1981. The effect of the thyroidal state on the immunological state of the chicken. *Dev. Comp. Immunol.* 5: 483-490.

Zijpp A.J. van der, J.J. Blankert, E. Egberts and M.G.J. Tilanus, 1988. Advances in genetic disease resistance in poultry. pg. 131-138 in: S. Korver, H.A.M. van der Steen, J.A.M. van Arendonk, H. Bakker, E.W. Brascamp and J. Dommerholt (compilers). *Advances in animal breeding.* Pudoc, Wageningen, The Netherlands.

Zijpp A.J. van der and F.R. Leenstra, 1980. Genetic analysis of the humoral immune response of White Leghorn chicks. *Poultry Sci.* 59: 1363-1369.

Thermal influences on antibody production and metabolism in chicken lines divergently selected for immune responsiveness.

General Abstract.

Introduction

The international character of the poultry industry requires that poultry bred under temperate conditions should be able to perform under tropical conditions as well. However, abrupt changes in environmental temperature or continuous high temperatures may affect production traits, immune responsiveness, and thus disease resistance.

Improved disease resistance can be obtained by breeding for high immune responsiveness. However, if the effects of a stressor, e.g. high temperature, is be different in the successive selection lines, the selective advantage can be lost.

Two mechanisms might cause such a genotype x environment interaction. First, the stress susceptibility may be increased after the selection for high responsiveness. Second, the body weight, and thus possibly metabolic rate, may be different between the selection lines. This could imply different thermal requirements of the birds, and thus different reactions to changed environmental temperatures.

In the present studies, the effects of acute thermal stress and continuous high temperature on antibody production to sheep red blood cells (SRBC) were investigated in young chickens, which had been selected for either high (H) or low (L) antibody titers to SRBC for several generations. Also, the influence of infused corticosterone, the "stress"hormone that is held responsible for immunosuppression, was studied. The general aim of the studies was to investigate the occurrence of genotype x environment interactions and the causes for such interactions.

Chapter I

To study the effects of acute thermal stress on antibody production, three experiments were carried out. The chickens were subjected to an acute heat stress treatment (4 periods of ½ h at 42°C, with ½ h intervals) 24 h before an immunization with SRBC. Experiments reported in literature described considerable immunosuppressive action of such treatments. This treatment could give therefore a good opportunity to study the possible differences in response between the selection lines.

Immunizations were given intramuscularly (i.m.) and intravenously (i.v.) (with different doses). Only in one experiment was a relatively small, but

significant, immunosuppressive effect found in the H line, but not in the L line. The immune response in the H line was always considerably higher than in the L line.

Chapter II

To quantify the impact of the heat stress treatment on the birds, changes in a number of physiological parameters were studied in the birds, which were subjected to the treatment described in chapter I.

During the heat stress treatment cloacal temperature and plasma corticosterone were increased. Changes in numbers of circulating leukocytes, and plasma albumin and fibrinogen concentrations were not changed during the heat stress, but changed during the following days in which the immune response was developing. Differences in these changes between i.m. and i.v. immunized birds were found, but H and L line were not different.

Chapter III

In chapter III an experiment is described, in which the chickens were placed in climate chambers. In this experiment, an acute thermal stress was given, comparable to that described in chapters I and II, but the effects of prolonged heat on the birds were also studied. After acclimation to high temperature, the influence of acute stress diminishes, and endocrine and metabolic changes can affect antibody production. The environmental temperature was constant either 25° or 35°C or it fluctuated daily between 15-25°C or 25-35°C. At the time the immunization with SRBC was given, some of the chickens were exchanged between the different temperatures (acute stress).

At the high temperature, the chickens were hyperthermic, showed depressed growth, and *post mortem* lower weights of lymphoid organs were found equally in acclimated and non-acclimated chickens. Birds moved from 25° to the 35°C environment (CH) had a lower immune response than those that remained at 25° (CC). The birds which were already in the 35° environment before immunization (HH) mounted higher immune responses. The responses in birds which were kept in the chambers with fluctuating temperatures, showed less pronounced differences, suggesting a smaller impact of these temperatures, or a better acclimation of the birds to the temperature. These effects were the same in H and L line chickens.

Chapter IV

A number of experiments are described in chapter IV, in which the ontogeny of lymphoid organs (important for the immune system), and the number of plaque-forming-cells (the precursors of the antibody-secreting cells) in the spleen were studied. Aim of this study was to find morphological differences between the H and L line that might determine the difference in immune

responsiveness. It was found that the spleen was already heavier in the H line than in the L line, before immunization. Also, higher numbers of plaque-forming-cells were found in the spleen of birds from the H line than in those of L line birds after immunization. These differences contribute to the differences in antibody production after immunization.

Chapter V

Because the effects of acute heat stress on antibody production, described in chapters I and III, were smaller than expected, a study with direct application of corticosterone was done. Corticosterone is the hormone presumed to cause immunosuppression after stress. Direct administration of corticosterone might reduce the variability in the response measured after thermal stressors, and thus provide a more precise comparison of stress effects between the lines. In chapter V this experiment is described in which the birds were infused with a corticosterone solution (CS).

Major effects of the CS treatment on plasma corticosterone, growth, leukocytes and lymphoid organs were found. The antibody titers to SRBC were unaffected, in the H and L line.

In chapters VI, VII and VIII the effects of the treatments already described in chapter III (CC, HH and CH) and V (CS infusion) were studied more extensively. Changes in metabolic rate, growth, energy and protein turnover, caused by the different temperature treatments might have comparable effects on antibody production as obtained by selection. These treatments were studied in relation to changes in endocrine factors (corticosterone, growth hormone, somatomedine, thyroid hormones T_3 and T_4) and energy metabolism. Therefore these studies were performed in the climate-respiration chambers.

Chapter VI

Because an average weight difference exists between the H and L line (L line is usually about 7% heavier) the influence of differences in metabolic rate between the lines on antibody production was studied.

Heat production was recorded at different temperatures in both lines. Heat production was somewhat higher in the L line than in the H line in one of two experiments, but no lower critical temperature could be estimated in either line. Thermo-neutrality was maintained by adjusting feed intake.

During the immune response only minor differences in energy metabolism were detected between the two lines. These could not unanimously be related to the height of the immune response.

Chapter VII

Chickens were subjected to a CC, CH or HH treatment, as described in chapter III. The effects of high temperature on metabolism were very evident. Decreased feed intake, growth rate, heat production and fat deposition were found. In the CH treatment also a decreased protein retention was found during the balance period immediately after the temperature change. Effects on cloacal temperature and lymphoid organs were as reported before (Chapter III). The effects of the temperature treatments on antibody production were not as impressive as found in the experiment in chapter III. Plasma corticosterone and thyroid hormones were not affected, somatomedine was increased and growth hormone decreased in hot environments. Differences in metabolic traits, caused by the different environmental temperatures were not related to differences in antibody titers, and differences in antibody titers between the H and L line were not reflected in differences in metabolic rate.

Chapter VIII

Finally, the CC and HH treatments were compared to CS infusion. Some effects of the infused corticosterone and HH treatment were similar. Decreased feed intake, growth and heat production were found in both treatments. Also lymphoid organs were negatively influenced in either treatment. But no increased plasma corticosterone levels were evident in HH treatment. T_4 concentration was decreased in CS infused birds; T_3 was lower in CS infused and was the lowest in HH treated birds. The energy balance was higher in CS infused birds immediately after immunization. Fat deposition was higher in CS infused birds. A small stimulatory effect of the HH treatment on antibody production was found in the L line birds only. Similar to chapter VII, no clear relations between endocrine parameters, energy metabolism and antibody production were found. It was questioned whether the CS infusion is valuable as an experimental model for continuous (heat) stress, because of the marked differences in a number of the measured characteristics.

Discussion

In the discussion it is argued that the differences between H and L line in antibody production are very steady.

From the experiments, it is evident that severe heat stress, prolonged heat and corticosteroids do affect the birds: growth, body temperature, corticosterone and other hormones, lymphoid development are changed, but equally in both lines. The selection did not result in a "high stress susceptibility line".

Moreover, both lines are apparently rather stress resistant, with regard to antibody production. Acute heat stress, prolonged severe heat and even

corticosterone infusion could only marginally affect antibody titers. If the antibody production was affected by the experimental treatment, the changes were usually very similar in the H and L line. The absence of genotype x environment (heat stress) effects was therefore apparent.

The influence of metabolic rate, as measured here, on antibody production was rather small. Some changes in fat deposition during the balance period immediately after the immunization were found, which could indicate a shift in energy distribution between fat and protein, during the immune response. But no direct relation between metabolic rate, influenced by selection or environmental temperature with endocrine regulation and antibody production was found. It was also concluded therefore that the difference in bodyweight between the selection lines is rather based on linked genes than physiologic meaningful relations between the immune system and energy metabolism.

Temperatuurs invloeden op antilichaam produktie en metabolisme in op immuun respons geselekteerde kippelijnen.

Samenvatting.

Inleiding

Het internationale karakter van de pluimvee-industrie vereist dat kippen, welke gefokt zijn onder gematigde klimatoligische omstandigheden, ook onder tropische omstandigheden moeten kunnen produceren. Plotselinge temperatuurs-veranderingen en hoge temperaturen kunnen echter de produktie en het immuunsysteem, en daarmee de ziekteresistentie, beinvloeden.

Verhoogde ziekteresistentie kan onder andere verkregen worden door te selekteren op een hoge immuunrespons. Wanneer deze voordelen van een verhoogde resistentie onder gematigde omstandigheden echter verloren gaan als gevolg van b.v. hoge temperaturen, is de waarde hiervan beperkt.

Er zijn twee mogelijke mechanismen waardoor dergelijke genotype x milieu interakties op zouden kunnen treden. Op de eerste plaats kan de stress-gevoeligheid van de kippen welke op een hoge respons geselekteerd zijn verhoogd zijn. Op de tweede plaats kan, als gevolg van een gemiddeld gewichtsverschil tussen de hoge en de lage selektielijn, de temperatuurs-behoefte van de dieren veranderd zijn en daarmee de reaktie op veranderende omgevingstemperaturen.

In de beschreven experimenten werd de invloed van "akute hittestress" (plotselinge temperatuursveranderingen) en konstant hoge temperaturen op antilichaamproduktie bestudeerd. Hierbij werden jonge kippen gebruikt, welke gedurende een aantal generaties geselekteerd waren op een hoge ("H") of lage ("L") antilichaamproduktie nadat zij geimmuniseerd waren met schapen rode bloed cellen (SRBC). Behalve temperatuursinvloeden is ook gekeken naar de invloed van corticosteron, toegediend via een infuus. Corticosteron is het "stress"hormoon, dat waarschijnlijk een belangrijke rol speelt in het veroorzaken van immuunsuppressie. Algemeen doel van alle experimenten was het bestuderen van het vóórkomen van genotype x milieu interakties en de oorzaken ervan.

Hoofdstuk I

Teneinde de invloed van akute hittestress op antilichaamproduktie te bestuderen werden drie experimenten gedaan. De kuikens werden aan een "hitte behandeling" blootgesteld van 4 periodes van een ½ uur bij 42°C, met telkens een ½ tussentijd, 24 uur voor ze geimmuniseerd werden met SRBC. Uit de literatuur was al bekend dat een dergelijke behandeling een goede methode was

om immuunsuppressie op te wekken. Daarom zou deze methode geschikt moeten zijn om de mogelijke verschillen in respons tussen de selektielijnen te bestuderen.

De immunisaties werden zowel in de spieren (intramusculair, i.m.) als rechtstreeks in de aderen (intraveneus, i.v.) gegeven, met verschillende doses. Slechts in één experiment werd een, relatief klein maar signifikant, immuunsuppressief effekt gevonden in de H lijn, doch niet in de L lijn. De hoogte van de immuunrespons was in de H lijn altijd belangrijk hoger dan in de L lijn.

Hoofdstuk II

Om een indruk te kunnen krijgen over hoe "zwaar" de hittestress was, zoals die gebruikt was in hoofdstuk I, en of deze belasting verschillend was tussen de twee lijnen, werden een aantal fysiologische parameters gemeten.

Tijdens de hitte behandeling waren de rektaal gemeten temperatuur en het corticosteron gehalte in het bloed verhoogd. Tijdens deze behandeling werden er geen veranderingen in de aantallen witte bloedcellen en plasma albumine en fibrinogeen concentraties gevonden. Deze parameters veranderden echter wel in de daarop volgende dagen tijdens het ontwikkelen van de immuunrespons. Er werden in deze kenmerken geen verschillen gevonden tussen de H en L lijn, maar wel tussen i.v. of i.m. geimmuniseerde dieren.

Hoofdstuk III

In hoofdstuk III wordt een experiment beschreven, waarin de kuikens in klimaatkamers geplaatst waren. In dit experiment werd een akute hittestress gebruikt, vergelijkbaar met die in hoofdstukken I en II, maar werd ook de invloed van kontinue hitte bestudeerd. Na aanpassing aan de hoge temperatuur neemt de stress invloed af en kunnen hormonale en metabole veranderingen mogelijk de immuunrespons beinvloeden. De ingestelde omgevingstemperatuur was 25°of 35°C of fluktueerde dagelijks tussen 15-25°C of 25-35°C. Op het moment dat de immunisatie met SRBC werd gegeven, werd een deel van de kuikens overgeplaatst van de ene temperatuur naar de andere (akute stress).

Bij de hoge temperaturen hadden de kuikens konstant een verhoogde rektaal temperatuur, groeiden slechter en hadden, na slachten, lagere gewichten van de lymfoïde organen. Bij de kuikens welke overgeplaatst waren van 25° naar 35°C (CH behandeling) werd een lagere immuunrespons gemeten dan bij die welke konstant bij 25°C gehouden werden (CC controle). Een hogere respons werd echter juist gemeten bij kuikens welke konstant in de 35°C omgeving zaten (HH behandeling). De kuikens in de kamers met dagelijks fluktuerende temperaturen vertoonden kleinere afwijkingen in de hoogte van de respons. Dit duidt op een geringere invloed van deze temperaturen, of een betere aanpassing van de kuikens aan de omgeving. Alle gemeten effekten waren hetzelfde in de H en de L lijn.

Hoofdstuk IV

In hoofdstuk IV wordt een aantal experimenten beschreven, waarin de ontogenie van een aantal lymphoïde organen (belangrijk voor het immuunsysteem), en het aantal plaque-vormende-cellen (de voorlopers van de antilichaam uitscheidende cellen) in de milt worden bestudeerd.
Het doel van deze studie was om morfologische verschillen tussen de H- en de L-lijn te vinden die het verschil in immuunrespons kunnen bepalen. Er werd gevonden dat de milt al voor immunisatie zwaarder is in de H dan in de L lijn. Tevens werden er grotere aantallen plaque-vormende cellen gevonden in de milt van H lijn kuikens na immunisatie. Deze verschillen dragen bij aan het verschil in antilichaamproduktie na immunisatie.

Hoofdstuk V

Omdat de verschillen in antilichaamproduktie, na de acute hittestress zoals beschreven in de hoofdstukken I en III, kleiner waren dan verwacht, werd een experiment uitgevoerd met rechtstreekse toediening van corticosteron. Corticosteron is het hormoon dat een belangrijke bijdrage levert aan de immuunsuppressie veroorzaakt na stress. Rechtstreekse toediening van corticosteron kan de variabiliteit in de gemeten respons na hittestress verminderen en kan dus een nauwkeuriger vergelijking van de stresseffekten geven tussen de lijnen. In dit hoofdstuk wordt dit experiment beschreven, waarbij corticosteron werd toegediend door middel van een infuus (CS-infuus).

Duidelijke effekten van de CS-behandeling werden gevonden op de plasma corticosteron concentratie, groei, aantallen leukocyten en ontwikkeling van lymfoïde organen. De antilichaam-respons tegen SRBC was niet beinvloed, noch in de H, noch in de L lijn.

In de hoofdstukken VI, VII en VIII worden de behandelingen, zoals beschreven in hoofdstukken III (CC, HH en CH) en V (CS-infuus) uitgebreider bestudeerd. Veranderingen in metabolisme, groei, energie- en eiwithuishouding, zoals veroorzaakt door de verschillende temperatuurbehandelingen, kunnen vergelijkbare effekten hebben op de antilichaamproduktie, als verkregen door selektie. Daarom werden deze behandelingen bestudeerd in relatie tot hormoonhuishouding (corticosteron, groeihormoon, somatomedine, en de schildklier hormonen T_3 en T_4) en energiemetabolisme. Deze studies werden daarom uitgevoerd in de klimaat-respiratiecellen.

Hoofdstuk VI

Omdat er een gemiddeld gewichtsverschil bestaat tusen de H en L lijn (de L lijn is meestal zo'n 7% zwaarder) werd de invloed van verschillen in metabole aktiviteit vergeleken tussen de lijnen.

De warmteproduktie van de kuikens werd geregisteerd bij verschillende omgevingstemperaturen. In één experiment werd een iets hogere warmteproduktie gevonden in de L lijn, maar niet in het tweede experiment. In geen van de experimenten kon de onderste kritieke temperatuur vastgesteld worden, noch bij de H, noch bij de L lijn. De kuikens handhaafden hun thermoneutraliteit door de voeropname te verhogen bij lagere temperaturen.

Tijdens de immuunrespons werden slechts geringe verschillen in energiehuishouding vastgesteld, welke niet éénduidig aan verschillen in de immuunrespons konden worden gerelateerd.

Hoofdstuk VII

Kuikens werden blootgesteld aan een CC, CH of HH behandeling, net zoals in hoofdstuk II. Er waren duidelijke invloeden van deze behandelingen op de energiehuishouding. Verlaagde voeropname, groei, warmteproduktie en vetdepositie werden gevonden als gevolg van de CH en HH behandeling. In de CH behandeling werd eveneens een lagere eiwitsynthese gevonden in de periode onmiddellijk na de temperatuursverandering. De effekten op rektale temperatuur en lymfoïde organen waren net als in hoofdstuk III. De effekten op de immuunrespons waren minder duidelijk dan in hoofdstuk III. De concentraties van plasma corticosteron en schildklierhormonen waren niet beinvloed, maar de concentratie van somatomedine was verhoogd en van groeihormoon verlaagd als gevolg van de hoge temperatuur. Verschillen in metabolisme kenmerken, veroorzaakt door de behandelingen, konden niet gerelateerd worden aan verschillen in immuunrespons. Omgekeerd waren verschillen in immuunrespons tussen de H en L lijn niet terug te voeren tot verschillen in metabole kenmerken.

Hoofdstuk VIII

Tot slot werden de CC en HH behandelingen vergeleken met de CS-infuus. Sommige effekten van de CS infuus en de HH behandeling waren zeer vergelijkbaar. Lagere voeropname, groei en warmteproduktie worden in beide behandelingen gevonden. Ook de ontwikkeling van lymfoïde organen is minder in beide behandelingen. Maar het plasma corticosteron niveau is niet verhoogd bij de HH behandeling, terwijl bij de CS infuus T_4 lager en T_3 hoger waren dan bij de CC behandeling. T_3 was nog lager bij HH behandelde kuikens. De energie balans was hoger direkt na de immunisatie. Vetdepositie was hoger in CS geinfuseerde dieren. In de HH behandeling werd een geringe stijging gevonden in de immuunrespons, alleen bij de L lijn kuikens. Vergelijkbaar met hoofdstuk VII, werd er geen duidelijke relatie gevonden tussen gemeten hormonen, het op deze wijze gemeten metabolisme en de antilichaam respons. Het is de vraag of een op deze wijze toegediende CS infuus bruikbaar is als

experimenteel model voor kontinue (hitte) stress, vanwege de toch duidelijke verschillen in een aantal van de gemeten parameters.

Diskussie

In de diskussie wordt er op ingegaan dat de verschillen in immuunrespons tussen de H en L lijn erg konstant zijn.

Uit deze experimenten is het duidelijk dat de hittestress en het corticosteron infuus de kuikens beslist beinvloeden: groei, lichaamstemperatuur, plasma corticosteron en andere hormonen, lymfoïde organen worden beinvloed, maar altijd in gelijke mate in de H en L lijn. De selektie heeft niet geresulteerd in een lijn met hogere stress gevoeligheid.

Bovendien lijken beide lijnen relatief resistent te zijn voor stress-invloeden, voor wat betreft hun antilichaam produktie. Zowel akute stress, als langdurige hitte en zelfs rechtstreeks toegediende corticosteron hadden slechts marginale effekten op de hoogte van de immuunrespons. En wanneer een van de behandelingen een invloed had op de respons, dan waren de effekten veelal vergelijkbaar in de hoge en lage lijn. Daarom is er geconcludeerd dat er geen noemenswaardige genotype x milieu (hitte) interaktie aanwezig is bij deze lijnen.

De invloed van energiehuishouding, zoals hier gemeten, op de immuunrespons is klein. In een aantal gevallen werd een verandering in de vetdepositie gevonden, in de periode direkt na de immunisatie. Dit kan duiden op een verschuiving in de verdeling van energie tussen eiwit- en vetaanzet, tijdens de immuunrespons. Maar geen rechtstreekse relatie tussen verschillen in metabole aktiviteit, als gevolg van de selektie of ten gevolge van de verschillende behandelingen, met hormonale regulatie of antilichaam produktie was aantoonbaar. Daaruit volgt dan ook dat verschillen in gewicht tussen de selektielijnen veeleer een gevolg zijn van genetische koppeling, dan dat ze berusten op fysiologische betekenisvolle relaties tussen immuunsysteem en energiehuishouding.

Curriculum vitae

Richard Alfred Donker werd geboren op 26 juli 1957 te 's Gravenhage. De kleuter- en lagere school werden suksesvol doorlopen te Rijswijk (ZH). Het diploma Atheneum-B werd behaald in 1976 aan de Openbare Dalton Scholengemeenschap Voorburg-Leidschendam te Voorburg. Aansluitend werd de studie zoötechniek aan de Landbouwhogeschool begonnen. Deze studie werd in maart 1984 afgesloten met als doktoraal hoofdvakken Veefokkerij en Gezondheids- en ziekteleer der huisdieren en als bijvak Erfelijkheidsleer.

Na zijn afstuderen is hij werkzaam geweest als wetenschappelijk medewerker in tijdelijke dienst bij achtereenvolgens: de vakgroep Tropische Veehouderij van de Landbouwhogeschool, de vakgroep Veefokkerij van de Landbouwhogeschool (met detachering bij het Instituut voor Veeteeltkundig Onderzoek "Schoonoord" te Zeist) en bij het Instituut voor Veeteeltkundig Onderzoek "Schoonoord" te Zeist.

In januari 1986 werd hij aangesteld als promotie-assistent bij de vakgroep Veehouderij van de Landbouwhogeschool (thans: Landbouwuniversiteit), op het onderzoeksprojekt "De invloed van milieu op de expressie van genetische verschillen in immuunrespons bij kippen", hetgeen resulteerde in dit proefschrift.

Sinds 1 maart 1989 is hij als wetenschappelijk medewerker verbonden aan het Proefstation voor de Rundveehouderij te Lelystad.