The effect of environmental temperature
on immune response and metabolism
of the young chicken

aan mijn ouders,
aan Sandra en Floortje

Promotor : dr. C.C. Oosterlee, hoogleraar in de veeteeltwetenschap
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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON IMMUNE RESPONSE AND METABOLISM OF THE YOUNG CHICKEN

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op woensdag 1 december 1982
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

STELLINGEN

1. De immuunrespons wordt beïnvloed door de omgevingstemperatuur.
   Regnier, J.A., K.W. Kelley, and C.T. Gaskins, 1980. Poultry Sci. 59: 985-990.
   Thaxton, P., 1978. Poultry Sci. 57: 1430-1440.
   Dit proefschrift.

2. De veronderstelling, dat er voor pluimvee geen thermoneutrale zone bestaat, is onjuist.
   Van Kampen, M., B.W. Mitchell, and H.S. Siegel, 1979. J. Agri. Sci. Camb. 92: 219-226.

3. Het verschil tussen de totaal en 2-mercapto-ethanol (2-ME) resistente titer (log_2-waarde) is niet gelijk aan de 2-ME gevoelige titer.
   Sebaldt, R.J., 1976. Immunochemistry 13: 473.

4. Vaccinaties kunnen verliezen ten gevolge van infectie-ziekten wel reduceren, maar niet elimineren.
   Gavora, J.S., and J.L. Spencer, 1978. Wld's Poultry Sci. J. 34: 137-148.

5. Door alle milieufactoren, die fysiologisch niet optimaal zijn, als stressoren te beschouwen, gaat men voorbij aan het belang van gewenning aan ongunstige omstandigheden.
   Dit proefschrift.

6. Door onvoldoende kennis van de variatie in temperatuursbehoefte van onze landbouwhuisdieren gaat veel energie verloren.

7. De verdeling van onderzoeksinspanning tussen aandachtsvelden binnen de vakgroep Veehouderij dient een afspiegeling te zijn van de onderlinge verhouding van de problemen in de praktische veehouderij.

8. In verband met de voedselvoorziening in de tropen verdient onderzoek naar voersamenstellingen met een relatief lage eiwit-energie verhouding aanbeveling.

9. Behandeling van motorisch gehandicapte kinderen dient persoon-gericht en niet handicap-gericht te zijn.

10. De in de reclame gebruikte aanduidingen voor kuikenpoten, zoals "TV-kluiven" en "drumsticks", geven slechts enkele van de gebruiksmogelijkheden weer.

11. Bij het formuleren van proefplannen zien de meeste onderzoekers zichzelf niet als de beperkende factor.

Proefschrift van A.M. Henken
The effect of environmental temperature on immune response and metabolism of the young chicken

Wageningen, 1 december 1982

Voorwoord

Dit proefschrift bestaat uit een bundeling van zes artikelen. Deze zijn het resultaat van een onderzoek dat uitgevoerd is in het kader van een driejarig promotie-assistentschap bij de vakgroep Veehouderij van de Landbouwhogeschool. Ik ben mij ten volle bewust dat het zonder de hulp en begeleiding van een groot aantal mensen niet mogelijk geweest was dit onderzoek ook inderdaad binnen drie jaar af te ronden.

Mijn promotor, prof.dr. C.C. Oosterlee, en andere begeleiders, dr.ir. M.W.A. Verstegen en mw.dr.ir. A.J. van der Zijpp, hebben ieder op eigen wijze gezorgd voor een inspirerende begeleiding bij de opzet van de experimenten, de bewerking van de resultaten en het gereedmaken van de artikelen en dit proefschrift. De discussies met dr. J.H. Boon, drs. J.L. Grondel, dr.ir. A. Kloosterman, ir. T. van der Lende en dr. H.S. Siegel heb ik zeer op prijs gesteld. De hulp die ik gekregen heb van ing. W. van der Hel en M.G.B. Nieuwland mag hier niet onvermeld blijven. Ook ir. H.A. Brandsma, W. Hazeleger, J.M. van der Linden, mw.ing. J.C. Roestenburg, een aantal doctoraalstudenten (A.M.J. Groote Schaarsberg, ir. G.R. Kleinhout, ir. E.F. Knol en ir. G. Wensink) en stagiaires zullen in dit proefschrift een deel van hun werk of studie herkennen.

Het personeel van de proefaccommodatie "De Haar" heeft op juiste wijze de proefdieren verzorgd en was te allen tijde bereid om te helpen. Dit laatste geldt ook voor de algemene diensten van Zodiac.

De presentatie en publicatie van resultaten is zeker niet de minst belangrijke fase van een onderzoek. Ik wil daarom op deze plaats ook mw. G.J. Gijsbertse-Huiberts bedanken voor het zorgvuldige typewerk en W. Heije voor de nauwgezette verzorging van de figuren.

Tenslotte wil ik iedereen bedanken die aan dit proefschrift heeft bijgedragen, maar niet met name is genoemd.

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INTRODUCTION TO THE PAPERS

Climatic conditions are involved in the aetiology of many infectious diseases in man and domestic animals (Crawford, 1962; Hudson et al., 1974; Anonymous, 1977; Tromp, 1980). Epidemiological studies revealed seasonal cycles in disease outbreaks and morbidity rates of infections of especially the respiratory tract, partially associated with environmental temperature (Hope-Simpson, 1967; Truijen, 1967; Tielen, 1974; Truppi et al., 1975). However, results of such studies are difficult to interpret. Thermal effects on disease outbreaks and morbidity rates may be the result of thermal effects on disease resistance which are confounded with effects on pathogen survival and transmission (Hudson et al., 1974).

Results of research with experimental infections in animals showed that thermal influences on disease resistance may occur (Marshall, 1959; Kovacs et al., 1974; Shimizu and Shimizu, 1979; Simensen et al., 1980). However, the mechanism by which such influences are exerted is not understood. Therefore, attention is focused on individual components of disease resistance, especially on the immune response being a major component (Roitt, 1980).

The effect of different temperature-treatments on the immune response was investigated using the chicken-SRBC (sheep red blood cell) model (Thaxton and Siegel, 1970, 1972 and 1973; Subba Rao and Glick, 1970 and 1977). Results of these model studies showed that thermal conditions affect the immune response to SRBC. They suggested also that effects of environmental temperature on the immune response are associated with increased metabolic rate.

Environmental temperature has pronounced effects on the metabolic rate of domestic animals, depending on the demand that is made upon the animal's capacities to maintain homeothermia (Verstegen, 1971; Mount, 1974; Curtis, 1981; Van Kampen, 1981). In Figure 1 is given the relationship between heat production, evaporative and non-evaporative heat loss and deep-body temperature in homeotherms over the range of environmental temperatures between the zones of hypo- and hyperthermia. Many data on poultry suggest that there is no distinct zone of thermoneutrality in the chicken, but only a point of flexure or narrow range of environmental temperatures at/in which metabolism is minimal (Van Kampen, 1981). Below as well as above the zone of minimal metabolism (outside zone CE), heat production is increased.

Environmental temperatures below the zone CE were shown to enhance the humoral immune response (St. Rose and Sabiston, 1971; Sabiston and St. Rose, 1976; Subba Rao and Glick, 1977; Blecha and Kelly, 1979). This cold induced increase of the

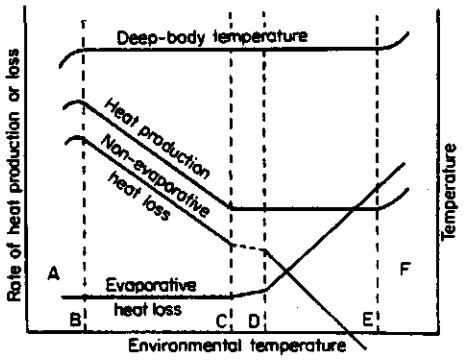

Fig. 1: Relationship between heat production, evaporative and non-evaporative heat loss and deep-body temperature in a strict homeotherm. A, zone of hypothermia whose border is defined by B; F, zone of hyperthermia whose border is defined by E; C, lower critical temperature; D, temperature of marked increase in evaporative loss (upper critical temperature); CD, zone of minimal thermoregulatory effort; CE, zone of minimal metabolism (after Mount, 1974)

humoral immune response can be expected if thermal effects on immune responsiveness are associated with thermal effects on metabolic rate.

Environmental temperatures above the zone CE have been reported to reduce the humoral immune response (Thaxton et al., 1968; Thaxton and Siegel, 1970 and 1973; Subba Rao and Glick, 1970 and 1977). This reduction in immune responsiveness was thought to result from the action of stress associated hormones, which were shown to have a regressive influence on the humoral immune response. This indicates that the applied high environmental temperatures have been extreme (in zone F, where deep-body temperature is affected). Extreme low environmental temperatures which cause a change in deep-body temperature (zone A) have been reported to reduce the immune response too (Siegel, 1980). If thermal effects on immune responsiveness are associated with increased metabolic rate, moderate high environmental temperatures (near point E in zone F) can be expected to increase the humoral immune response similarly to the increase at moderate low environmental temperatures.

Thus, thermal effects on the humoral immune response may depend on the demand upon animal's capacities to maintain homeothermia. This means that, when studying effects of environmental temperature on immune responsiveness, a quantification of the thermal demand upon thermoregulatory capacities must be made simultaneously. Data on energy balance characteristics (a.o. energy retention and heat production) will provide information about the zone wherein the applied

environmental temperatures fall (see Figure 1). Data on protein and fat gain will provide information about the severity of the imposed conditions. At extreme conditions blood levels of corticosteroids are increased. Corticosteroids were reported to increase fat deposition at the expense of protein (Siegel, 1980). Therefore, thermal effects on immune response and metabolism were studied simultaneously in this research.

An immune response itself may affect metabolism. It was shown that an immune response influences blood levels of corticosterone and thyroxine (Besedovsky et al., 1975). Corticosterone and thyroxine are both associated with metabolic rate. The first two papers deal with this aspect. Chickens were immunized by intramuscular injection of SRBC in the thighs. The effect on heat production of injection per se is reported in the first paper and the effect of the ensueing immune response on energy metabolism in the second paper.

The third and fourth paper deal with the effect of high (30-40 C) and low (10-20 C) environmental temperatures on immune response and metabolism. It is clear that extreme environmental temperatures which cause a change in deep-body temperature do not reflect the conditions, which occur normally in practice. Therefore, less extreme temperatures were used in this part of the research. The deviation from thermoneutrality was about 10 C. This deviation was large enough to affect metabolic rate and performance characteristics, but not large enough to be stressfull (i.e. between the zones of hypo- and hyperthermia).

Effects on immune response and metabolic rate of environmental temperatures which are more below thermoneutrality (near zone A) than the above mentioned are discussed in the fifth paper. The experiments described in this paper were performed in order to assess more precisely the environmental temperature below which the humoral immune response will be reduced.

In the experiments described in the sixth paper, thermal effects on metabolic rate were investigated separately in order to determine to which extent the temperatures applied in the various experiments deviated from thermoneutrality. Metabolic rate was used as parameter to compare the thermoneutral and deviating conditions.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON IMMUNE RESPONSE AND METABOLISM OF THE YOUNG CHICKEN

1. Effect of intramuscular injection on heat production

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ABSTRACT

The effect of intramuscular (IM) injection, per se, on heat (H) production was investigated in ad lib.- and restricted-fed pullets, held at normal (21 C) and low (10 C) environmental temperatures. At 21 C IM injection, per se, decreased H-production of ad lib.- and restricted-fed pullets for 2 to 4 hr after injection. This effect was significant ($P < 0.01$) in ad lib.-fed pullets, held at 21 C as well as at 10 C. Thus, when studying the effect of an immune response on the energy metabolism, an effect may be confounded with the effect of the IM injection per se, when the measurements are made during the first 2 to 4 hr following injection.

INTRODUCTION

In 1979 studies were initiated on the effects of environmental temperature on the humoral immune response and the energy and protein metabolism of the young chicken. In a series of four experiments the effect of immunization on heat (H) production was investigated in ad lib.- and restricted-fed pullets, held at normal and low environmental temperatures. Handling, blood sampling, immunization, etc. of the pullets are associated with such experiments. Some effects of handling, with and without blood sampling, on growth have been reported by McPherson et al. (1961), Reichmann et al. (1978), Gross and Siegel (1979). In preliminary studies Zaalmink (1979) measured metabolic rate in pullets 3 - 5 days after immunization; however, the immediate response of injection or immunization on metabolic rate was not measured. Thus, the effect of injection, per se, is not clear. In this paper we report the effect of intramuscular (IM) injection, with and without antigen, on H-production. The effects of environmental temperature and feeding regimen on the humoral immune response will be given elsewhere (Henken et al., in preparation).

MATERIALS AND METHODS

Pre-experimental conditions

Warren SSL pullets were vaccinated during the first 21 days after hatching as follows: day 1 - Marek's disease, IM; day 2 - infectious bronchitis, intraocularly (IO); day 8 - Newcastle disease, IO; day 21 - infectious bursal

disease, IO. During the three week-rearing period environmental temperatures were gradually lowered from ± 35 C to ± 21 C. The light regimens were gradually changed from 20 hr light per day and 4 hr dark (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum. At 21 days of age the pullets were weighed and placed in one of two climate respiration chambers (dimensions: 58 cm l. x 27 cm w. x 24 cm h.). Four pullets were placed in each chamber. From 21 days of age onwards the pullets were continously exposed to the experimental conditions.

Experimental conditions

Experiment 1 - In this experiment the pullets in one chamber were immunized with sheep red blood cells (SRBC), while the pullets in the other were sham-immunized with phosphate buffered saline (PBS). Environmental temperatures in both chambers were maintained at 21 ± .5 C. Feed and water were available ad libitum.

Experiment 2 - In this experiment the difference in treatment between chambers was ad lib. vs restricted feeding. All pullets in both chambers were immunized with SRBC. The feeding level of the restricted-fed pullets was assumed to provide the maintenance requirements in terms of metabolizable energy (ME_m). Using chickens of the same breed, Van Boekel and Zaalmink (1979) found, that the maintenance requirement in KJ ME per kg body weight per day changed according to the equation: 1108 - 0.595 G (G = g body weight). Water availability was limited to the restricted-fed pullets for 2 - 3 hr after feeding. Environmental temperatures in both chambers were maintained at 21 ± .5 C.

Experiment 3 - This experiment was the same as the previous one, except that environmental temperatures in both chambers were maintained at 10 ± .5 C in this experiment. The feeding level of the restricted-fed pullets was adjusted to meet the increased heat requirement at 10 C. This additional amount was estimated from results of Barott and Pringle (1946) and Farrell and Swain (1977).

Experiment 4 - In this experiment immunization was compared with non-injection. All pullets in both chambers were restricted fed and held at 21 ± .5 C. The daily amount of feed was determined as 80% of the daily intake of the ad lib.-fed pullets in Experiments 1 and 2. Feeding level was adjusted according to body weight. Water was available ad libitum.

In all four experiments the light regimens were 12 L : 12 D (light switched on at 0730 hr) and the relative humidity (RH) 70 ± 5%. The daily amount of feed

for the restricted-fed pullets was offered in two equal portions (0900 and 1600 hr). Feed contained 88.1% dry matter, 20.8% crude protein and the gross energy content (GE) was 16.26 $KJ.g^{-1}$. The contrasts between groups within experiments are summarized in Table 1.

Table 1: Contrasts between groups within experiments

Experiment	(C)	Contrasts
1	(21)	immunization vs sham-immunization
2	(21)	ad lib. - vs restricted-feeding
3	(10)	ad lib. - vs restricted-feeding
4	(21)	immunization vs non-injection

Experimental procedure within experiments

In Figure 1 the schedule of all experiments is given. The pullets were allowed 5 days of adaptation (A) to the chambers before the experimental periods started at 26 days of age.

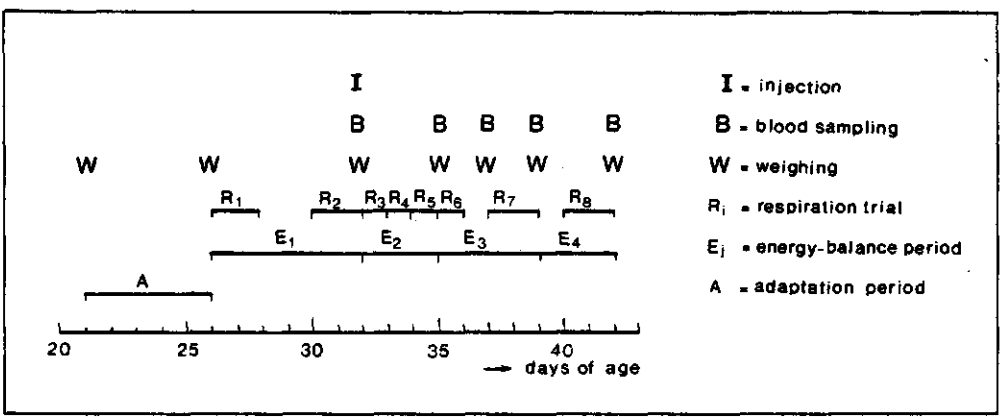

Fig. 1: Experimental schedule

Experiments started with weighing (W) and an energy-balance period (E_1) before injection. During this 6-day period two 48-hr respiration trials (R_1 and R_2) were performed. At 32 days of age, after weighing and drawing $\pm$ 1 ml blood

(B) from the ulnar vein, the pullets were injected (I) with 1 ml packed SRBC, $\pm$ 3 x 10^{10} cells (immunized groups) or with 1 ml PBS, pH = 7.2 (sham-immunized group). The doses were injected IM in two equal portions, one into each thigh (Van der Zijpp and Leenstra, 1980). From the non-injected pullets (Experiment 4) no blood samples were drawn: they were only weighed. Immediately after injection and weighing a balance period (E_2) of 3 days began, during which three 24-hr respiration trials (R_3, R_4 and R_5) were performed. At 35 days of age, after drawing blood and weighing, a new balance period (E_3) of 4 days was started with one 24-hr (R_6) and one 48-hr respiration trial (R_7). R_7 was started at 37 days of age, after drawing blood and weighing. At 39 days of age, after drawing blood and weighing, a final balance period (E_4) of 3 days was conducted with one 48-hr respiration trial (R_8). The experiments ended after drawing the final blood samples and the final weighing at 42 days of age.

Balance measurements

In each balance period (E_1 to E_4) the following parameters were measured: feed and ME intake, mean body weight and rate of gain. H-production (R_1 to R_8) was determined from measurements of oxygen consumption and carbon dioxide production, using a formula derived from the one proposed by Romijn and Lokhorst (1961): H (KJ) = 16.20 x liters CO_2 consumed + 5.00 x liters CO_2 produced.

The H-production data are expressed in watt ($J.sec.^{-1}$) per kg body weight^p. We used p = 1, instead of p = 0.75 as is usual for older animals (Kleiber, 1965). Results from Kuenzel and Kuenzel (1977) and our own results (Henken et al., in preparation) indicate that it is preferable to use p = 1 in young chickens.

In Experiments 3 and 4 activity was measured during R_1 to R_8, using ultrasound activity detectors* (Wenk and Van Es, 1976). In Experiment 2 one pullet of the restricted-fed group died (cannibalism) on 36 days of age. From this group only data of E_1, E_2 and R_1 to R_6 were used. In Experiment 4 no H-production measurements were performed during R_5.

Statistics

H-production data were regressed on days of age within experimental groups and deviations from the expected regression values on the day of injection (R_3) were tested (Snedecor and Cochran, 1967). Activity data were expressed as per-

* Solfan intrusion detectors, model 3325.

centage of the highest value within the specific day and experimental group.

RESULTS

Body weight, rate of gain and feed intake

Data on body weight (at 26 days of age), rate of gain and feed intake (from 26 to 42 days of age) are given in Table 2. Feed intake of the ad lib.-fed pullets was about 9% higher at 10 C than at 21 C (Experiment 1, 2 and 3). Low environmental temperature reduced daily gain in ad lib.-fed pullets by about 34% as compared to those, held at 21 C (Experiment 1, 2 and 3).

Table 2: Mean body weight (g) at 26 days of age, rate of gain.animal^{-1}.day^{-1} (g) and feed intake.animal^{-1}.day^{-1} (g) from 26 to 42 days of age and standard deviations for each experimental group

Experiment	Group	Body weight at 26 days of age, g	(SD)	Rate of gain, g.animal^{-1}.day^{-1}	(SD)	Feed intake, g.animal^{-1}.day^{-1}	(SD)
1	PBS, ad lib.	280.4	(5.5)	21.2	(1.9)	51.0	(8.0)
	SRBC, ad lib.	299.8	(5.4)	20.1	(2.5)	49.5	(8.2)
2	SRBC, restr.	261.0	(14.1)	3.6	(4.9)	23.2	(1.3)
	SRBC, ad lib.	317.3	(11.7)	18.2	(2.0)	46.2	(2.1)
3	SRBC, restr.	215.2	(13.1)	3.9	(1.4)	29.7	(1.8)
	SRBC, ad lib.	243.5	(21.1)	13.0	(1.6)	53.3	(5.5)
4	SRBC, restr.	253.7	(13.7)	9.6	(1.1)	31.4	(2.5)
	Non-inj., restr.	250.6	(12.1)	6.5	(2.4)	30.7	(2.3)

H-production and activity

In Table 3 the equations for linear regression of H-production on days of age are given for each experimental group. The regression coefficients (b's) are negative (except for the restricted-fed pullets in Experiment 2), indicating decreasing H-production per kg body weight with increasing age. The actual H-production at 32 days of age (R_3), the day of injection, was below the values estimated from the regression equations. Therefore, the daily pattern in H-production before and after injection was investigated.

Table 3: Linear regression equations of H-production ($W.kg^{-1}$) on days of age ($26 \leq x \leq 42$) and correlation coefficients for each experimental group

Experiment	Group	Linear regression	Corr. coeff.
1	PBS, ad lib.	18.39 - 0.18x	- 0.84**
	SRBC, ad lib.	18.16 - 0.19x	- 0.85**
2	SRBC, restr.	6.53 + 0.27x	+ 0.64
	SRBC, ad lib.	24.35 - 0.37x	- 0.80**
3	SRBC, restr.	21.71 - 0.11x	- 0.49
	SRBC, ad lib.	23.88 - 0.11x	- 0.53
4	SRBC, restr.	19.65 - 0.20x	- 0.89**
	Non-inj., restr.	21.49 - 0.20x	- 0.38

** $P < 0.01$

In Figures 2, 3, 4 and 5 the pattern of H-production during the first 6 hr after the measurements started (± 0930 hr), is shown for Experiments 1, 2, 3 and 4, respectively, as the mean before injection (R_1 and R_2), A; on the day of injection (R_3), B; and on the day after the day of injection (R_4), C (see also Figure 1). When starting a respiration trial or energy-balance period, feed was provided immediately. From Figures 2 to 5, it can be seen that the H-production pattern on the day of injection (B) in all experiments was different from A or C. This difference was most pronounced during the first 2 to 4 hr after injection. To quantify these deviations H-production data during the first 3 hr after the start of R_1 to R_8 were regressed on days of age. In Table 4 are the correlation and regression coefficients of those regression lines and the absolute and relative deviations on R_3. In all experimental groups, except one, the 3-hr heat produced on R_3 was below the value of the regression line. In 3 of the 4 ad lib.-fed groups this deviation was significant ($P < 0.01$: Experiments 1 and 3).

The activity data during the first 6 hr after the measurements started on the day of injection in Experiment 3 and 4, are given in Figure 6 and 7 respectively. Restricted-fed pullets became active more rapidly after injection than those fed ad libitum (Experiment 3). Non-injected pullets became active immediately after the measurements started, while the activity of the injected birds remained at a low level for about 2 hr.

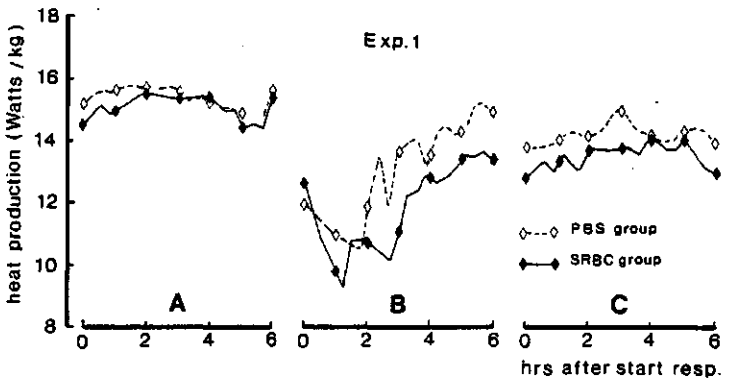

Fig. 2: H-production during the first 6 hr after the respiration measurements started in Experiment 1 as the mean before injection (A), on the day of injection (B), and on the day after the day of injection (C)

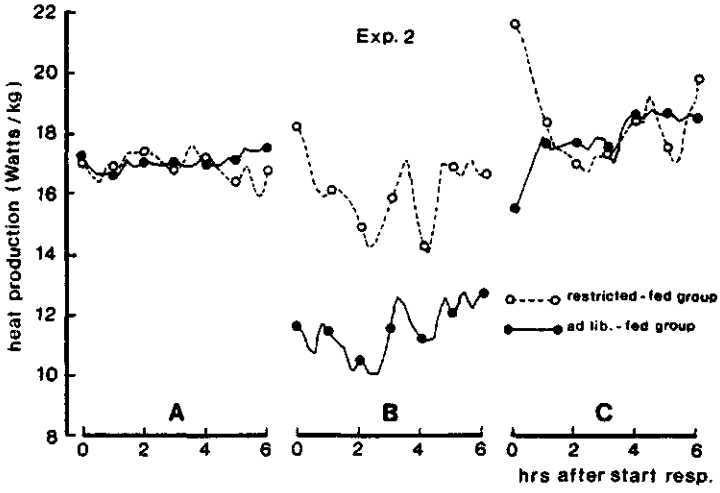

Fig. 3: H-production during the first 6 hr after the respiration measurements started in Experiment 2 as the mean before injection (A), on the day of injection (B), and on the day after the day of injection (C)

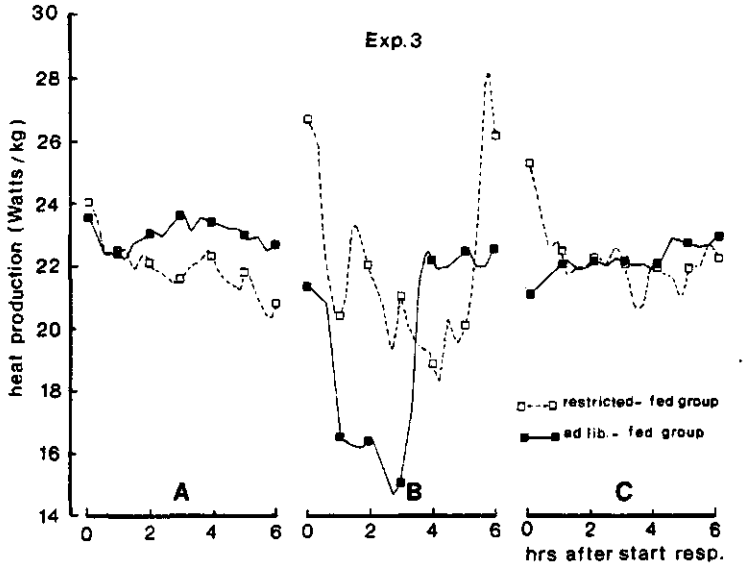

Fig. 4: H-production during the first 6 hr after the respiration measurements started in Experiment 3 as the mean before injection (A), on the day of injection (B), and on the day after the day of injection (C)

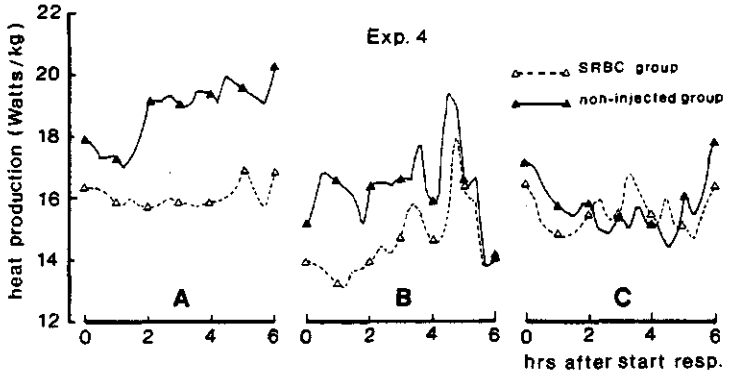

Fig. 5: H-production during the first 6 hr after the respiration measurements started in Experiment 4, as the mean before injection (A), on the day of injection (B), and on the day after the day of injection (C)

Table 4: Regression and correlation coefficients from the linear regression of 3-hr H-production on days of age and the absolute ($W.kg^{-1}$) and relative (%) deviations on R_3 from the expected values for each experimental group

Experiment	Group	Regr. coeff.	Corr. coeff.	Deviation on R_3 from regression, $W.kg^{-1}$ (%)
1	PBS, ad lib.	- 0.25	- 0.96**	- 2.76 (20)**
	SRBC, ad lib.	- 0.28	- 0.96**	- 3.28 (25)**
2	SRBC, restr.	+ 0.22	+ 0.73*	- 2.29 (15)
	SRBC, ad lib.	- 0.43	- 0.78	- 4.40 (30)
3	SRBC, restr.	- 0.22	- 0.83**	+ 0.26 (1)
	SRBC, ad lib.	- 0.06	- 0.47	- 5.26 (25)**
4	SRBC, restr.	- 0.19	- 0.73*	- 2.08 (13)
	Non-inj., restr.	- 0.22	- 0.66*	- 1.28 (7)

* $P < 0.05$
** $P < 0.01$

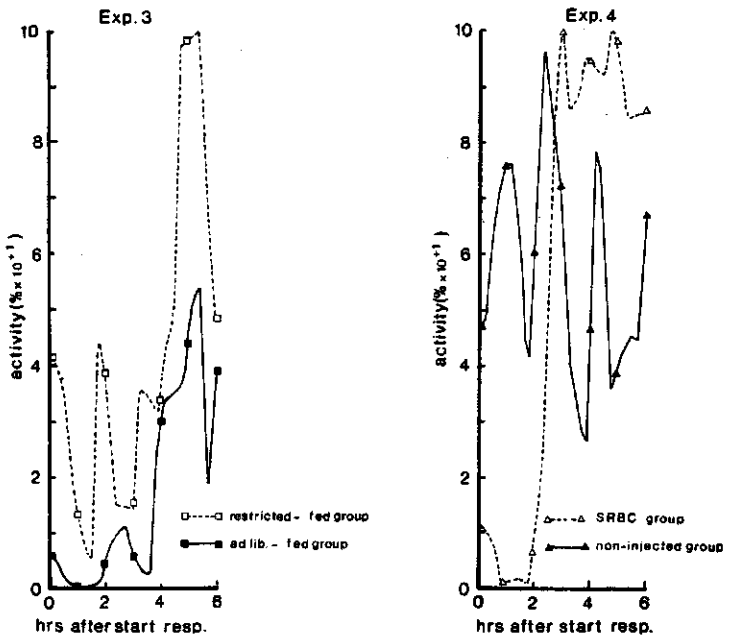

Fig. 6 and 7: Activity, as percentage of the highest value within day and experimental group, during the first 6 hr after the respiration measurements started on the day of injection in Experiment 3 resp. 4

DISCUSSION

Effect of an immune response on metabolic rate and performance characteristics can only be assessed if effects of handling and injection, as such, are known. Therefore it is important to investigate these phenomena. The data of the ad lib.-fed groups in Experiment 1 and 2 indicate that there is an effect of IM injection, per se, on H-production immediately after injection. Injection of pullets decreases H-production at least 2 to 4 hr after injection. As a consequence, the heat produced on the day of injection (R_3), as a whole, is about 10% below the value that is predicted for non-injected pullets. In restricted-fed pullets, the effect of injection is less pronounced (Experiment 2 and 4). In addition to the effect of feed restriction, there is, in Experiment 3, the effect of low environmental temperature. In this third experiment there was a decrease in H-production on the day of injection in the ad lib.-fed group, but not in the restricted-fed group.

In restricted-fed birds, H-production is usually lower than in those fed ad libitum. In Experiment 2, however, pullets were severely restricted and Wenk and Van Es (1980) showed that feed restriction may considerably increase activity. The data in Table 3 show that from 28 days of age onwards the heat produced by the restricted-fed pullets is higher than by those fed ad libitum.

H-production in Experiment 3 was considerably increased to a similar level in the restricted- as well as the ad lib.-fed group. This indicates that the low environmental temperature determines heat production in both groups because a large difference of feeding level did not result in a different metabolic rate. Ad lib.-fed pullets gained faster and had higher body weights; however, the 16-day experimental period did not cause a great difference in heat exchange properties because no effect of age on H-production was found in either group.

To explain the phenomenon of decreased H-production after IM injection, activity data are important. These data (Figures 6 and 7) indicate that activity decreased after injection. The peak activity of ad lib.-fed pullets occurred later than that of the restricted-fed pullets (Experiment 3). Non-injected pullets showed activity immediately after the measurements started, while in the SRBC-injected pullets there was a very low level of activity for about 2 hr after injection (Experiment 4). Thus, it appears that there is reduced mobility for several hours after injection, and thus H-production during that time is also reduced. This effect may be due to the used route of injection, i.e. IM in the thighs. The difference in activity after injection between restricted-

and ad lib.-fed pullets, may be caused by the hunger of the restricted-fed birds which will be more actieve in search for food. When environmental temperature is low then the difference between ad lib. and restricted feeding becomes more pronounced.

In conclusion, the effect of eliciting an immune response by IM immunization on the energy metabolism of pullets is confounded with the effect of IM injection per se when the measurements include the first 2 to 4 hr after injection. Reduced activity, and, as a consequence, decreased H-production during the first 2 to 4 hr after IM injection are associated with this effect.

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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

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ABSTRACT

The effect of an immune response, following injection with sheep red blood cells (SRBC) in pullets, on energy balance characteristics and on protein and fat gain was investigated. Controls were sham-immunized with phosphate buffered saline (PBS). Feed intake, body weight, rate of gain and metabolizability of gross energy were not significantly ($P > 0.10$) affected by SRBC-immunization. The magnitude and composition of energy gain were influenced by SRBC-immunization. SRBC-immunized pullets retained significantly ($P < 0.01$) more energy, deposited significantly ($P < 0.05$) more fat and had a significantly ($P < 0.01$) lower maintenance requirement of metabolizable energy than PBS-injected pullets during day 1 to 5 after injection. During day 6 to 10 after injection, SRBC-immunized pullets deposited significantly ($P < 0.05$) less fat and significantly ($P < 0.10$) more protein than PBS-injected pullets. Effects of vaccination on metabolic rate and performance characteristics may be comparable with these effects of SRBC-immunization.

INTRODUCTION

Although vaccination against many infectious diseases is performed routinely in practice, information on the effects of an immune response on performance characteristics is scarce. Vaccination against Marek's disease in one-day old pullets has been reported to reduce feed intake significantly ($P < 0.05$) during the first 12 weeks after hatching (Lee and Reid, 1977). This decrease in feed consumption may have been caused by associated effects of the used vaccin. Apart from associated effects of vaccins, vaccination may influence metabolism by inducing an immune response. Besedovsky et al. (1975) have shown in rats, that an immune response itself affects blood levels of corticosterone and thyroxine. At the peak of the immune response to sheep red blood cells (SRBC), corticosterone concentrations were increased, while thyroxine levels were decreased. Corticosterone and thyroxine are both associated with metabolic rate.

In this paper, we report the results of a study on the effects of the immune response to SRBC on energy balance characteristics and on protein and fat gain. Controls were sham-immunized with phosphate buffered saline (PBS), because it was shown that injection, per se, affects metabolic rate (Henken et al., 1982).

MATERIALS AND METHODS

Pre-experimental conditions

Pullets (Warren SSL) were subjected to the following vaccination scheme during the first 21 days after hatching: day 1 - Marek's disease, intramuscularly (IM); day 2 - infectious bronchitis, intraocularly (IO); day 8 - Newcastle disease, IO; day 21 - infectious bursal disease, IO.

During this three week-rearing period, environmental temperature was gradually lowered from about 35 C to about 25 C. The light regimen was gradually changed from 20 hr light and 4 hr dark per day (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum.

At 21 days of age, pullets were randomly assigned to one of two climate respiration chambers. 240 Pullets were placed in each chamber, divided into 4 experimental groups of 60 pullets each. From 21 days of age on the pullets were continuously exposed to the experimental conditions.

Experimental conditions

Environmental temperatures in both chambers (dimensions: 6 m l. x 4 m w. x 2.2 m h.) were maintained at 24.7 C (SD: 0.3 C) and the relative humidity at 70.7% (SD: 4%). The light regimens in both chambers were 12 L : 12 D; light (about 42 lux at animal level) was switched on at 0730 hr. Feed and water were available ad libitum. Feed contained 87.45% of dry matter, 23.61% of crude protein and the gross energy content (GE) was 17.16 $KJ.g^{-1}$. Within both chambers, each group of 60 pullets was housed in a "balance cage" (dimensions: 2.4 m l. x 0.8 m w.).

Experimental procedure

The experimental schedule is given in Figure 1. The pullets were allowed 4 days of adaptation (A) to the experimental conditions before the experiment started at 25 days of age. The experiment started with a 6-day balance period (B1) before injection. During this 6-day period two 48-hr respiration trials (R1 and R2) were performed. At 32 days of age the pullets in one chamber were injected (I) with 1 ml packed SRBC ($\pm$ 3 x 10^{10} cells). The pullets in the other chamber were injected with 1 ml PBS (pH = 7.2). The doses were injected

IM in two equal portions, one into each thigh. At 33 days of age the first 4-day balance period after injection (B2) was started, followed by another one (B3) starting at 38 days of age. During each of these two balance periods after injection, two 48-hr respiration trials were performed (R3 and R4 resp. R5 and R6).

At 24, 31, 37 and 42 days of age total body weight (BW) per experimental group of 60 pullets was determined. Just before, and at day 5 and 10 after injection (at 32, 37 and 42 days of age) about 1 ml blood (S), per pullet, was drawn from the ulnar vein of 20 pullets in each experimental group. Each pullet was bled only once. The heparinized blood samples were centrifuged and the plasma harvested and stored at -20 C until total haemagglutinin antibody titers were determined.

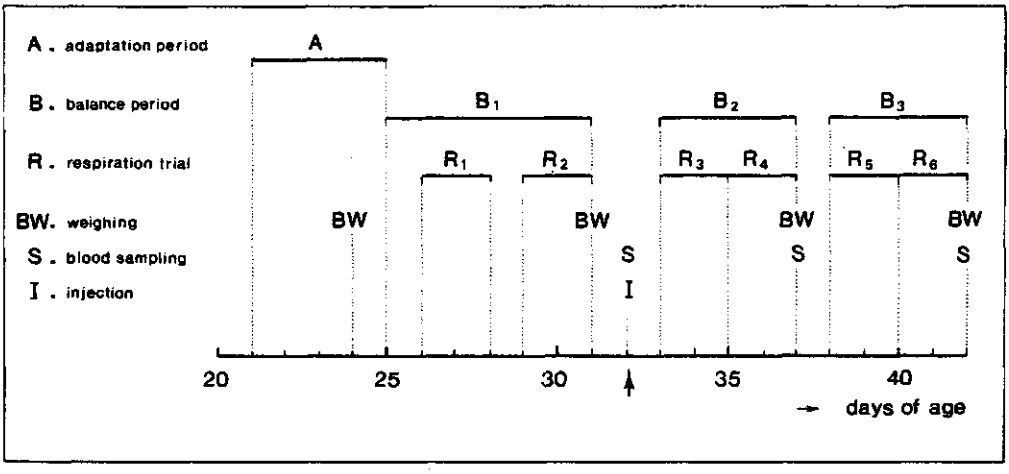

Fig. 1: Experimental schedule

Balance measurements

The following parameters were measured per experimental group in each balance period (B1 to B3): feed and metabolizable energy (ME) intake, protein retention (N-retention x 6.25), mean body weight and rate of gain. Metabolizability of GE (ME%) was determined from GE intake and energy found in the excreta. In R1 to R6, heat production (H) was determined per chamber during each consecutive 18 min. from measurements of oxygen consumption and carbon dioxide production, using a formula derived from the one proposed by Romijn and Lokhorst (1961): H (KJ) = 16.20 x liters CO_2 consumed + 5.00 x liters CO_2 produced.

Activity measurements per chamber were made during each consecutive 6 min. in R1 to R6, using ultra-sound activity detectors (Wenk and Van Es, 1976).

Haemagglutinin assay

Total haemagglutinin antibody titers were determined as described by Van der Zijpp and Leenstra (1980).

Statistics

Data on feed intake, rate of gain and protein retention are expressed as g per day per kg body weight ($g.d^{-1}.kg^{-1}$). Data on ME intake and heat production are expressed as watt ($J.sec.^{-1}$) per kg body weight ($W.kg^{-1}$). Heat production associated with activity was determined per respiration period by regression of heat on activity in a similar way as described by Wenk and Van Es (1976). The intercept in this equation represents the activity free heat production ($\bar{H}$).

Heat ($W.kg^{-1}$) produced within a chamber per balance period was divided between experimental groups according to the ratio of the feed intake ($g.d^{-1}.kg^{-1}$) of each group to the mean feed intake per chamber.

For each experimental group, the difference between ME intake and H was taken as the energy retention (RE: $W.kg^{-1}$). The difference between RE and energy gained as protein was used to determine the fat retention ($g.d^{-1}.kg^{-1}$). Values for the energy content of 1 g protein and fat were based on the constants proposed by Blaxter et al. (1964). The maintenance requirements in terms of metabolizable energy (ME_m) were calculated, using 0.68 (Chwalibog and Thorbek, 1975; Wenk and Van Es, 1980) as the efficiency of energy gain from ME intake above maintenance.

Differences in balance characteristics between SRBC- and PBS-injected pullets were tested within balance periods. The following model was used:

$$\underline{Y}_{ijk} = \mu + B_i + I_{j:i} + \underline{e}_{ijk}$$

$\underline{Y}_{ijk}$ represented the value of the kth group

μ = experimental mean

B_i = effect of the ith balance period (i = 1, 3)

$I_{j:i}$ = effect of injection (j = 1, 2: SRBC or PBS) in the ith balance period

e_{ijk} = remainder

Although significances may be influenced to some extent, the effect of balance periods (B_i) was added to the model as a main effect.

Differences in total antibody titers between SRBC- and PBS-injected pullets were tested within blood sampling day.

RESULTS

A summary of the results is given in Table 1.

Feed intake, body weight and rate of gain

Mean feed intake ($g.d^{-1}.kg^{-1}$), body weight (g) and rate of gain ($g.d^{-1}.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets are shown in Figure 2. There were no significant ($P > 0.10$) differences in performance between SRBC- and PBS-injected pullets within balance periods. Differences between balance periods were significant ($P < 0.01$).

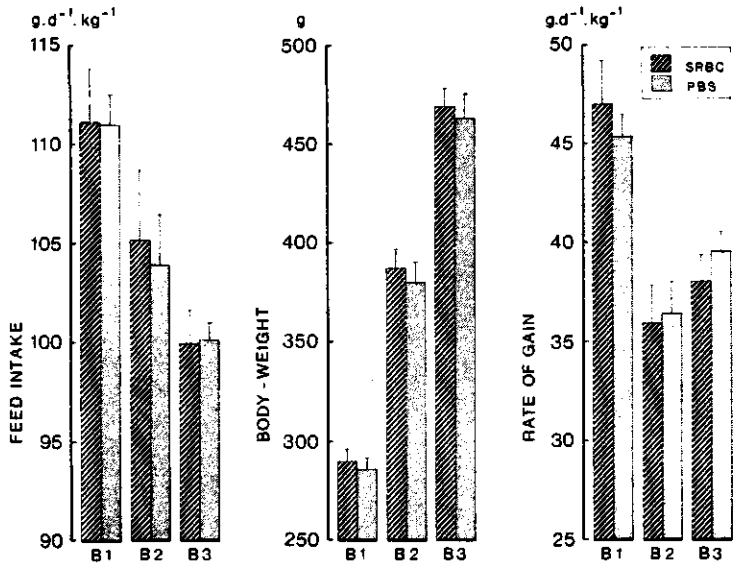

Fig. 2: Means and standard deviations of feed intake ($g.d^{-1}.kg^{-1}$), body weight (g) and rate of gain ($g.d^{-1}.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets

Table 1: Analyses of variance (mean squares) between balance periods (B_i) and between SRBC- and PBS-injected pullets within balance periods ($I_{j:i}$) of feed intake ($g.d^{-1}.kg^{-1}$), body weight (g), rate of gain ($g.d^{-1}.kg^{-1}$), ME%, ME ($W.kg^{-1}$), H ($W.kg^{-1}$), $\bar{H}$ ($W.kg^{-1}$), RE ($W.kg^{-1}$), ME_m ($W.kg^{-1}$), $\bar{ME}_m$ ($W.kg^{-1}$), protein ($g.d^{-1}.kg^{-1}$) and fat ($g.d^{-1}.kg^{-1}$) retention

Source of variation	df	Feed	Body weight	Rate of gain	ME%	ME	H	$\bar{H}$	RE	ME_m	$\bar{ME}_m$	Protein	Fat
B_i	2	230.22^{***}	63154.58^{***}	218.21^{***}	1.86	5.17^{***}	6.04^{***}	3.42^{***}	0.40^{***}	6.73^{***}	3.83^{***}	1.95^{*}	4.19^{***}
$I_{j:1}$	1	0.02	36.12	5.45	0.63	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00
$I_{j:2}$	1	2.83	98.56	0.44	0.05	0.06	0.21^{*}	0.04	0.50^{***}	0.63^{***}	0.29^{***}	0.79	1.11^{**}
$I_{j:3}$	1	3.23	64.75	4.43	2.42	0.07	0.11	0.04	0.00	0.13^{*}	0.03	2.33^{*}	0.63^{**}
Remainder	18	5.67	84.40	2.68	0.97	0.12	0.06	0.03	0.01	0.04	0.02	0.57	0.14

* $P < 0.10$
** $P < 0.05$
*** $P < 0.01$

Metabolizability (ME%), metabolizable energy (ME) intake and heat production (H)

Overall mean ME% was 75.45 (SD: 1.03). Differences in ME% within or between balance periods were not significant ($P > 0.10$). Therefore this mean ME% was used to calculate the ME intake per experimental group per balance period. Mean ME intake and H ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets are shown in Figure 3. Differences in H between balance periods were significant ($P < 0.01$). In B2, the SRBC-immunized pullets produced significantly ($P < 0.10$) less heat (about 3%) than the PBS-injected pullets. In B3, H was not significantly ($P > 0.10$) affected by SRBC-injection. Significances of differences in ME intake were conform to those of feed intake.

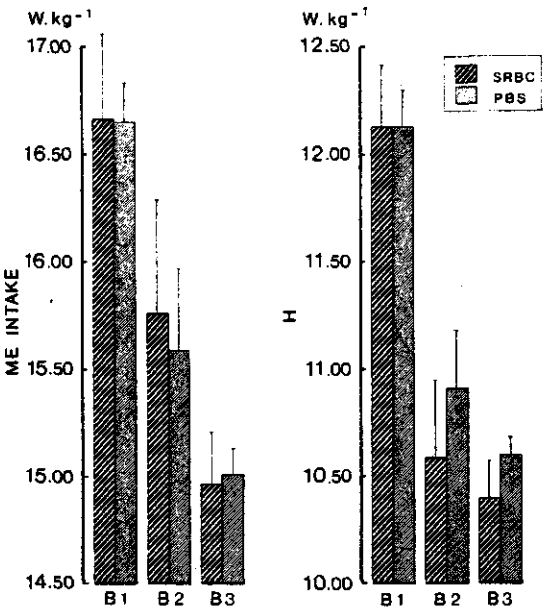

Fig. 3: Means and standard deviations of ME intake ($W.kg^{-1}$) and H ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets

Energy retention (RE) and maintenance requirement (ME_m)

Mean RE and ME_m ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected

pullets are shown in Figure 4.
Differences in RE and ME_m between balance periods were significant ($P < 0.01$). In B2, SRBC-immunized pullets had a significantly ($P < 0.01$) higher RE (about 10.8%) and lower ME_m (about 6.4%) than PBS-injected pullets. In B3, these differences were smaller, but still significant ($P < 0.10$) concerning ME_m (about 3%).

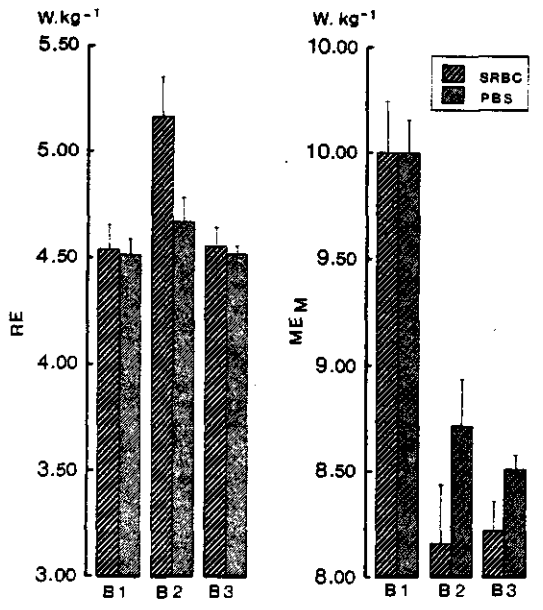

Fig. 4: Means and standard deviations of RE ($W.kg^{-1}$) and ME_m ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets

Activity free heat production ($\bar{H}$) and maintenance requirement ($\bar{M}E_m$)

Mean $\bar{H}$ and $\bar{M}E_m$ ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets are shown in Figure 5. Differences in $\bar{H}$ and $\bar{M}E_m$ between balance periods were significant ($P < 0.01$). Within balance periods, there were no significant ($P > 0.10$) differences in $\bar{H}$ between SRBC- and PBS-injected pullets. SRBC-injected pullets produced less heat, due to activity, than PBS-injected pullets during B1 to B3: the difference was the greatest in B3 (about 12.3%). Mean $\bar{H}$

was 73.1% (SD: 1.2%) of H. In B2, SRBC-injected pullets had a significantly ($P < 0.01$) lower $\bar{M}\bar{E}_m$ than PBS-injected pullets (about 6.5%). Mean $\bar{M}\bar{E}_m$ was 66.5% (SD: 1.5%) of ME_m.

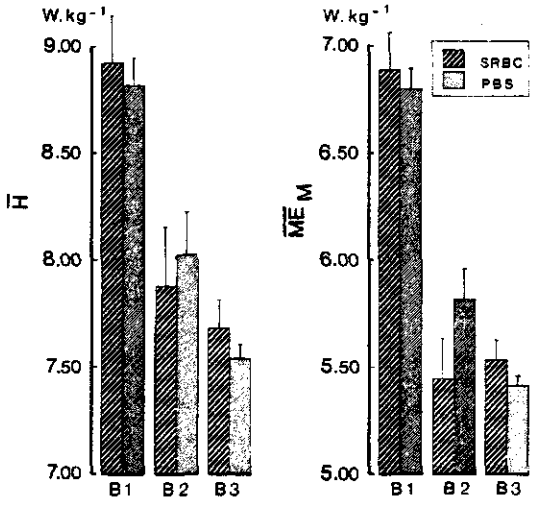

Fig. 5: Means and standard deviations of $\bar{H}$ ($W.kg^{-1}$) and $\bar{M}\bar{E}_m$ ($W.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets

Protein and fat retention

Mean protein and fat retention ($g.d^{-1}.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets are shown in Figure 6. Differences in protein and fat retention between balance periods were significant ($P < 0.01$). In B2, SRBC-immunized pullets retained significantly ($P < 0.05$) more fat than PBS-injected pullets (about 17.3%). In B3, SRBC-immunized pullets retained significantly ($P < 0.05$) less fat (about 14.6%) and significantly ($P < 0.10$) more protein (about 10.8%) than PBS-injected pullets.

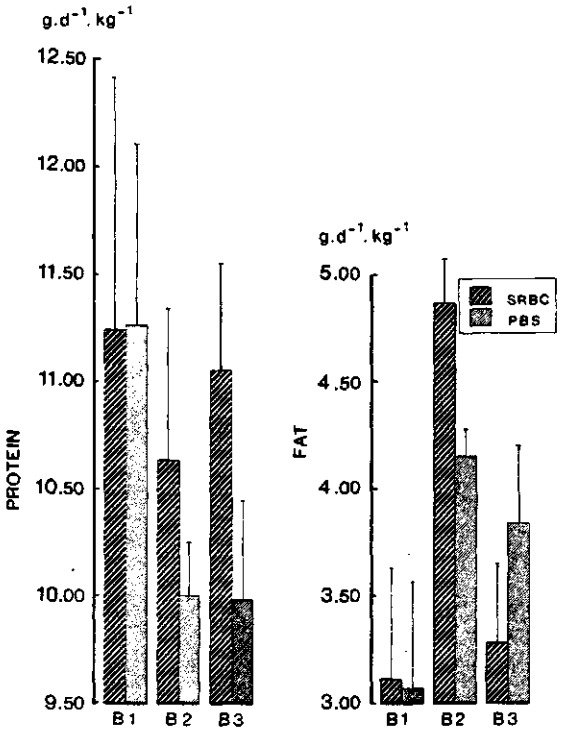

Fig. 6: Means and standard deviations of protein and fat deposition ($g.d^{-1}.kg^{-1}$) per balance period for the SRBC- and PBS-injected pullets

Antibody titers

In Figure 7 are shown the mean total $\log_2$ anti-SRBC antibody titers at day 0, 5 and 10 after injection (at 32, 37 and 42 days of age) for the SRBC- and PBS-injected pullets. At day 5 and 10 after injection, differences in antibody titers were significant ($P < 0.01$).

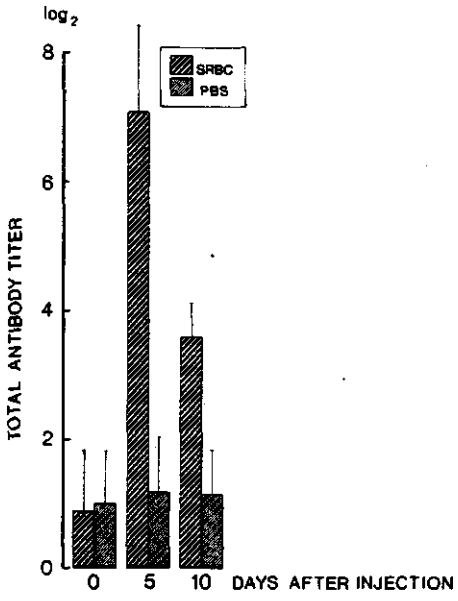

Fig. 7: Mean and standard deviation of total anti-SRBC antibody titers (log_2) at day 0, 5 and 10 after injection for the SRBC- and PBS-injected pullets

DISCUSSION

An immune response itself may affect metabolic rate and performance characteristics. Therefore, it is important to investigate this phenomenon. Before injection, differences in balance data between the future SRBC- and PBS-injected pullets were not significant ($P > 0.10$).

Results of this experiment show that feed intake, body weight and rate of gain were not significantly affected by SRBC-immunization. Although differences were not significant, SRBC-immunized pullets were eating more while gaining less than PBS-injected pullets during B2, the first balance period after injection. During this period, SRBC-immunized pullets produced significantly ($P < 0.10$) less heat, had a significantly ($P < 0.01$) higher energy retention and deposited significantly ($P < 0.05$) more fat than PBS-injected pullets. Thus it seems that SRBC-immunization causes a shift in metabolism in favour of fat

deposition during this period, in which antibody titers were increasing. These effects are consonant with the effects of corticosteroids on metabolism. Corticosteroids cause a shift in metabolism, which seems to favor fat deposition and to increase protein catabolism (Brown et al., 1958; Nagra and Meyer, 1963; Siegel, 1980). Protein deposition was depressed in SRBC-immunized pullets compared to the relative high amount of energy deposited as fat tissue. Because the efficiency of net energy gain was assumed to be the same for SRBC- and PBS-injected pullets, SRBC-immunized pullets, with the higher RE, had a significantly ($P < 0.01$) lower ME_m than PBS-injected pullets during B2.

During B3, when antibody titers were decreasing, SRBC-immunized pullets deposited significantly ($P < 0.10$) more protein and significantly ($P < 0.05$) less fat than PBS-injected pullets. Apparently there is a compensation for the relative low amount of energy deposited as protein during B2.

Fat deposition is, energetically, more efficient than protein deposition (Petersen, 1970), which is in agreement with the lower heat production of the SRBC-injected pullets during B2. During B3, heat production data of the SRBC-injected pullets are confounded with the decreased heat, due to activity, compared to PBS-injected pullets. If corrected for activity, SRBC-injected pullets, with the higher protein deposition, produced more heat than PBS-injected pullets. Overall mean $\overline{ME}_m$ was 5.95 $W.kg^{-1}$. i.e. 403.5 $KJ.d^{-1}.kg^{-0.75}$, which is about the same as the value of 400 $KJ.d^{-1}.kg^{-0.75}$ reported by Wenk and Van Es (1980). Effects of vaccination may be comparable with these effects of SRBC-immunization on energy metabolism.

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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

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ABSTRACT

The effect of environmental temperature on the humoral immune response of pullets following injection of sheep red blood cells (SRBC) was investigated. For this purpose 4 experiments were performed in 2 climate respiration chambers. In each experiment a standard temperature regimen of constant 25 C was maintained in one chamber. The temperature regimens in the other chamber were as follows: a constant temperature of 15 C (Experiment 1) and 35 C (Experiment 3) and a temperature, which fluctuated between 10-20 C (Experiment 2) and between 30-40 C (Experiment 4). Total haemagglutinin anti-SRBC antibody titers at day 5 after injection were increased significantly ($P < 0.01$) at 10-20 C, 35 C and 30-40 C compared to the antibody titers of pullets at 25 C. 2-Mercapto-ethanol resistant antibody titers at day 5 after injection were increased significantly ($P < 0.05$) at 35 C and 30-40 C. The effect of feeding level and thermal acclimation on antibody titers was investigated within each temperature regimen. Restricted-feeding at low or high temperatures increased antibody titers at day 5 after injection. Exchange of pullets, just before injection, to low or high temperatures decreased total antibody titers at day 5 after injection.

INTRODUCTION

It is well known that environmental temperature may alter susceptibility of chickens to infectious diseases. The humoral immune response following injection of sheep red blood cells (SRBC) is often used as a model to study the nature of this effect (Thaxton et al., 1968; Thaxton and Siegel, 1970 and 1973; Subba Rao and Glick, 1970 and 1977). The effect of environmental temperature on immune responsiveness may depend on the demand that is made upon the animal's capacities to maintain homeothermia. To cope with a particular thermal environment, there are two general types of regulatory processes: specific and non-specific (Siegel, 1980). At extremely high or low temperatures the regulatory response will be mainly a non-specific, stress, response. At moderate temperatures a specific regulatory response may be more prevalent. Heat production has to be changed at any temperature deviating from thermoneutrality. This acclimation may be realized by changing feed intake (Farrell and Swain, 1977) and/or by extra thermoregulatory heat production (Barott and Pringle, 1946). Measurements of thermal demand can thus best be made at various feeding levels. Therefore also effects of temperature on immune responsiveness should be measured at various feeding

levels. The frequency and/or duration of temperature-treatments has been reported to influence the effect of temperature on immune responsiveness (Thaxton, 1978). This indicates that the effect of thermal environment on immune responsiveness may depend on the degree of acclimation at the moment of immunization. Thus, effects of environmental temperature on immune responsiveness should be measured in acclimated as well as in non-acclimated animals.

It is clear that thermal conditions, which cause a change in body temperature, do not reflect the conditions, which occur normally in practice. Therefore the relation of environmental temperature and the humoral immune response was investigated at conditions which were less extreme.

In this paper we report the effects of constant and fluctuating temperatures on the humoral immune response following injection of SRBC. These effects were investigated in ad lib.- and restricted-fed, in acclimated and non-acclimated pullets during 4 experiments.

MATERIALS AND METHODS

Pre-experimental conditions

Pre-experimental conditions were the same in all 4 experiments. Environmental temperatures were gradually lowered from about 35 C to about 25 C during the first 3 weeks after hatching. The light regimens were gradually changed from 20 hr light and 4 hr dark per day (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum. The pullets (Warren SSL) were subjected to the following vaccination scheme during the first 21 days after hatching: day 1 - Marek's disease, intramuscularly (IM); day 2 - infectious bronchitis, intraocularly (IO); day 8 - Newcastle disease, IO; day 21 - infectious bursal disease, IO. At 21 days of age the pullets were randomly assigned to one of eight groups of 60 animals each. Four groups were placed inside each of two climate respiration chambers (Verstegen et al., in preparation).

Experimental conditions

Within each chamber (dimensions: 6 m l. x 4 m w. x 2.2 m h.) each group of 60 pullets was housed in a cage with a surface area of about 2 m^2. The light regimens were 12 L : 12 D. Light (about 42 lux at animal level) was switched on at 0730 hr. In Table 1 are shown the environmental temperatures per chamber in

all 4 experiments. In each experiment a standard temperature regimen of constant 25 C was maintained in one chamber. In Experiment 2 and 4 the temperature in chamber 1 was fluctuating between the indicated limits as is shown in Figure 1. The relative humidity (RH) was held at about 70% if the temperature was less or equal to 30 C. If the temperature was above 30 C, the absolute humidity was held at about 19 g H_2O per kg dry air, i.e. the water content of the air at 30 C with a RH of 70%.

Table 1: Environmental temperatures (C) in Experiment 1 to 4

Experiment	Chamber 1	2
1	25	15
2	10 - 20	25
3	25	35
4	30 - 40	25

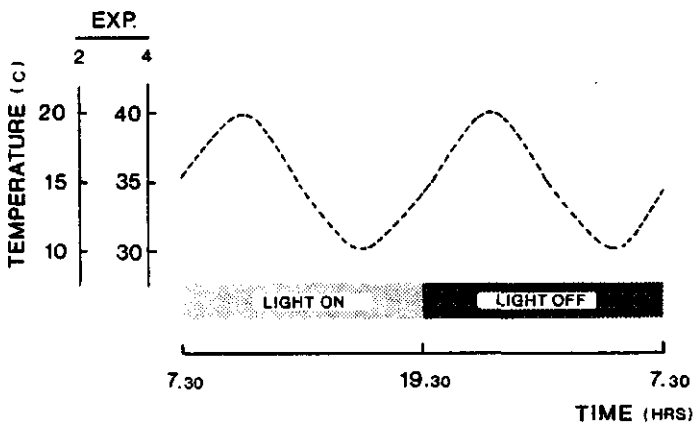

Fig. 1: Temperature fluctuation (Experiment 2 and 4) and light regimen (Experiment 1 to 4) per day

Treatments within chambers

The groups (1 to 4) within each chamber were assigned to one of the following treatments:

Group 1: Ad lib. feeding, injection with phosphate buffered saline (PBS);
Group 2: Restricted feeding, injection with SRBC;
Group 3: Ad lib. feeding, injection with SRBC;
Group 4: Ad lib. feeding, exchange with the same group in the other chamber prior (about 2 hr) to injection with SRBC.

From 21 days of age onwards, restricted-fed pullets (group 2) were given about 80% of the estimated ad lib. intake at each temperature. This estimation was based on results of previous experiments. Feed contained 89.2% of dry matter, 23.5% of crude protein and the gross energy content was 16.77 $kJ.g^{-1}$. Water was available ad lib. in all experiments. No antibiotics were given to the pullets. In Experiment 3 and 4 feed to the restricted-fed pullets at 25 C was withheld during about one day before injection.

Injection

At 32 days of age the pullets were injected with 1 ml PBS (group 1) or with 1 ml packed SRBC (group 2 to 4), about 3×10^{10} cells. The doses were IM injected in two equal portions, one into each thigh.

Blood sampling

Just before, and at day 5 and 10 after injection (at 32, 37 and 42 days of age) about 1 ml blood, per pullet, was drawn from the ulnar vein of 20 pullets in each group. Each pullet was bled only once. The heparinized blood samples were centrifuged and the plasma harvested and stored at -20 C until haemagglutinin antibody titers were determined.

Immunological parameters

Haemagglutinin antibody titers - Total and 2-mercapto-ethanol resistant (2-ME_r) antibody titers ($\log_2$) were determined as described by Van der Zijpp and Leenstra (1980). Repeatability was tested in one experiment, using samples of all three blood sampling days and all groups. Repeatability of total and

2-ME_r titer determinations was 0.98 and 0.97 respectively.

Number of plaque forming spleen cells - At day 5 after injection, 6 pullets of the groups 3 and 4 in both chambers were killed and their spleen used for a direct haemolytic plaque assay. A spleencellsuspension was prepared as described by Sato and Glick (1970). The plaque assay was performed according to the monolayer technique (Cunningham, 1965), using microscope slides prepared as described by Majoor et al. (1975). Pooled goose serum was used as complement source after absorption of natural anti-SRBC antibodies. The number of plaque forming cells per 10^6 viable leukocytes (PFC) was determined and transformed to $\log_e$ PFC. Weights of bursa of Fabricius and spleen were determined and expressed as percentage of body weight.

Statistics

The data on antibody titers were analysed within experiment and blood sampling day, using the Harvey (1977) LSML 76 program. The effects of immunization, feeding level and exchange on antibody titers were tested within each temperature regimen (or chamber). This strategy was chosen because of significant ($P < 0.01$) differences in antibody titers between experiments at the standard temperature regimen and because differences in antibody titers between temperatures within experiments were apparent only at day 5 after injection. The following model was used:

$\underline{Y}_{ijk} = \mu + a_i + b_{j:i} + \underline{e}_{ijk}$

$\underline{Y}_{ijk}$ represented the titer-value of the kth animal

μ = mean titer (per experiment and blood sampling day)

a_i = effect of temperature ($i = 1, 2$)

$b_{j:i}$ = effect of treatment within chamber ($j = 1, 4$)

$\underline{e}_{ijk}$ = remainder.

The effect of antibody titers at a previous blood sampling day on the titers at day 5 and 10 after immunization was tested. At random combinations within group 2 to 4 for titer-values at day 0, 5 and 10 after immunization were made. The linear regressions on covariable antibody titer at a previous blood sampling day (day 0 for day 5 and day 0 and 5 for day 10 after immunization) were not significant ($P > 0.05$).

The effect of temperature and exchange on $\log_e$ PFC and relative weights of spleen and bursa of Fabricius was tested within each experiment with a Duncan's multiple range test if differences in $\log_e$ PFC and spleen- and bursa-weights

were significant at P = 0.05, using the SPSS analysis of variance (Nie et al., 1975).

RESULTS

Antibody titers of the SRBC-immunized pullets at day 0, 5 and 10 after injection are shown in Figure 2 as means per experiment. Effects of SRBC-immunization on antibody titers at day 5 and 10 after injection were significant ($P < 0.01$) at all temperatures.

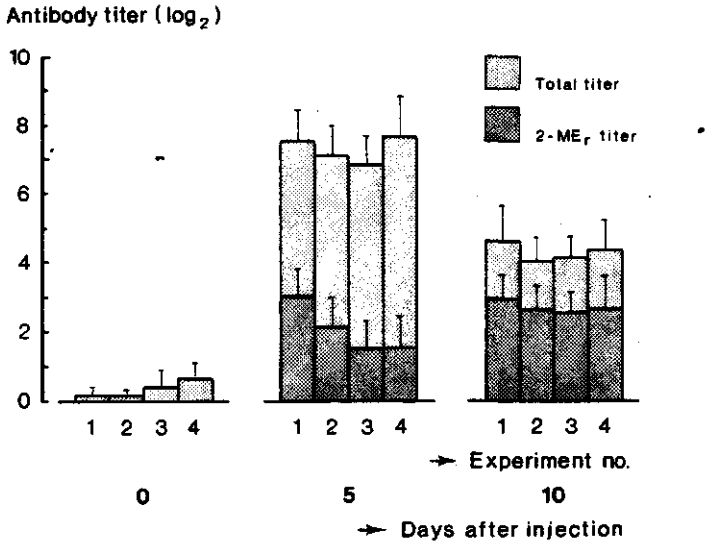

Fig. 2: Antibody titers of SRBC-immunized pullets at day 0, 5 and 10 after injection in Experiment 1 to 4 (mean and SD)

A summary of the results at day 5 after injection is given in Table 2. Differences between temperatures in antibody titers at day 0 and 10 after injection were not significant ($P > 0.05$).

All antibodies at day 0 after injection belonged most probably to the IgM type of antibodies, because no agglutination took place after 2-ME treatment (Osler, 1978). This holds too for the antibodies of the PBS-injected pullets at day 5 and 10 after injection.

Table 2: Analyses of variance (mean squares) for total and 2-ME_r (between brackets) antibody titers at day 5 after injection of Experiment 1 to 4

Source of variation	Df	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Temperature (T)	1	1.11 (0.06)	6.89** (0.41)	3.00** (1.81*)	11.34** (3.36*)
At T = 25 C					
Immunization	1	729.76** (140.30**)	648.45** (66.05**)	540.00** (29.05**)	670.67** (28.64**)
Feeding level	1	2.25 (2.50*)	2.26 (2.02)	0.24 (1.02)	8.28** (4.83**)
Exchange	1	0.02 (0.21)	0.40 (2.64*)	3.57* (0.83)	2.08 (0.42)
At T ≠ 25 C					
Immunization	1	597.24** (135.75**)	736.75** (74.82**)	671.68** (42.08**)	798.62** (46.91**)
Feeding level	1	0.16 (0.40)	0.22 (4.22**)	0.40 (2.50*)	2.60 (1.37)
Exchange	1	2.27 (0.01)	0.21 (0.53)	4.03* (3.68**)	1.83 (0.06)
Remainder	152	0.72 (0.49)	0.64 (0.61)	0.64 (0.43)	0.98 (0.63)

* $P < 0.05$
** $P < 0.01$

Effect of environmental temperature

Mean total and 2-ME_r antibody titers at day 5 after injection of the SRBC-immunized pullets at each temperature in Experiment 1 to 4 are shown in Figure 3. There was no significant effect of constant low temperature on antibody titers (Experiment 1). The low fluctuating temperature (Experiment 2) increased total titers by 7.0% ($P < 0.01$). The constant high temperature (Experiment 3) increased total titers by 6.7% ($P < 0.01$) and 2-ME_r titers by 6.4% ($P < 0.05$). The high fluctuating temperature (Experiment 4) increased total titers by 9.4% ($P < 0.01$) and 2-ME_r titers by 28.1% ($P < 0.05$).

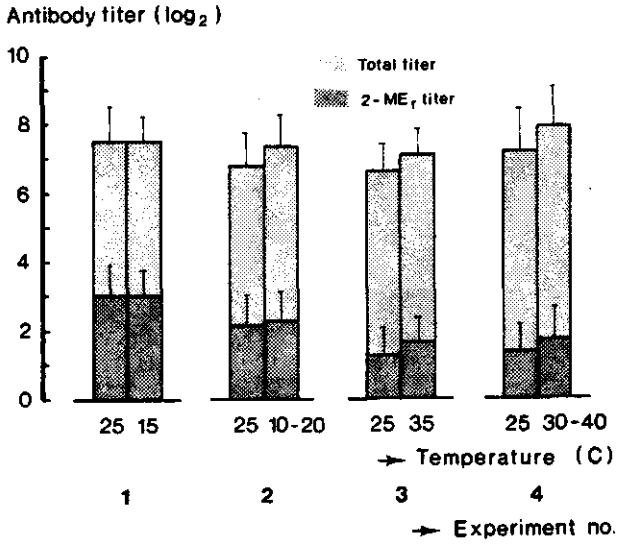

Fig. 3: Antibody titers of SRBC-immunized pullets at day 5 after injection at each temperature regimen in Experiment 1 to 4 (mean and SD)

The plaque forming cells counts (log_e PFC) are shown in Figure 4 per experiment and temperature. Differences in log_e PFC and spleen- and bursa-weights (not shown) between groups were not significant. Although differences were not significant, log_e PFC at 25 C was smaller than at the other temperatures (except in Experiment 1).

Effect of feeding level

Differences in antibody titers at day 5 after injection between restricted- and ad lib.-fed, SRBC-immunized pullets at each temperature are given in Table 3.

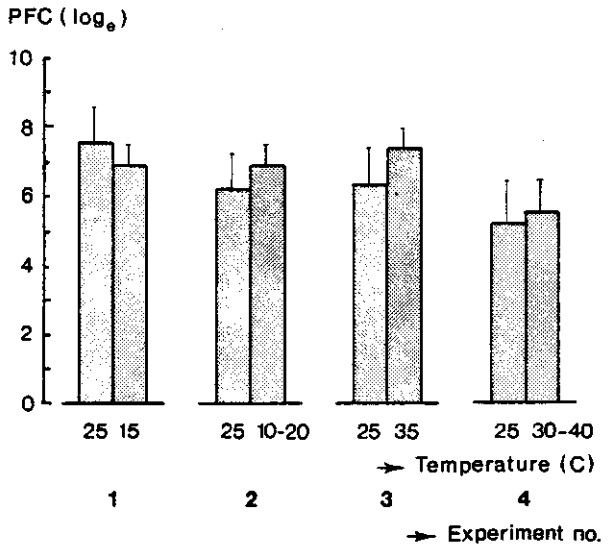

Fig. 4: Numbers of plaque forming spleencells of SRBC-immunized pullets at day 5 after injection at each temperature regimen in Experiment 1 to 4 (mean and SD)

Table 3: The increasing (+) or reducing (-) effects of restricted feeding on total and 2-ME$_r$ (between brackets) antibody titers at day 5 after injection compared to the antibody titers of ad lib.-fed pullets at each temperature (T)

Experiment	Differences in antibody titers at T = 25 C	T ≠ 25 C
1	- 0.48 (- 0.50*)	+ 0.12 (+ 0.20)
2	+ 0.48 (+ 0.45)	+ 0.15 (+ 0.65**)
3	+ 0.16 (- 0.32)	+ 0.20 (+ 0.50*)
4	+ 0.91** (+ 0.70**)	+ 0.51 (+ 0.37)

* $P < 0.05$
** $P < 0.01$

Effects of feed restriction on antibody titers at 25 C (Experiment 1 and 2) were not significant (except for 2-ME_r titers in Experiment 1). Feed restriction combined with feed withdrawal just before immunization at 25 C (Experiment 3 and 4) increased antibody titers, although significantly ($P < 0.01$) only in Experiment 4. Feed restriction at the other temperatures increased antibody titers in all experiments. This effect was significant with respect to the 2-ME_r titers at the fluctuating low temperature ($P < 0.01$) and the constant high temperature ($P < 0.05$).

At day 0 and 10 after injection there were no significant effects of feeding level on antibody titers.

Effect of exchange

At the day of injection there were no significant effects of exchange on antibody titers.

Differences in antibody titers at day 5 after injection between acclimated and exchanged, SRBC-immunized, pullets at each temperature are given in Table 4.

Table 4: The increasing (+) or reducing (-) effects of exchange on total and 2-ME_r (between brackets) antibody titers at day 5 after injection compared to the antibody titers of acclimated pullets at each temperature (T)

Experiment	Differences in antibody titers at T = 25 C	Differences in antibody titers at T ≠ 25 C
1	+ 0.04 (- 0.38)	- 0.41 (+ 0.02)
2	- 0.17 (+ 0.44*)	- 0.12 (+ 0.12)
3	- 0.52* (- 0.25)	- 0.55* (- 0.28)
4	+ 0.85* (+ 0.52*)	- 0.37 (- 0.06)

* $P < 0.05$
** $P < 0.01$

Exchange from constant low temperature to 25 C (Experiment 1) did not affect antibody titers at day 5 after injection significantly. Exchange from low fluctuating temperature to 25 C (Experiment 2) increased 2-ME_r titers signifi-

cantly ($P < 0.05$). Exchange from constant high temperature to 25 C (Experiment 3) reduced total titers at day 5 after injection significantly ($P < 0.05$). Exchange from high fluctuating temperature to 25 C (Experiment 4) increased antibody titers at day 5 after injection significantly ($P < 0.05$). Exchange from 25 C to the other temperatures reduced total titers at day 5 after injection in all experiments. This effect was significant ($P < 0.05$) in Experiment 3.

Differences in antibody titers at day 10 after injection between acclimated and exchanged, SRBC-immunized pullets at each temperature are given in Table 5. Exchange to 25 C reduced total (Experiment 2, 3 and 4) and 2-ME_r (Experiment 2 and 4) antibody titers at day 10 after injection. This effect was significant with respect to total titers in Experiment 3 and 4 ($P < 0.01$) and 2-ME_r titers in Experiment 2 and 4 ($P < 0.05$). Exchange from 25 C to the constant low temperature (Experiment 1) increased antibody titers at day 10 after injection significantly ($P < 0.01$).

Table 5: The increasing (+) or reducing (-) effects of exchange on total and 2-ME_r (between brackets) antibody titers at day 10 after injection compared to the antibody titers of acclimated pullets at each temperature (T)

Experiment	Differences in antibody titers at T = 25 C	Differences in antibody titers at T ≠ 25 C
1	+ 0.14 (0.00)	+ 1.00** (+ 0.76**)
2	- 0.38 (- 0.54*)	- 0.21 (- 0.21)
3	- 0.52** (0.00)	+ 0.22 (+ 0.04)
4	- 0.57** (- 0.56*)	+ 0.46 (+ 0.14)

* $P < 0.05$
** $P < 0.01$

DISCUSSION

Stress has been reported to increase protein catabolism (Brown et al., 1958; Nagra and Meyer, 1963) and to reduce weights of lymphoid organs such as spleen and bursa of Fabricius (Siegel, 1980). Data on protein gain showed that protein retention on day 1-5 and 6-10 after injection was not significantly affected at

the constant or fluctuating, low or high temperatures compared to the protein retention at 25 C (Henken et al., in preparation). Moreover, there was no significant thermal effect on weights of spleen or bursa of Fabricius. Therefore it is concluded that the thermal conditions in the present experiments have not been extreme.

Effect of environmental temperature

In 3 out of 4 experiments moderate low and high temperatures significantly ($P < 0.01$) increased total antibody titers at day 5 after injection. At these temperatures 2-ME_r antibody titers were also increased, although not always significantly.

Cold induced immuno-enhancement has been reported by Subba Rao and Glick (1977). They postulated that the hypothalamic-hypophyseal-thyroid axis (HHT-axis) may be involved. In Experiment 1, no immuno-enhancement was found at 15 C. Apparently the 10 C difference in temperature did not effect the HHT-axis of acclimated pullets to such extent that effects were reflected in antibody titers. However, the exchanged pullets (from 25 C to 15 C) had at day 10 after injection higher antibody titers ($P < 0.01$) than pullets, which were acclimated to 15 C at the moment of immunization. At the low fluctuating temperature a significant increase in total titers at day 5 after injection was found. The difference in effect of constant low and fluctuating low temperature on antibody titers at day 5 after injection may reflect the difference in acclimation to a constant or fluctuating temperature.

Heat induced immuno-suppression is well documented (Subba Rao and Glick, 1970 and 1977; Thaxton et al., 1968; Thaxton and Siegel, 1970 and 1973). The hypothalamic-hypophyseal-adrenocortical axis was suggested to be involved. Glick (1967), Subba Rao and Glick (1970) and Thaxton et al. (1968) reported the regressive influence of stress associated hormones on antibody mediated immunity. However, in Experiment 3 and 4, high temperatures caused a significant increase in antibody titers at day 5 after injection. This discrepancy of effects of high temperature may be caused by the less extreme thermal conditions in the present experiments. This high temperature induced immuno-enhancement may be caused by the changed metabolic rate (via HHT-axis) at above thermoneutral, but not stressfull, conditions.

The data suggest a positive relationship between the humoral immune response and metabolic rate within certain temperature limits. If a regressive effect of

temperature on the humoral immune response is to be expected than more extreme conditions than the present will be needed. Therefore it can be concluded that temperatures between 15 and 35 C are not very stressfull for pullets of 3 to 6 weeks of age. Similarly they seem to cope with temperature fluctuation between 10-20 and 30-40 C without considerable stress.

Effect of feeding level

Effects of restricted feeding on antibody titers at 25 C are not clear. Restricted feeding at the other temperatures increased antibody titers at day 5 after injection in all cases. At non-thermoneutral conditions metabolic rate is determined by environmental temperature and not by feed intake. At these conditions restricted-fed pullets must produce more thermoregulatory heat than ad lib.-fed pullets. Thus, effects of restricted feeding will be comparable with effects of decreasing environmental temperature. The increasing effect of feed restriction on antibody titers in these data is in agreement with this.

Effect of exchange

Significant effects of exchange on antibody titers were found at day 5 as well as at day 10 after injection. Exchange from 25 C to the other temperatures reduced total antibody titers at day 5 after injection in all cases. This reduction was significant by the exchange to 35 C (Experiment 3). Effects of exchange to 25 C on antibody titers at day 5 after injection seem to be dependent of the original temperature, because significant decreasing (Experiment 3) as well as increasing (Experiment 4) effects were found. However, exchange to 25 C seem to reduce antibody titers at day 10 after injection. Acclimation to 15 C may have caused the increased antibody titers at day 10 after injection in Experiment 1.

The pullets may have been stressed at the day of injection by the sudden change of thermal environment. This may be the reason of the reducing effect of exchange on antibody titers in some cases. Transport itself to the other chamber was unlikely to be stressfull, because pullets needed not to be handled.

With respect to the humoral immune response it can be concluded that, if a regressive effect of environmental temperature is to be expected, a change in temperature at the moment of immunization may be more important than the absolute temperature itself.

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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

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ABSTRACT

The effect of environmental temperature on some aspects of energy and protein metabolism of 3-6 weeks old pullets was investigated in a combined immunological and physiological study. Four experiments were performed in two climate respiration chambers. In each experiment a constant standard temperature of 25 C was maintained in one chamber. The temperature regimens in the other chamber were: a constant temperature of 15 C (Experiment 1) and 35 C (Experiment 3) and a temperature, which fluctuated between 10-20 C (Experiment 2) and between 30-40 C (Experiment 4). Four groups of 60 pullets were held at each temperature regimen. Treatments differed between groups in: 1. type of injection (phosphate buffered saline or sheep red blood cells); 2. feeding level (ad libitum vs about 80% of ad libitum); 3. length of acclimation period.

At low temperatures feed conversion (g feed/g growth) was higher ($P < 0.05$) than at 25 C. Feed intake was increased by 12.9% (at 15 C) and 10.5% (at 10-20 C) compared to intake at 25 C. Growth rate and protein gain were not significantly affected by low temperatures.

High temperatures reduced ($P < 0.05$) feed intake (15.9% at 35 C and 14.9% at 30-40 C) and growth rate (12.3% at 35 C and 12.5% at 30-40 C) compared to 25 C. Protein gain and feed conversion were not significantly affected by high temperatures.

Restricted feeding reduced ($P < 0.05$) growth rate (Experiment 1 to 4) and increased ($P < 0.05$) feed conversion (Experiment 3 and 4). Differences due to type of injection and degree of acclimation were not significant.

INTRODUCTION

Homeothermic animals will keep their body temperature constant within a small range of variation under different thermal conditions. At thermoneutrality this is achieved by regulation of heat loss by physical processes. At non-thermoneutral temperatures extra chemical energy will be used for thermoregulation (Curtis, 1981). The amount of energy available for production will decrease at these temperatures if feed intake is not affected. Thus, retained energy will be reduced as a consequence of increased heat loss at nonthermoneutral temperatures. Apart from effects on the amount of energy retained, the composition of energy gain may be affected as well (Swain and Farrell, 1975). Acclimation to nonthermoneutral temperatures is achieved by changing feed in-

take and/or by extra thermoregulatory heat production (Kleiber and Dougherty, 1934; Davis et al., 1973; Van Kampen, 1981). This acclimation is dependent on the duration of nonthermoneutral conditions. Therefore measurements of thermal demand can best be made at various feeding levels and degrees of acclimation.

For the most efficient and/or most profitable production effects of environmental temperature on performance need to be known. Discussions on optimal temperatures however should also take into account climatic effects on disease resistance. The humoral immune response following injection of sheep red blood cells (SRBC) is often used as a model to investigate these effects (Thaxton, 1978).

In 1979 studies were initiated on the effects of environmental temperature on the humoral immune response and on the energy and protein metabolism of the young chicken. In a series of 4 experiments the thermal effect on the humoral immune response to SRBC in restricted- and ad lib.-fed, in acclimated and non-acclimated pullets was investigated (Henken et al., in preparation). In this paper we report the thermal effect on some aspects of energy and protein metabolism.

MATERIALS AND METHODS

Pre-experimental conditions

Pre-experimental conditions, from hatch to 21 days of age, were the same in all 4 experiments. Environmental temperatures were gradually lowered from about 35 C to about 25 C and light regimens were gradually changed from 20 hr light and 4 hr dark (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum. The pullets (Warren SSL) were vaccinated for Marek's disease (day 1), infectious bronchitis (day 2), Newcastle disease (day 8), and infectious bursal disease (day 21). At 21 days of age the pullets were randomly assigned to one of eight groups of 60 animals each. Four groups were placed in each of two climate respiration chambers.

Experimental conditions

Pullets were held at the experimental conditions from 21 to 42 days of age. Within each chamber (dimensions: 6 m l. x 4 m w. x 2.2 m h.) each group of 60 pullets was housed in a "balance" cage with a surface area of 2 m^2. The

light regimens were 12 L : 12 D with lights on at 0730 hr. The environmental temperatures are shown in Table 1. In each experiment a constant standard temperature of 25 C was maintained in one chamber. In Experiment 2 and 4 the temperature in chamber 1 fluctuated as shown in Figure 1. Relative humidity (RH) was held at about 70% if the temperature was below 30 C. If the temperature was above 30 C, the absolute humidity was held at about 19 g H_2O per kg dry air, i.e. the water content of the air at 30 C with a RH of 70%.

Table 1: Environmental temperatures (C) in Experiment 1 to 4

	Chamber	
Experiment	1	2
1	25	15
2	10 - 20	25
3	25	35
4	30 - 40	25

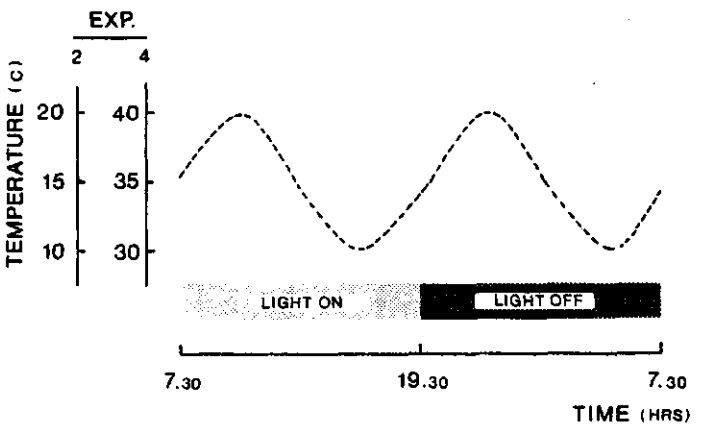

Fig. 1: Temperature fluctuation (Experiment 2 and 4) and light regimen (Experiment 1 to 4) per day

Treatments within chamber

The groups (1 to 4) within each chamber were assigned to one of the following treatments:

Group 1: Ad lib. feeding, injection with phosphate buffered saline (PBS);
Group 2: Restricted feeding, injection with SRBC;
Group 3: Ad lib. feeding, injection with SRBC;
Group 4: Ad lib. feeding, exchange with the same group in the other chamber prior (about 2 hr) to injection with SRBC.

Restricted-fed pullets were given about 80% of the estimated ad lib. intake at each temperature. This estimation was based on results of previous experiments. Diet composition, calculated and determined analyses are given in Table 2. A high protein diet was chosen because it was assumed that:

1. Effects of an immune response on growth and performance characteristics are only independent of protein supply if energy and protein gain are maximal.
2. Effects of environmental temperature on immune responsiveness can only be accurately assessed with non-limiting protein supply even at the low feed intakes at high temperatures.

Water was available ad libitum. Feed to the restricted-fed pullets at 25 C in Experiment 3 and 4 was withheld during one day before injection to study the effects of feed withdrawal at the moment of immunization on immune responsiveness. Feed withdrawal took place after ending the first balance measurements and one day before the second balance measurements were started.

Experimental procedure

The experimental procedure was similar for all experiments. After a 4-day adaptation period, experiments started at 25 days of age with a 6-day balance period before injection (B1). At 32 days of age the pullets were injected with 1 ml packed SRBC (groups 2 to 4) or 1 ml PBS (group 1). At 33 days of age the first 4-day balance period after injection (B2) was started, followed by another one (B3) starting at 38 days of age. At 24, 31, 37 and 42 days of age total body weight per group was determined. Experiments ended at 42 days of age.

Table 2: Diet composition and analytical data

Ingredients	%
Yellow corn	55.0
Corngluten feed (23%)	9.2
Soybean meal (50%)	25.9
Herring meal	2.0
Meat meal	4.0
Soybean oil	0.8
Dicalcium phosphate	0.5
DL-methionine (98%)	0.1
Vitamin premix[a]	0.5
Mineral premix[b]	2.0
Calculated analyses	
Metabolizable energy, MJ/kg	12.09
Crude protein	23.3
Lysine	1.25
Methionine	0.50
Methionine + cystine	0.89
Crude fat	4.9
Linolic fat	2.1
Crude fiber	2.7
Calcium	1.07
Available phosphorus	0.53
Analysed	
Gross energy, MJ/kg	16.77
Crude protein, 6.25 x N	23.5
Dry matter	89.2

[a] To supply the following vitamins per kilogram feed: vitamin A palmitate, 9000 IU; vitamin D, 1800 IU; vitamin E, 5 IU; vitamin K_3, 1.5 mg; thiamin, 1 mg; riboflavin, 5 mg; pyridoxin, 1 mg; cyanocobalamin, .01 mg; choline chloride, 350 mg; folic acid, .5 mg; niacin, 30 mg; D-pantothenic acid, 7.5 mg; (ethoxyquin, 75 mg).

[b] To supply the following minerals and trace elements per kilogram feed: calcium, 5.3 g; phosphorus, 1.4 g; sodium, 1 g; chloride, 1.5 g; copper, 10 mg; sulphur, 70 mg; zinc, 14 mg; manganese, 60 mg; potassium, .24 mg; iodine, .76 mg; iron, 52 mg.

Balance measurements

In each balance period the following parameters per group were measured: feed intake, metabolizability of gross energy intake (ME%), protein gain (N-retention x 6.25), mean body weight and growth rate. ME% was determined from gross energy intake and energy found in the excreta.

Statistics

Data on feed intake, growth rate and protein gain are expressed as g per day per kg body weight ($g.d^{-1}.kg^{-1}$). Data of B1 to B3 were used to calculate per group a mean level (M-value = (B1 + B2 + B3)/3), and a linear (L-value = B3 - B1) and quadratic (Q-value = 2B2 - B1 - B3) component of all measured balance characteristics. This strategy was chosen because data of B1 to B3 are not independent. The M-, L- and Q-values are mutually independent. The M-value gives a mean level of the balance data in an experiment, while the L- and Q-values provide information about how the balance characteristics change during an experiment. The M-, L-, and Q-values were each analyzed per experiment with the following model, using the SPSS analysis of variance (Nie et al., 1975):

$\underline{Y}_{ijk} = \mu + T_i + G_j + \underline{e}_{ijk}$

$\underline{Y}_{ijk}$ represented the M-, L- or Q-value of the kth group
μ = experimental mean
T_i = effect of environmental temperature (i = 1, 2)
G_j = effect of treatment (j = 1, 4) over temperatures
$\underline{e}_{ijk}$ = remainder

The effect of treatment (G_j) was estimated using data of both temperatures within experiments because of confounding effects of exchange and acclimation within temperatures. The sum of squares due to effect of treatment was subdivided into sum of squares belonging to effects of immunization, feeding level and exchange. Analysis per experiment was chosen because of significant ($P < 0.05$) differences in some balance data between experiments at 25 C. These were associated with differences in body weight at the start of the experiments.

RESULTS

A summary of the results with respect to the analyses of variance of M-values is presented in Table 3. With respect to the analysis of L-values, only differences in body weight changes were significant ($P < 0.05$), due to depressed growth rates at high temperatures (Experiment 3 and 4) or restricted feeding (Experiment 1 to 4). There were no significant effects of temperature, immunization, feeding level or exchange on Q-values.

Table 3: Analyses of variance (mean squares) of M-values of balance data in Experiment 1 to 4

Exp. no.	Source of variation	Df	Feed intake	ME%	Growth rate	Protein gain	Body weight	Feed conversion
1	Temperature	1	339.95	1.77	0.15	0.00	3.88	0.21*
	Immunization	1	2.10	0.01	3.26	0.18	132.94	0.01
	Feeding level	1	291.69	1.11	18.76*	6.20**	842.18*	0.02
	Exchange	1	1.39	0.20	13.00	0.34	265.64	0.06
	Remainder	3	37.04	0.35	1.41	0.18	73.86	0.01
2	Temperature	1	217.15	0.07	0.28	1.06	113.18	0.12*
	Immunization	1	2.10	0.01	0.13	0.16	2.04	0.00
	Feeding level	1	225.09	0.84	31.88**	6.65**	1250.07*	0.00
	Exchange	1	1.35	1.39	0.13	0.00	0.01	0.00
	Remainder	3	25.27	1.10	0.88	0.11	94.09	0.01
3	Temperature	1	427.49*	3.01	36.94*	1.89	1568.84	0.02
	Immunization	1	2.67	2.94	0.48	0.88	100.90	0.00
	Feeding level	1	397.88*	1.59	166.27**	4.13	5182.93*	0.26*
	Exchange	1	0.13	2.59	0.00	0.03	30.37	0.00
	Remainder	3	29.41	1.78	1.47	1.03	369.06	0.02
4	Temperature	1	375.38*	0.12	36.42**	0.12	1255.00	0.02
	Immunization	1	0.00	0.47*	0.46	0.09	3.19	0.00
	Feeding level	1	394.79*	3.74**	197.86**	5.15**	3699.67	0.45*
	Exchange	1	0.11	0.03	1.69	0.19	35.06	0.01
	Remainder	3	20.37	0.02	0.78	0.08	423.91	0.04

* $P < 0.05$
** $P < 0.01$

Effect of environmental temperature

The M-values of balance data per temperature regimen are given in Table 4. At low environmental temperatures (Experiment 1 and 2) significantly ($P < 0.05$) more feed was used per g growth than at 25 C. Growth rate and protein gain were not significantly affected by temperatures due to a compensation in feed intake of 12.9% (at 15 C) and 10.5% (at 10-20 C). High temperatures (Experiment 3 and 4) significantly ($P < 0.05$) reduced feed intake (by 15.9 resp. 14.9%) and growth rate (by 12.3 resp. 12.5%) compared to 25 C. Protein gain and feed conversion were not significantly affected by high temperatures. The "overall" decrease in feed intake per degree Celcius increase (Experiment 1 to 4) was 1.29 $g.d^{-1}.kg^{-1}$. Protein gain expressed as percentage of protein intake amounted to 36.4 $\pm$ 3.8% (μ and SD with $n = 8$) and was inversely related ($r = -0.93$: $P < 0.01$) to feed intake, being about 41% at the high and 31% at the low temperatures.

Table 4: M-values of balance data for each temperature regimen in Experiment 1 to 4 (mean and SE)

Exp. no.	Temp.	Feed intake $g.d^{-1}.kg^{-1}$	ME%	Growth rate $g.d^{-1}.kg^{-1}$	Protein gain $g.d^{-1}.kg^{-1}$	Body weight g	Feed conversion g Feed . g^{-1} Growth
1	25	101.38 (4.77)	75.23 (0.26)	37.40 (1.46)	9.61 (0.56)	362.38 (10.15)	2.71 (0.10)
	15	114.42 (3.33)	74.29 (0.36)	37.68 (1.07)	9.57 (0.54)	363.77 (4.33)	3.04 (0.03)*
2	25	99.63 (4.46)	75.60 (0.63)	38.02 (1.47)	9.59 (0.48)	374.51 (10.33)	2.62 (0.03)
	10-20	110.05 (2.34)	75.41 (0.25)	38.39 (0.86)	8.86 (0.61)	382.03 (4.60)	2.87 (0.04)*
3	25	91.93 (4.15)	76.14 (0.33)	34.84 (2.33)	9.00 (0.22)	388.72 (12.88)	2.65 (0.09)
	35	77.31 (4.85)*	74.92 (0.96)	30.55 (2.97)*	8.02 (0.79)	360.72 (19.21)	2.57 (0.13)
4	25	91.87 (3.46)	75.81 (0.42)	34.06 (3.16)	8.90 (0.43)	385.56 (14.16)	2.75 (0.20)
	30-40	78.17 (5.10)*	75.56 (0.42)	29.80 (2.62)**	8.77 (0.59)	360.51 (14.73)	2.65 (0.10)

* $P < 0.05$
** $P < 0.01$

Effect of immunization

The M-values of balance data for the PBS- and SRBC-injected pullets are given in Table 5. In Experiment 4, SRBC-immunized pullets had a significant ($P < 0.05$) lower ME% than PBS-injected pullets (75.00 vs 75.68%). This relatively small difference in ME% has become significant due to the very low remainder mean square in Experiment 4 as can be seen in Table 3. Because pullets were injected between B1 and B2 (at 32 days of age) effects of the type of injection (PBS or SRBC), if present, should have become apparent in the L- or Q-values. However, analyses of L- and Q-values showed that there were no significant effects of immunization on the measured balance characteristics.

Effect of feeding level

Data from groups 1 and 3 were used to determine the relation between feed intake and body weight (BW) at the standard, low and high temperatures. Feed intake ($g.d^{-1}.kg^{-1}$) changed with body weight (g) according to the linear regression equations shown in Figure 2. The realized feed restrictions at 25 C, at 15 and 10-20 C, and at 35 and 30-40 C were resp. 81.8%, 88.9% and 77.0% of the calculated ad lib. intake at each temperature.

Table 5: M-values of balance data for PBS- and SRBC-injected pullets in Experiment 1 to 4 (mean and SE)

Exp. no.	Inj.	Feed intake $g.d^{-1}.kg^{-1}$	ME%	Growth rate $g.d^{-1}.kg^{-1}$	Protein gain $g.d^{-1}.kg^{-1}$	Body weight g	Feed conversion g Feed . g^{-1} Growth
1	PBS	112.46 (9.04)	74.35 (0.98)	40.36 (0.26)	10.48 (0.33)	379.46 (7.14)	2.79 (0.24)
	SRBC	111.00 (7.32)	74.47 (0.40)	38.56 (0.24)	10.06 (0.03)	367.94 (1.22)	2.88 (0.17)
2	PBS	108.29 (6.00)	75.62 (0.42)	39.07 (0.20)	9.96 (0.02)	384.80 (3.93)	2.77 (0.17)
	SRBC	106.84 (6.33)	75.70 (0.13)	39.44 (0.20)	9.56 (0.44)	386.23 (4.30)	2.71 (0.15)
3	PBS	87.98 (9.33)	74.00 (1.75)	34.99 (2.60)	8.40 (1.06)	396.03 (19.70)	2.51 (0.08)
	SRBC	89.62 (9.72)	75.66 (0.44)	35.68 (1.73)	9.34 (0.25)	385.99 (15.72)	2.50 (0.15)
4	PBS	89.19 (7.92)	75.68 (0.22)	34.84 (2.84)	9.52 (0.14)	386.26 (24.86)	2.56 (0.02)
	SRBC	89.14 (7.74)	75.00 (0.17)*	35.52 (2.22)	9.22 (0.19)	388.05 (18.25)	2.53 (0.04)

* $P < 0.05$

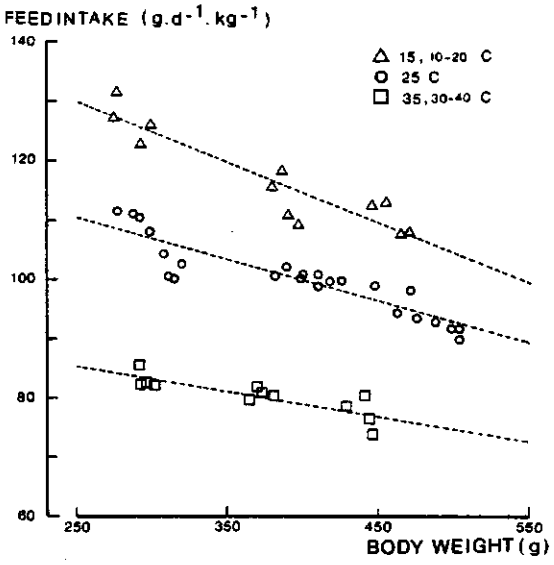

Fig. 2: Linear regression of feed intake on body weight (BW). Feed intake ($g.d^{-1}.kg^{-1}$) changed with body weight (g) according to: 154.46 - 0.10 BW ($r = -0.90$) at 15, 10-20 C; 128.13 - 0.07 BW ($r = -0.90$) at 25 C; 95.26 - 0.04 BW ($r = -0.81$) at 35, 30-40 C

The M-values of balance data for the ad lib.- and restricted-fed pullets are given in Table 6. Restricted feeding significantly depressed growth rate, protein gain (except in Experiment 3) and body weight (except in Experiment 4). Metabolizability of gross energy intake was somewhat higher in restricted-fed pullets, although not significantly. In Experiment 3 and 4 restricted-fed pullets gained less efficiently than ad lib.-fed pullets.

Table 6: M-values of balance data for ad libitum- and restricted-fed pullets in Experiment 1 to 4 (mean and SE)

Exp. no.	Feed. level	Feed intake $g.d^{-1}.kg^{-1}$	ME%	Growth rate $g.d^{-1}.kg^{-1}$	Protein gain $g.d^{-1}.kg^{-1}$	Body weight g	Feed conversion g feed . g^{-1} Growth
1	Ad lib.	111.39 (3.02)	74.54 (0.29)	38.42 (0.76)	10.10 (0.19)	369.00 (4.23)	2.90 (0.09)
	Restricted	97.44 (9.50)	75.40 (0.53)	34.88 (1.25)*	8.06 (0.10)**	345.30 (6.60)*	2.79 (0.17)
2	Ad lib.	107.90 (2.28)	75.32 (0.27)	39.36 (0.15)	9.75 (0.18)	385.49 (1.71)	2.74 (0.06)
	Restricted	95.65 (8.39)	76.06 (1.13)	34.74 (1.11)**	7.64 (0.52)**	356.62 (12.45)*	2.75 (0.15)
3	Ad lib.	88.69 (3.52)	75.27 (0.65)	35.33 (0.87)	8.92 (0.33)	389.42 (6.97)	2.51 (0.05)
	Restricted	72.40 (8.60)*	76.30 (0.80)	24.80 (3.09)**	7.26 (1.14)	330.64 (25.96)*	2.92 (0.02)*
4	Ad lib.	89.07 (2.92)	75.29 (0.15)	34.80 (1.12)	9.24 (0.12)	385.45 (8.34)	2.56 (0.04)
	Restricted	72.85 (9.52)*	76.87 (0.10)**	23.32 (1.32)**	7.39 (0.32)**	335.78 (15.48)	3.11 (0.23)*

* $P < 0.05$
** $P < 0.01$

Effect of exchange

The M-values of balance data for the acclimated and exchanged pullets are given in Table 7. Analyses of variance of M-, L- and Q-values showed that there were no significant effects of exchange on the measured balance characteristics. Feed intake of exchanged pullets during the second balance period (B2) was intermediate to feed intakes of acclimated pullets at each of both original temperatures which explains the smaller standard errors.

Table 7: M-values of balance data for acclimated and exchanged pullets in Experiment 1 to 4 (mean and SE)

Exp. no.	Degree of accl.	Feed intake $g.d^{-1}.kg^{-1}$	ME%	Growth rate $g.d^{-1}.kg^{-1}$	Protein gain $g.d^{-1}.kg^{-1}$	Body weight g	Feed conversion g Feed . g^{-1} Growth
1	Acclimated	111.73 (4.76)	74.41 (0.43)	39.46 (0.54)	10.27 (0.18)	373.70 (4.44)	2.83 (0.12)
	Exchanged	110.71 (0.22)	74.80 (0.03)	36.34 (0.70)	9.76 (0.38)	359.58 (4.18)	3.05 (0.06)
2	Acclimated	107.56 (3.59)	75.66 (0.18)	39.25 (0.16)	9.76 (0.21)	385.52 (2.41)	2.74 (0.09)
	Exchanged	108.57 (0.12)	74.64 (0.46)	39.56 (0.37)	9.73 (0.47)	385.44 (2.96)	2.74 (0.03)
3	Acclimated	88.80 (5.52)	74.81 (0.89)	35.33 (1.29)	8.87 (0.52)	391.01 (10.69)	2.51 (0.07)
	Exchanged	88.48 (1.60)	76.20 (0.53)	35.32 (1.18)	9.03 (0.02)	386.23 (5.36)	2.51 (0.04)
4	Acclimated	89.17 (4.52)	75.34 (0.23)	35.18 (1.49)	9.37 (0.13)	387.16 (12.60)	2.53 (0.03)
	Exchanged	88.88 (2.22)	75.18 (0.02)	34.05 (2.14)	8.99 (0.16)	382.03 (8.50)	2.62 (0.10)

DISCUSSION

Effect of environmental temperature

Thermoregulatory capacity develops rapidly after hatching and is relatively mature at 2 to 3 weeks of age (Osbaldiston, 1968; Wekstein and Zolman, 1970; Freeman, 1976). From that age on chickens are able to cope with a wider range of environmental temperatures (Barott and Pringle, 1946; Osbaldiston, 1968). Regulation of feed intake will be a major mechanism for thermal acclimation within this range. At low environmental temperatures feed intake is stimulated to withstand the increased thermal demand. Although growth rate may even be greater feed conversion (g feed.g^{-1} growth) will be increased due to higher maintenance requirements and change in energy used for fat and protein deposition in terms of metabolizable energy (Prince et al., 1965; Farrell and Swain, 1977). This corresponds with our results as reported in Table 4. At high environmental temperatures growth rate is depressed due to decreased feed intake while feeding efficiency itself is not affected as is also shown by Adams et al. (1962).

Protein gain was not significantly affected by temperature in the present experiments. It has been shown that protein anabolism is relatively independent of environmental temperature (Kubena et al., 1972; Swain and Farrell, 1975). The latter authors showed that lower temperatures may cause a decline in carcass

fat while protein content remained constant and apparently was independent of the temperature at which the chickens were grown. In the present experiments this is also found and therefore the percentage of ingested protein which is retained, is increased at the higher temperatures. Reducing effects of high temperatures on protein gain may be found with diets containing less protein as the diet used in the present experiments.

Effect of immunization

There were no significant differences in the measured balance characteristics due to type of injection. This corresponds with observations of Henken and Brandsma (1982). However, they also measured heat production and determined energy gain and fat deposition. They reported that fat deposition was increased during day 1 to 5 after immunization and decreased during day 6 to 10. A temporary change in levels of corticosteroids and/or thyroxine due to the induced immune response was suggested to be involved. In the present experiments heat productions were not measured because there were different treatments within each climate respiration chamber.

Effect of feeding level

Restricted feeding significantly reduced growth rate and protein gain (except in Experiment 3) compared to ad lib. feeding. This indicates that feed intake of restricted-fed pullets was too low for maximum protein gain. In Experiment 1 and 2 feed conversion was not significantly affected by restricted feeding. In Experiment 3 and 4 restricted-fed pullets used significantly more feed per g growth than ad lib.-fed pullets. This may be due to the extreme low feed intake of the restricted-fed pullets at high temperatures (about 63.5 $g.d^{-1}.kg^{-1}$).

Effect of exchange

No significant differences were found in the balance data due to differences in thermal acclimation. Pullets seem to adapt very fast to their new thermal environment (Harrison and Biellier, 1969). Effects of exchange may have occurred on the day of exchange only during which no measurements were done. A possible temporary effect on feed intake or growth rate may explain why exchanged pullets

tended to have somewhat smaller body weights.

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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

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ABSTRACT

The effect of low environmental temperature on the humoral immune response to sheep red blood cells (SRBC) was investigated in relation to energy metabolism. Two experiments were done with 3-6 weeks old pullets using two climate respiration chambers. During both experiments a standard temperature regimen of constant 25 C was maintained in one chamber, while the temperature in the other chamber was constant 10 C (Experiment 1) or fluctuated between 5-15 C (Experiment 2). The relative humidity was held at 70% at all temperature regimens. Feed allowance was such that growth rates at the two temperatures within experiment were expected to be similar.

Indeed, growth rate and body weight were not significantly ($P > 0.05$) influenced by temperature in Experiment 1. Feed consumption at 10 C was 32.7% above that at 25 C. At 5-15 C feed consumption was 34.1% higher than at 25 C, while growth rate was reduced ($P < 0.05$) by 8%. At day 0 and 10 after injection, differences in haemagglutinin anti-SRBC antibody titers ($\log_2$) between temperatures within experiment were not significant. 2-Mercapto-ethanol resistant (2-ME_r) antibody titers at day 5 after injection were increased by 26% ($P < 0.05$) at 10 C compared to 25 C. The fluctuating low temperature reduced 2-ME_r antibody titers at day 5 after injection by 19% ($P < 0.05$). Differences in total antibody titers at day 5 after injection between temperatures were not significant, but they tended to be reduced by low temperatures. No significant thermal effects on plaque forming cell counts and relative weights (% of body weight) of spleen and bursa of Fabricius were found. Fat gain during the period between day of injection (day 0) and peak antibody titers (day 5) was significantly increased at the low temperatures. Thermal effects on protein gain were not significant, but more protein was deposited at 25 C than at the other temperature regimens during this periods after injection.

The immunological and physiological data indicate that the imposed thermal conditions approach cold tolerance limits for 3-6 weeks old, restricted-fed, pullets with respect to humoral immune responsiveness.

INTRODUCTION

Moderate high and low environmental temperatures have been shown to enhance the humoral immune response to sheep red blood cells (SRBC) in acclimated pullets (Henken et al., 1983a). It was stated that if a regressive influence of

thermal conditions on immune responsiveness is to be expected, temperatures needed to be more extreme, i.e. below 15 or above 35 C. Thus, the effect of environmental temperature on the humoral immune response may be dependent on the demand upon animal's capacities to maintain homeothermia. This indicates that, when studying the effect of thermal conditions on immune responsiveness, a quantification of the demand of those conditions upon thermoregulatory capacities must be made simultaneously. Energy balance characteristics (a.o. metabolic rate) seem to be appropriate for this.

The present experiments were performed in order to assess more precisely the environmental temperature below which the humoral immune response will be reduced. Controls were held at 25 C. Feed allowance was restricted in such a way that growth rates were similar despite the large differences in thermal conditions at which the pullets were held.

MATERIALS AND METHODS

Pre-experimental conditions

Two experiments were performed. Pre-experimental conditions were the same in both experiments. Environmental temperatures were gradually lowered from about 35 C to about 25 C during the first 3 weeks after hatching. Photoperiod length gradually decreased from 20 hr : 4 hr dark (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum. The pullets (Warren SSL) were subjected to the following vaccination scheme during the first 21 days after hatching: day 1 - Marek's disease, intramuscularly (IM); day 2 - infectious bronchitis, intraocularly (IO); day 8 - Newcastle disease, IO; day 21 - infectious bursal disease, IO. At 21 days of age the pullets were randomly assigned to one of eight groups of 60 animals each. Four groups were placed inside each of two climate respiration chambers.

Experimental conditions

Within each chamber (dimensions: 6 m l. x 4 m w. x 2.2 m h.) each group of 60 pullets was housed in a "balance" cage with a surface area of about 2 m^2. The light regimens were 12 L : 12 D with lights (about 42 lux at animal level) on at 0730 hr. The environmental temperature regimens are given in Table 1. In each experiment a standard temperature regimen of constant 25 C was maintained

in one chamber. In Experiment 2 the temperature in chamber 2 fluctuated as shown in Figure 1. The relative humidity was held at about 70% at all temperatures. From 21 days of age pullets were given about 90% of the ad lib. intake that is predicted for the relevant thermal conditions (see Henken et al., 1982). The 10 and 5-15 C environments were assumed to be identical with respect to feed intake. Diet composition was the same as reported by Henken et al. (1983b). Water was available ad libitum in both experiments.

Table 1: Environmental temperatures (C) per chamber in Experiment 1 and 2

	Chamber	
Experiment	1	2
1	25	10
2	25	5 - 15

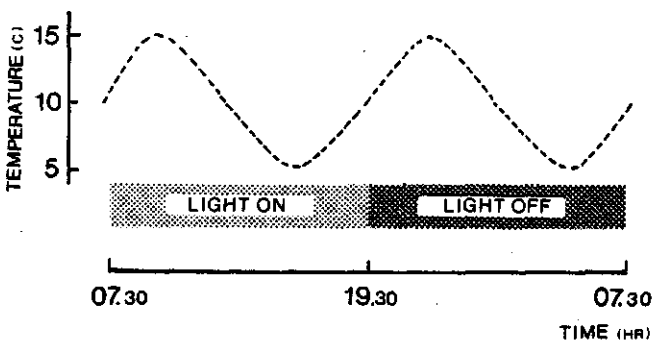

Fig. 1: Temperature fluctuation (Experiment 2) and light regimen (Experiment 1 and 2) per day

Experimental procedure

The experimental procedure was similar for both experiments (Figure 2). After a 4-day adaptation period (A), experiments started at 25 days of age with

a 6-day balance period before injection (B1). During this 6-day period two 48-hr respiration trials (R1 and R2) were performed. At 32 days of age all pullets in both chambers were immunized (I) with 1 ml packed SRBC (about 3 x 10^{10} cells). The doses were IM injected in two equal portions, one into each thigh. At 33 days of age the first 4-day balance period after injection (B2) was started, followed by another one (B3) starting at 38 days of age. During each of these two balance periods after injection, two 48-hr respiration trials were performed (R3 and R4 resp. R5 and R6). Experiments ended at 42 days of age.

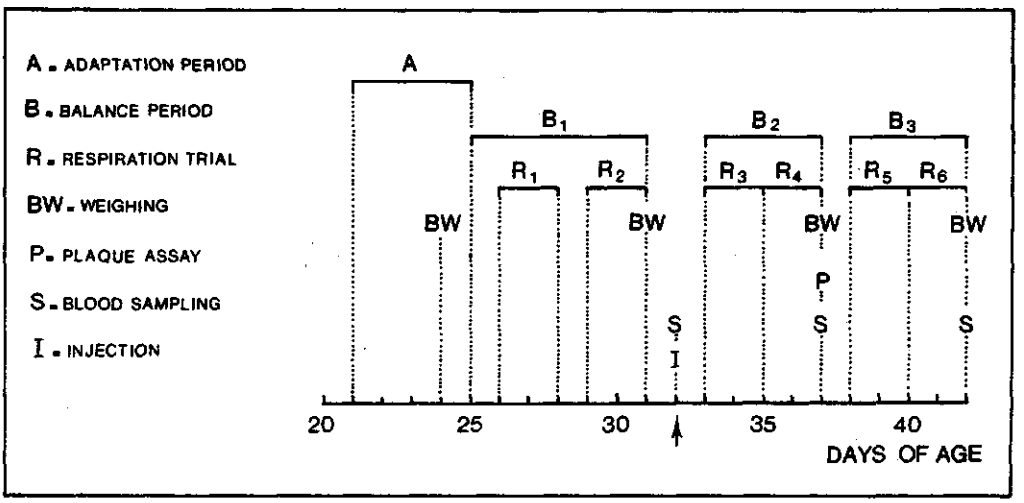

Fig. 2: Experimental schedule

Immunological measurements

Haemagglutinin antibody titers - Just before, and at day 5 and 10 after injection (at 32, 37 and 42 days of age) about 1 ml blood, per pullet, was drawn from the ulnar vein of 20 pullets in each experimental group (Figure 2). Each pullet was bled only once. The heparinized blood samples were centrifuged and the plasma harvested and stored at -20 C until a haemagglutinin assay was performed. Total and 2-mercapto-ethanol resistant (2-ME_r) antibody titers (log_2) were determined as described by Van der Zijpp and Leenstra (1980).

Number of plaque forming spleencells - At day 5 after injection (at 37 days of age) 3 pullets from each experimental group were killed and their spleen used for a direct haemolytic plaque assay (Figure 2). The number of plaque forming cells per 10^6 viable leucocytes (PFC) was determined and transformed to log_e PFC (Henken et al., 1983a). Weight of bursa of Fabricius and spleen were

determined and expressed as percentage of body weight.

Balance measurements

The following parameters were measured per group in each balance period (B1 to B3): feed and metabolizable energy (ME) intake, protein retention (N-retention x 6.25), mean body weight and growth rate. At 24, 31, 37 and 42 days of age total body weight per group was determined (Figure 2). Metabolizability (ME%) of gross energy (GE) was determined from GE intake and energy found in the excreta. Heat production (H) was determined per chamber during each consecutive 18 min. in R1 to R6 from measurements of oxygen consumption and carbon dioxide production, using a formula derived from the one proposed by Romijn and Lokhorst (1961):
H (KJ) = 16.20 x liters O_2 consumed + 5.00 x liters CO_2 produced.

Statistics

The data on antibody titers were analysed within experiment with the following model, using the Harvey (1977) LSML 76 program:

$\underline{Y}_{ijk} = \mu + S_i + T_{j:i} + \underline{e}_{ijk}$

$\underline{Y}_{ijk}$ represented the titer-value of the kth animal
μ = experimental mean
S_i = effect of blood sampling day (i = 1, 3)
$T_{j:i}$ = effect of temperature regimen (j = 1, 2) within blood sampling day
$\underline{e}_{ijk}$ = remainder

Analysis per experiment was chosen because of significant differences in antibody titers between experiments at the standard temperature regimen. The effect of environmental temperature on $\log_e$ PFC and relative weights of spleen and bursa of Fabricius was analysed with a T-test, using the SPSS program (Nie et al., 1975).

Data on feed intake, growth rate and protein retention are expressed as gram per day per kilogram body weight ($g.d^{-1}.kg^{-1}$). Data on ME intake and heat production are expressed as watt ($J.sec.^{-1}$) per kilogram body weight ($W.kg^{-1}$). For each experimental group the difference between ME intake and mean heat production per balance period per chamber was taken as the energy retention (RE). The difference between RE and energy gained as protein was used to determine the fat deposition ($g.d^{-1}.kg^{-1}$). Values for the energy content of 1 gram protein and fat were based on the constants proposed by Blaxter et al. (1964).

Data of the balance periods B1 to B3 were each separately analyzed per experiment with a T-test, using the SPSS program (Nie et al., 1975). The results of B2, the balance period between day of immunization and peak antibody titers, are presented in the following together with data on protein and fat gain in all three balance periods.

RESULTS

Feed consumption, body weight and growth rate

Data on feed consumption, body weight and growth rate are presented in Figure 3. Growth rate and body weight in Experiment 1 were not significantly ($P > 0.05$) affected by temperature. Feed consumption at 10 C was 32.7% above that at 25 C. At 5-15 C (Experiment 2) feed consumption was 34.1% higher than at 25 C, while growth rate was reduced ($P < 0.05$) by 8%, but pullets at 5-15 C still had a higher body weight (+ 5.2 g) than pullets at 25 C.

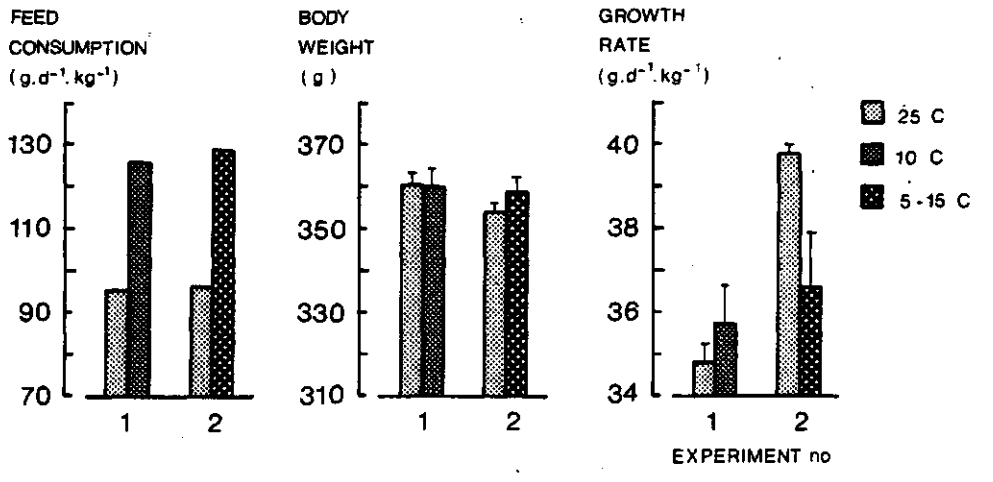

Fig. 3: Feed consumption, body weight and growth rate in the balance period between day of injection and peak antibody titers per temperature regimen in Experiment 1 and 2 (mean and SD)

Antibody titers and plaque forming cell counts

Results of the analyses of variance on antibody titers are given in Table 2. Antibody titers (mean and SD) are presented in Figure 4 per temperature regimen in Experiment 1 and 2 for all three blood sampling days. Differences in antibody titers between blood sampling days were significant ($P < 0.01$). At day 0 and 10 after injection, differences in antibody titers between temperatures were not significant. The low constant temperature of 10 C increased 2-ME_r titers at day 5 after injection by 26% ($P < 0.05$) compared to 25 C. The fluctuating low temperature (5-15 C) reduced 2-ME_r titers at day 5 after injection by 19% ($P < 0.05$). Differences between temperatures in total titers at day 5 after injection were not significant, but total titers tended to be reduced by low temperature in both experiments. Differences in $\log_e$ PFC and relative weights of spleen and bursa of Fabricius between temperatures were not significant (Figure 5).

Table 2: Analyses of variance (mean squares) of total and 2-ME_r antibody titers

Source of variation	df	Experiment 1		Experiment 2	
		total titer	2-ME_r titer	total titer	2-ME_r titer
Days after injection	2	1487.96**	192.82**	1793.02**	142.54**
Temperature: day 0	1	0.06	0.00	0.08	0.00
Temperature: day 5	1	0.49	2.87*	0.46	2.02*
Temperature: day 10	1	0.51	0.10	1.50	0.00
Remainder†	469	0.65	0.49		
	468			0.87	0.48

† in Experiment 1 and 2, 5 resp. 6 pullets died (cannibalism)

* $P < 0.05$

** $P < 0.01$

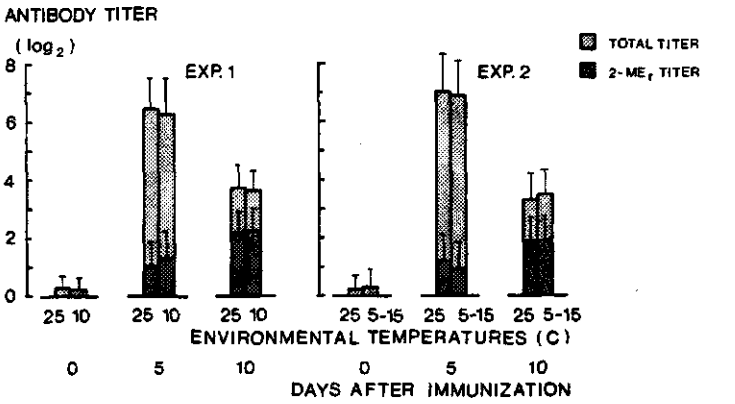

Fig. 4: Total and 2-ME$_r$ antibody titers at day 0, 5 and 10 after injection per temperature regimen in Experiment 1 and 2 (mean and SD)

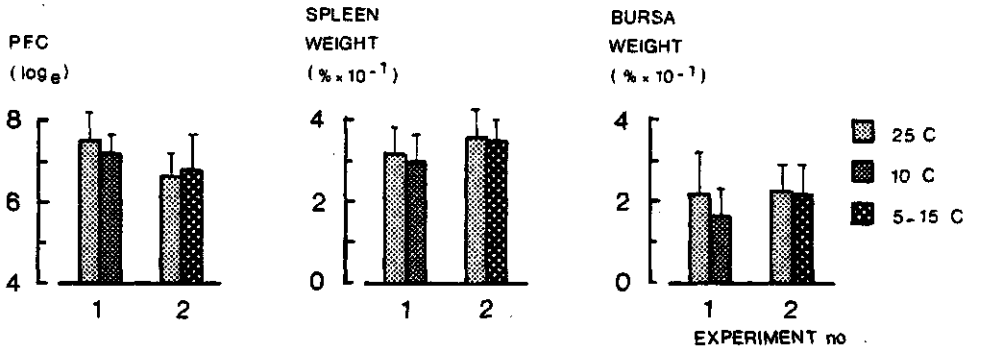

Fig. 5: Plaque forming cell counts and relative weights of spleen and bursa of Fabricius per temperature regimen in Experiment 1 and 2 (mean and SD)

Metabolizability of gross energy, heat production and energy gain

ME% was reduced ($P < 0.01$) by low temperature in both experiments (Experiment 1, 71.7 vs 74.3%: Experiment 2, 73.9 vs 76.3%). Heat production and energy gain were increased ($P < 0.01$) by low temperature in both experiments (Figure 6). At 10 C (Experiment 1) heat production and energy gain were increased by 33.3 resp. 13.2% compared to 25 C. In Experiment 2 these differences were 34.8 resp. 17.9%.

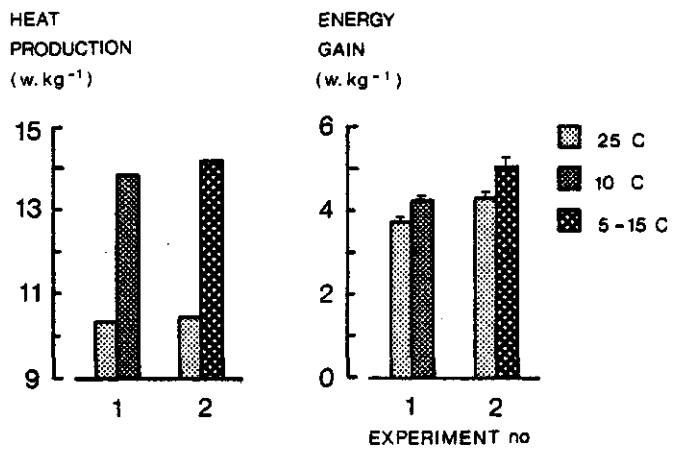

Fig. 6: Heat production and energy gain in the balance period between day of injection and peak antibody titers per temperature regimen in Experiment 1 and 2 (mean and SD)

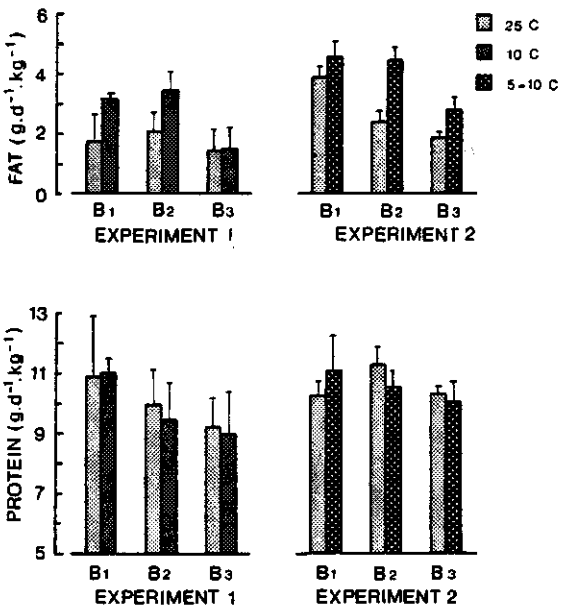

Fig. 7: Fat and protein gain in all balance periods per temperature regimen in Experiment 1 and 2 (mean and SD)

Protein and fat gain

Data on protein and fat gain of all three balance periods are presented in Figure 7. There was no significant effect of environmental temperature on protein and fat gain in B1, but both were higher at the lower temperatures. In B2 protein gain was reduced compared to B1; this reduction was most pronounced at the lower temperatures. In both experiments fat gain during B2 was significantly (Experiment 1, $P < 0.05$; Experiment 2, $P < 0.01$) increased at the low temperatures compared to 25 C. In B3 differences in protein gain between temperatures were not significant. In Experiment 2 fat gain in B3 was significantly ($P < 0.01$) higher (+ 50%) at 5-15 C than at 25 C.

DISCUSSION

Regressive effects of thermal conditions on the humoral immune response can be expected if the evoked regulatory response is mainly non-specific, i.e. a stress response, causing blood levels of corticosteroids to rise. Corticosteroids are thought to act by influencing the rate of synthesis of specific RNA and protein, resulting in lymphoid cell destruction and reduced antibody production (Thompson and Lippman, 1974; Gould and Siegel, 1980). In the present experiments total antibody titers at day 5 after SRBC-injection tended to be lower at the low, constant or fluctuating, temperature regimens compared to titers at 25 C. 2-ME_r titers at day 5 after injection were increased at 10 C and reduced at 5-15 C. Henken et al. (1983a) found that thermal conditions of about 15 and 35 C increased the humoral immune response to SRBC compared to 25 C. However in the present experiments total titers were not increased at 10 or 5-15 C. Therefore these results suggest that more extreme cold conditions than the present may revert the immune response to SRBC. An indication for such a cold induced reduction of the humoral immune response may be represented by the results at 5-15 C. In the literature it is reported that low environmental temperatures in most cases enhanced the humoral immune response (Subba Rao and Glick, 1977; Thaxton, 1978; Blecha and Kelley, 1979). Low temperatures which cause reduced body temperatures have been reported to reduce the immune response (Siegel, 1980). The apparent reduction in the humoral immune response at more extreme low environmental temperatures is in line with data on gain.

In general, fluctuating temperatures give similar results in performance of chickens as mean constant temperatures. In the present experiments feed allowance at 10 and 5-15 C was the same. The results of Experiment 1 showed that growth

rates at 25 and 10 C were similar, as was predicted. However, the performance of the pullets at 5-15 C was reduced compared to 25 C. Combining results of both experiments, it can be concluded that the pullets were more able to cope with 10 C than with 5-15 C.

The severity of the imposed cold conditions in the present experiments may also be reflected in the energy balance characteristics. As stated above, a non-specific response will prevail at extreme conditions, causing blood levels of corticosteroids to rise. Corticosteroids cause a change in energy expenditure, which seems to favor fat deposition and to increase protein catabolism (Brown et al., 1958; Nagra and Meyer, 1963; Siegel, 1980). During the first balance period after injection, pullets at 10 and 5-15 C deposited significantly more fat than pullets at 25 C, while protein gain was higher at 25 C in both experiments. This may confirm the extremity of the imposed thermal conditions because normally fat deposition is reduced at below thermoneutral temperatures in order to withstand the increased thermal demand. A more definite explanation could have been given if not growth rate but energy gain was equalized between temperatures. Energy gain was higher at the low temperatures. Therefore the comparison of fat gain between temperatures may be biased.

The combined immunological and physiological data indicate that the imposed thermal conditions approach cold tolerance limits for 3-6 weeks old, restricted-fed, pullets with respect to humoral immune responsiveness.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON IMMUNE RESPONSE AND METABOLISM OF THE YOUNG CHICKEN

6. Effect of environmental temperature on heat production in pullets in relation to feeding level

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ABSTRACT

The effect of environmental temperature on heat production (H) of 3-6 weeks old pullets was investigated. Two experiments were done with 8 pullets each using two climate respiration chambers. Both groups of 4 pullets within each experiment got a similar treatment procedure. They were fed ad libitum (Experiment 1) or were given 80% of the estimated ad libitum intake at each temperature (Experiment 2). Restricted-fed pullets were shown to have a lower critical temperature (T_{cr}) of 27.9 C. Below T_{cr} extrathermoregulatory H (about .345 $W.kg^{-1}.C^{-1}$) seemed to be independent of feeding level. Above T_{cr} H of ad libitum-fed pullets was decreasing at a faster rate (.215 $W.kg^{-1}.C^{-1}$) with increasing temperature than H of restricted-fed pullets (.075 $W.kg^{-1}.C^{-1}$). At night, when lights were off, T_{cr} was about 3 C lower than the calculated 24-hr value. At daytime T_{cr} was above 30 C or absent.

INTRODUCTION

Moderate low (10-20 C) and high (30-40 C) temperatures have been shown to enhance the humoral immune response following injection of sheep red blood cells (SRBC) in acclimated pullets (Henken et al., in preparation). It was concluded that regressive effects of thermal conditions on immune responsiveness can be expected if temperatures are more extreme. Thus, thermal effects on the humoral immune response may depend on the demand upon animal's capacities to maintain homeothermia. This indicates that, when studying climatic effects on immune responsiveness, a quantification of the thermal demand upon thermoregulatory capacities must be made simultaneously. This can best be made at various feeding levels, because regulation of feed intake is a major mechanism in poultry for thermal acclimation. In this paper we report results of two experiments which were done to study the effect of environmental temperature on heat production in ad libitum- and restricted-fed pullets. Thermal effects on the humoral immune response were investigated in parallel experiments, results of which will be given elsewhere (Henken et al., in preparation).

MATERIALS AND METHODS

Pre-experimental conditions

During the first 18 days after hatching, environmental temperatures were

gradually lowered from about 35 C to about 25 C. Photoperiod length gradually decreased from 20 hr : 4 hr dark (20 L : 4 D) to 12 L : 12 D. Feed and water were available ad libitum.

Experimental conditions

When 18 days old, the pullets (from a medium heavy layer breed) were weighed and 4 were placed in each of two climate respiration chambers (contents: 85 dm^3) with differences in body weight between chambers minimal. All pullets within each experiment got a similar treatment procedure. They were fed ad lib. (Experiment 1) or were given 80% of the estimated ad lib. intake at each temperature (Experiment 2). Feed contained 87.5% of dry matter, 23.9% of crude protein and the gross energy content was 17.3 $KJ.g^{-1}$. Water was available ad libitum. The light regimens were 12 L : 12 D with lights (about 14 lux) on at 0730 hr.

Experimental procedure

The pullets were allowed three days of adaptation to the chambers (at 25 C), before the experiments started at 21 days of age. From that age until 43 days of age, pullets were weighed and exchanged between chambers after each of eleven 2-day periods. At the end of each period, temperatures in both chambers were changed with 5 C beginning at 35 C in the first period until temperatures were 10 C and back in a similar way until 35 C was reached in the eleventh period. The relative humidity was held at about 70% except at 35 C where it was held at about 52%.

Balance measurements

Feed intake, mean body weight and growth rate were measured in each 2-day period. A 24-hr respiration trial was performed at every second day of each 2-day period (R1 to R11). Heat production (H) was determined per chamber during each consecutive 18 min. from measurements of oxygen consumption and carbon dioxide production, using a formula derived from the one proposed by Romijn and Lokhorst (1961): H (KJ) = 16.20 x liters O_2 consumed + 5.00 x liters CO_2 produced. Activity measurements were made per chamber during each consecutive 6 min. during R1 to R11, using ultra-sound activity detectors (Wenk and Van Es, 1976). Activity free heat production was determined by regression of heat production on activity.

Statistics

Analysis was done per experiment. Data on heat production were expressed as watt (J.sec.$^{-1}$) per kg body weight, pooled within respiration trial and regressed on environmental temperature. Two regression lines were determined using data of 10, 15 and 20 C (L1) resp. 30 and 35 C (L2). Thus, the lower critical temperature (T_{cr}) was supposed to be between 20 and 30 C. If the two lines crossed above 25 C, data at 25 C were added to L1 and vice versa. The validity of this procedure was checked by testing the differences in the regression coefficients of L1 and L2 (Kreyszig, 1970).

RESULTS

Feed intake, body weight and growth rate

When temperature decreased (from 35 to 10 C), feed intake of ad lib.-fed pullets increased continuously and growth rate was maximal at 20 C (Figure 1).

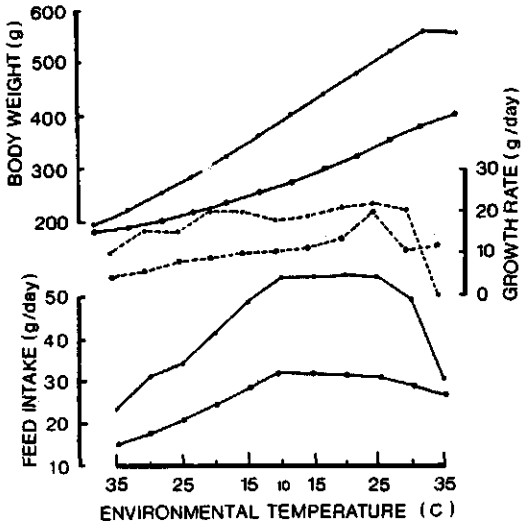

Fig. 1: Body weight, growth rate and feed intake of ad lib.- (0) and restricted-fed (●) pullets

When temperature increased from 10 to 25 C, growth rate of ad lib.-fed pullets increased progressively while feed intake remained constant. In the 10th (30 C) and 11th (35 C) period, feed intake and growth rate of ad lib.-fed pullets dropped considerably. Growth rate of restricted-fed pullets had a more constant pattern except for the sharp increase at 25 C (9th period) and drop thereafter.

Heat production

Heat productions in the temperature-decreasing part of the experiments were, except at 15 C, above those in the temperature-increasing part (Figure 2). Restricted-fed pullets had a T_{cr} of 27.9 C. This is about 2 C higher than the T_{cr} of ad lib.-fed pullets (Table 1). However, the difference in extrathermoregulatory heat below and above T_{cr} in ad lib.-fed pullets was significant only if activity free heat production data were used (Table 2).

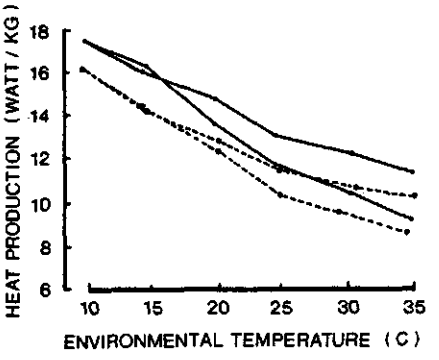

Fig. 2: 24 hr heat production of ad lib.- (O) and restricted-fed (●) pullets

Table 1: Critical temperatures (T_{cr}) and extrathermoregulatory heat ($W.kg^{-1}.C^{-1}$), using 24 hr data

Feed. level (Exp. no.)	Method	T_{cr}	b (below T_{cr})		b (above T_{cr})
Ad lib. (1)	without 25 C	26.8	- .344		- .215
	with 25 C	25.9	- .353		- .215
Restricted (2)	without 25 C	28.3	- .326	*	- .075
	with 25 C	27.9	- .337	**	- .075

* P < .05
** P < .01

Table 2: Critical temperatures (T_{cr}) and extrathermoregulatory heat ($W.kg^{-1}.C^{-1}$), using 24 hr activity free heat production data

Feed. level (Exp. no.)	Method	T_{cr}	b (below T_{cr})		b (above T_{cr})
Ad lib. (1)	without 25 C	24.3	- .387	**	- .109
	with 25 C	25.0	- .387	**	- .078
Restricted (2)	without 25 C	30.5	- .227	**	+ .034
	with 25 C	29.3	- .248	**	+ .034

** P < .01

Diurnal rhythm in heat production

In Figure 3A and 3B heat productions during daytime (1030-1530 hr) and at night (2100-0600 hr) are shown separately. In all respiration periods heat produced at daytime was above that produced at night. At daytime no significant T_{cr} could be calculated in ad lib.- nor in restricted-fed pullets (Table 3A and 3B). At night ad lib.-fed pullets had a T_{cr} of 23.1 C: this is about 4 C less than that of restricted-fed pullets. Heat production of restricted-fed pullets dropped continuously during the night at the lower temperatures (Figure 4). Extrathermoregulatory heat below T_{cr} is higher in the first (2100-0130 hr) part of the night than in the second (0130-0600 hr) part and attained a same level as that of ad lib.-fed pullets at night (~.401 vs -.406 $W.kg^{-1}.C^{-1}$).

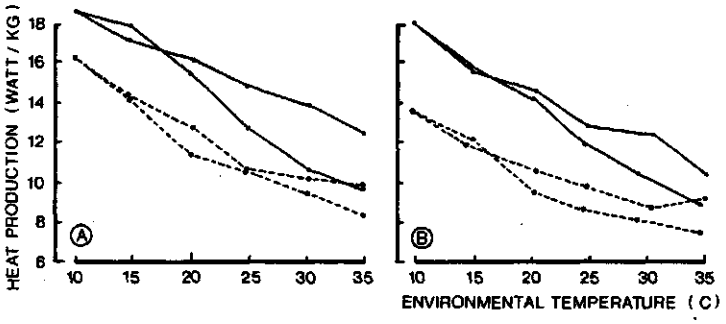

Fig. 3A and 3B: Heat production within day (O) and night (●) of ad lib.- (3A) and restricted-fed (3B) pullets

Table 3A: Critical temperatures (T_{cr}) and extrathermoregulatory heat ($W.kg^{-1}.C^{-1}$) during day and night at ad lib. feeding

Period	Method	T_{cr}	b (below T_{cr})		b (above T_{cr})
Day	without 25 C	-	- .291		- .277
	with 25 C	29.9	- .330		- .277
Night	without 25 C	23.2	- .406	**	- .146
	with 25 C	23.1	- .406	**	- .147

** P < .01

Table 3B: Critical temperatures (T_{cr}) and extrathermoregulatory heat ($W.kg^{-1}.C^{-1}$) during day and night at restricted feeding

Period	Method	T_{cr}	b (below T_{cr})		b (above T_{cr})
Day	without 25 C	30.5	- .312		- .173
	with 25 C	27.0	- .349		- .173
Night	without 25 C	25.6	- .342	**	+ .012
	with 25 C	27.2	- .300	**	+ .012

** P < .01

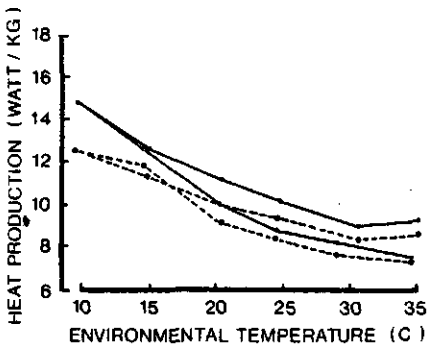

Fig. 4: Heat production in the first (0) and second (●) part of the night at restricted feeding

DISCUSSION

In the calculations based on 24 hr heat production data the regression coefficients below T_{cr} of ad lib.- and restricted-fed pullets resemble each other: below T_{cr} heat production is determined by temperature and not by feed intake (Kleiber, 1961). In restricted-fed pullets there is a very small decrease in heat production with increasing temperature above T_{cr} in contrast with ad lib.-fed pullets. Therefore T_{cr} can be found in the restricted feeding experiment ($T_{cr} \approx 28$ C). In the ad lib. feeding experiment T_{cr} is less dinstinct ($T_{cr} \approx 26$ C). This difference in T_{cr} between ad lib. and restricted feeding is in agreement with literature (Kleiber, 1961; Verstegen, 1971).

At daytime no T_{cr} was found in the present experiments. This indicates that either there is no T_{cr} at daytime or T_{cr} is above 30 C. Van Kampen et al. (1979) reported a T_{cr} of 32.2 in the light and 27.5 in the dark in White Rock males. The regression of heat on temperature may be linear over a small range of temperatures or may be curvilinear. When heat production is corrected for activity than the difference between day and night with respect to thermal demand and T_{cr} is considerably reduced.

At restricted feeding heat production diminishes continuously during the night at low temperatures in contrast to heat production of ad lib.-fed birds. Probably restricted-fed pullets are less challenged in the beginning of the night than later on in order to reduce heat loss. In the second part of the

night restricted-fed pullets, held at 10 C, seem to be unable to maintain metabolic rate and thus body temperature. Low temperatures which cause reduced body temperatures have been shown to affect immune responsiveness in chickens (Siegel, 1980). This is in agreement with our finding of reduced total antibody titers after injection of SRBC in restricted-fed pullets at 10 C.

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GENERAL DISCUSSION

The function of the immune system is to provide protection against invasion of foreign structures (micro-organisms, toxins). Recognition and ultimate disposition of these structures constitute the beginning and the aim of a complex process, involving phagocytosis, humoral and cell mediated immune responsiveness (Roitt, 1980).

This function is not fullfilled without consequences for the production traits of the animal. The results reported in the 2nd paper, show that SRBC-injected pullets were eating more while gaining less than PBS-injected pullets during the first balance period after injection. During this period, SRBC-injected pullets produced less heat, had a higher energy retention and deposited more fat than PBS-injected pullets. Thus it seems that an immune response causes a shift in metabolism in favour of fat deposition. This shift may be caused by a rise of the plasma corticosteroid level during an immune response, presumably due to the activity of lymphokines produced by immunoreactive lymphocytes (Nagra and Meyer, 1963; Besedovsky et al., 1975 and 1981; Siegel and Van Kampen, 1981; Siegel et al., 1983). It is important to investigate in that respect whether vaccinations induce similar metabolic responses and/or have an effect on growth traits.

From the results in the 3rd paper it is clear that the humoral immune response is influenced by environmental temperature. Moderate high and low environmental temperatures enhance the humoral immune response to SRBC in acclimated pullets (Figure 1). Extreme high environmental temperatures have been reported to reduce the humoral immune response (Thaxton et al., 1968; Thaxton and Siegel, 1970 and 1973; Subba Rao and Glick, 1970 and 1977), while severe cold mostly enhanced the humoral immune response (St. Rose and Sabiston, 1971; Sabiston and St. Rose, 1976; Subba Rao and Glick, 1977; Blecha and Kelley, 1979). These apparent contradictionary effects of heat and cold may be due to the fact that animals are more able to cope with cold than with heat (Mount, 1979). At low temperatures animals must produce heat, while at high temperatures they must get rid of heat which in itself will cause heat production to rise and so on. The upper lethal temperature is more near thermoneutrality than the lower lethal temperature. Low temperatures which cause reduced body temperatures, have been reported to reduce also the immune response (Siegel, 1980). This may be in agreement with our finding that the humoral immune response was somewhat reduced in non-acclimated pullets, even at moderate high and low temperatures (Table 1).

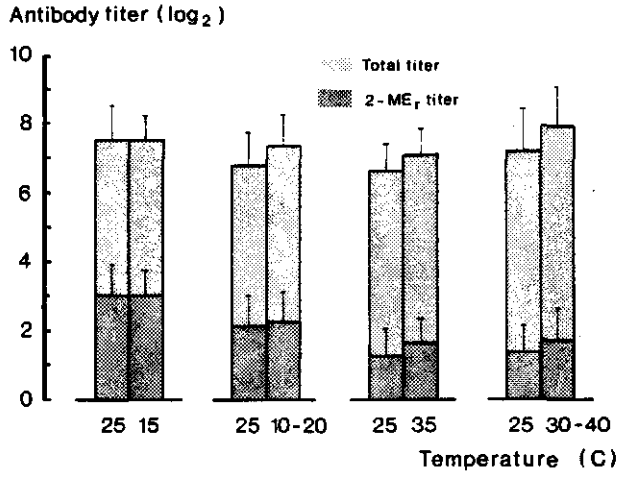

Fig. 1: Antibody titers of SRBC-immunized pullets at day 5 after injection at moderate high and low environmental temperatures (mean and SD)

Table 1. The increasing (+) or reducing (-) effects of exchange on total and 2-ME_r (between brackets) antibody titers at day 5 after injection compared to the antibody titers of acclimated pullets at each temperature (T)

Experiment	Differences in antibody titers at T = 25 C	Differences in antibody titers at T ≠ 25 C
25 vs 15 C	+ 0.04 (- 0.38)	- 0.41 (+ 0.02)
25 vs 10-20 C	- 0.17 (+ 0.44*)	- 0.12 (+ 0.12)
25 vs 35 C	- 0.52* (- 0.25)	- 0.55* (- 0.28)
25 vs 30-40 C	+ 0.85* (+ 0.52*)	- 0.37 (- 0.06)

* $P < 0.05$
** $P < 0.01$

In order to explain thermal influences on immune responsiveness one may consider which regulatory processes will be induced by changes in thermal conditions. Essentially, there are two general types of regulatory processes: specific and non-specific (Siegel, 1971 and 1980). In the following, this terminology of "specific" and "non-specific" is used in describing responses to changes in environmental temperature. Thus, specific responses refer to acclimation, while non-specific responses refer to a generalized effort of the animal to adapt itself irrespective of the particular environmental factor.

Specific responses to changes in environmental temperature are mediated by the hypothalamic-hypophyseal-thyroid axis (HHT-axis). In short, cold sensation activates the hypothalamus causing release of a thyrotropin releasing factor which passes down through the portal vessels to the adenohypophyse, where it stimulates the release of thyroid stimulating hormone (TSH). The TSH is transported by the blood stream to the thyroid gland. Here it promotes the synthesis and release of thyroxine and triiodothyronine, which elicit a progressive increase in metabolic rate. Another specific response may be the immediate and temporary rise of the plasma catecholamines (adrenaline and noradrenaline), mediated by the sympathetic nervous system innervating the adrenal medulla, and causing an immediate rise in metabolic rate. The autonomic nervous system as a whole is of great importance as control of heat loss by regulating peripheral blood flow, skin temperature and evaporation. However, the sympathetic nervous system can also considered to be a pathway of non-specific responses.

Non-specific responses to changes in environmental conditions are symptoms of activation of two systems: the sympathetic nervous system innervating the adrenal medulla (see above), and the hypothalamic-hypophyseal-adrenocortical axis (HHA-axis). The first is expressed by a rise of blood levels of catecholamines causing increases of blood glucose, blood pressure, muscle tone, nerve sensibility, respiration and pulse rate. The second is expressed by a rise of blood levels of corticosteroids due to increased synthesis and release by the adrenal cortex which is stimulated to do so by hypophyseal ACTH (adrenocorticotrophic hormone).

Effects of thermal conditions on immune responsiveness may depend on which regulatory processes, specific or non-specific, will prevail. When pullets were immunized immediately after changing of thermal environment, the humoral immune response tended to be reduced. This reduction may be caused by the immediate response of the sympathetic nervous system causing blood levels of catecholamines to rise. Besedovsky et al. (1979) investigated a possible immunoregulatory role for the autonomic nervous system. They reported that noradrenaline

strongly suppressed the in vitro induced immune response of murine spleen cells to SRBC, while local surgical denervation of the spleen in rats and general chemical sympathectomy combined with adrenalectomy increased the numbers of plaque forming spleencells. Pullets, acclimated to moderate high or low temperatures, had higher anti-SRBC antibody titers than pullets held at the standard temperature regimen of 25 C, which is reported to be near or at thermoneutrality (3rd and 6th paper). This indicates a positive relationship between immune responsiveness and metabolic rate which may be caused by the blood levels of the thyroid hormones. Thyroid and other growth-promoting and developmental hormones have been shown to influence immune responsiveness, although the mechanism of action is not understood (Pierpaoli and Sorkin, 1968; Baroni et al., 1969; Astaldi et al., 1972). It has been suggested that these hormones act through influencing intracellular concentrations of adenosine 3'5'-monophosphate (MacManus and Whitfield, 1969; Ishizuka et al., 1970; Rixon et al., 1970). The positive relationship between immune responsiveness and metabolic rate is further confirmed by the immuno-enhancing effect of restricted feeding (Table 2).

Table 2. The increasing (+) or reducing (-) effects of restricted feeding on total and 2-ME_r (between brackets) antibody titers at day 5 after injection compared to the antibody titers of ad lib.-fed pullets at each temperature (T)

Experiment	Differences in antibody titers at T = 25 C	T ≠ 25 C
25 vs 15 C	- 0.48 (- 0.50*)	+ 0.12 (+ 0.20)
25 vs 10-20 C	+ 0.48 (+ 0.45)	+ 0.15 (+ 0.65**)
25 vs 35 C	+ 0.16 (- 0.32)	+ 0.20 (+ 0.50*)
25 vs 30-40 C	+ 0.91** (+ 0.70**)	+ 0.51 (+ 0.37)

* $P < 0.05$
** $P < 0.01$

Thus, restricted feeding seems to accentuate effects of environmental temperature on the humoral immune response at nonthermoneutral conditions by increasing further the demand for thermoregulatory heat production. In order to investigate where, with respect to the humoral immune response, low temperature limits may

lie below which regressive effects of thermal conditions can be expected, we performed the experiments described in the fifth paper. Regressive effects can be expected if the induced regulatory response is mainly non-specific (HHA-axis). This may occur at extreme thermal conditions. The regressive effect is thought to result from the action of glucocorticosteroids which can pass the lymphocyte cell membrane and bind to cytoplasmic receptors to form a steroid-receptor complex which, if translocated to the nucleus, leads to altered rates of synthesis of specific RNA's and proteins (Thompson and Lippman, 1974; Gould and Siegel, 1980), resulting in cell destruction and reduced antibody production (Siegel, 1980). Total antibody titers in acclimated, restricted-fed, pullets tended to be reduced at extreme low temperatures (at 10 resp. 5-15 C) compared to titers at 25 C (Figure 2). 2-ME resistant titers were increased at 10 C and reduced at 5-15 C. If we compare these results with those reported in the 3rd paper, it can be concluded that the application of extreme temperatures in contrast with moderate temperatures does not increase the humoral immune response in acclimated pullets. An indication for a cold induced reduction of the humoral immune response can be found in the results at the extreme low fluctuating temperature (5-15 C).

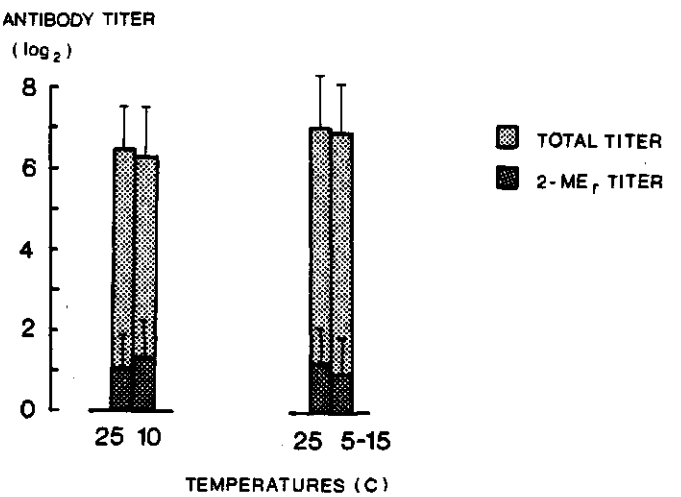

Fig. 2: Antibody titers of SRBC-immunized, restricted-fed, pullets at day 5 after injection at extreme low environmental temperatures (mean and SD)

In non-acclimated pullets even less extreme temperatures tended to reduce the humoral immune response. Thus, with respect to immune responsiveness, the degree of acclimation plays an important role. This role may be due to the change in basal metabolic rate (HHT-axis) in pullets which are held for a relatively long period at a certain temperature. In this way the demand for extrathermoregulatory heat production is decreased (Verstegen, 1971). If regressive effects on immune responsiveness are to be expected, a sudden temporary change in thermal conditions may be more important than the absolute temperature itself.

The results also indicate that it may be profitable to keep animals at less stringent regulated temperatures, although minima and maxima should be fixed at an appropriate level. Such fluctuating temperatures have no reducing effect on performance characteristics when compared to constant temperatures as is reported in the 4th paper. The results in the 6th paper show a diurnal rhythm in the lower critical temperature. Thus, a fluctuating temperature regimen can be used without increasing thermal demand for the animals and will lead to some reduction in energy costs.

The physiological results allow a rough estimation of the real values of B, C and E in Figure 3. These values are resp. about 6, 26 and 36 C for the pullets used in this research and were shown to depend on feeding level, degree of acclimation and time of day.

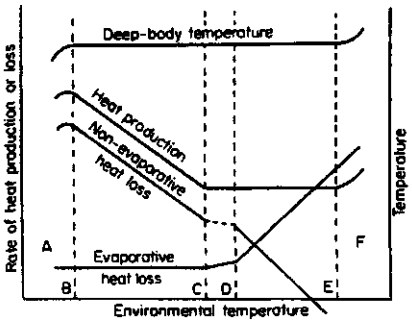

Fig. 3: Relationship between heat production, evaporative and non-evaporative heat loss and deep-body temperature in a strict homeotherm. A, zone of hypothermia whose border is defined by B; F, zone of hyperthermia whose border is defined by E; C, lower critical temperature; D, temperature of marked increase in evaporative loss (upper critical temperature); CD, zone of minimal thermoregulatory effort; CE, zone of minimal metabolism (after Mount, 1974)

The immunological results show that immune responsiveness follows a similar pattern as heat production. This means higher antibody titers if heat production is increased. In zone A and F, where deep-body temperature is affected, reduced antibody titers can be expected. The correlated response to temperatures of metabolic rate and immune responsiveness indicate that large differences between breeds in immune responsiveness at a certain temperature may exist, because values for B, C and E will be different.

It remains to be investigated whether thermal effects on the immune response are similar to thermal effects on disease resistance. Although differences in antibody titers between temperatures may appear to be small, the consequences of these differences for disease outbreaks and morbidity rates may be important. That applies especially to the enzootic diseases which result from unbalances between infection pressure and disease resistance (Oosterlee, 1978). In that respect more research is needed to effects on disease resistance of environmental temperatures which are near point B or E in Figure 3. At those temperatures thermal demand is increased to such extent that regressive effects on disease resistance can be expected. Research with challenge infections may be needed to study this.

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GENERAL ABSTRACT

The effect of environmental temperature on immune response and metabolism was studied in young chickens. Immunization was performed by injecting intramuscularly 0.5 ml packed SRBC (sheep red blood cells) in both thighs of 32 days old pullets (Warren SSL). The ensueing immune response was evaluated by determining haemagglutinin anti-SRBC antibody titers and the number of plaque forming spleencells. The pullets were kept in climate controlled respiration chambers allowing accurate regulation and maintenance of climatic conditions. Thermal demand of the imposed conditions was measured by determining metabolic rate and performance before as well as after immunization. The following three aspects were studied separately: 1. The effect of the immune response to SRBC on growth rate and energy gain; 2. The effect of environmental temperature on the immune response to SRBC; 3. The relation between thermal effects on the immune response to SRBC and energy metabolism.

The effect of the immune response to SRBC on metabolic rate and performance characteristics can only be accurately assessed if compared with an appropriate control-treatment. Because injection per se was shown to reduce heat production for at least 2 to 4 hr after injection, controls were sham-immunized with PBS (phosphate buffered saline). Feed intake, body weight, growth rate and metabolizability of gross energy were not significantly affected by SRBC-injection, but SRBC-injected pullets were eating more while gaining less than PBS-injected pullets. The magnitude and composition of energy gain were influenced by immunization. Immunized pullets retained significantly ($P < 0.01$) more energy, deposited significantly ($P < 0.05$) more fat and had a significantly ($P < 0.01$) lower derived maintenance requirement of metabolizable energy than sham-immunized pullets during day 1 to 5 after injection. During day 6 to 10 after injection, SRBC-injected pullets deposited significantly ($P < 0.05$) less fat than PBS-injected pullets. It remains to be investigated whether vaccinations have similar effects on metabolic rate and growth traits.

The effect of environmental temperature on the immune response to SRBC can only be described in terms of deviations from data gathered at a standard temperature. Such a standard temperature should be included in all research on this area, because significant differences in immunological data within temperature between experiments were found in all cases. In this research a 25 C environment was chosen as the standard control.

This standard temperature of 25 C was shown to be near or at the lower

critical temperature (T_{cr}) of the animals used in this research. The T_{cr} depended on feeding level and time of day. Restricted-fed (about 80% of ad lib. intake) pullets had a T_{cr} of 27.9 C. This is about 2 C above that of ad lib.-fed birds. However, the difference in thermoregulatory heat below and above T_{cr} in ad lib.-fed pullets was significant only if activity free heat production data were used. Below T_{cr} thermoregulatory heat (about 0.345 $W.kg^{-1}.C^{-1}$) seemed to be independent of feeding level. At night, with lights off, T_{cr} was about 3 C lower than the calculated 24-hr value. At daytime T_{cr} was above 30 C and could not be calculated.

The effect of moderate temperatures (about 10 C deviating from T_{cr}) on the immune response was investigated in 4 experiments, using 2 climate respiration chambers. In each experiment the standard temperature of constant 25 C was maintained in one chamber. The temperature regimens in the other chamber were as follows: a constant temperature of 15 or 35 C or a temperature, which fluctuated between 10 and 20 C (10-20 C) or between 30 and 40 C (30-40 C). Total antibody titers at day 5 after injection, the day of peak titers, were increased significantly ($P < 0.01$) at 10-20 C, 35 C and 30-40 C compared to the antibody titers at 25 C. 2-Mercapto-ethanol resistant antibody titers at day 5 after injection were increased significantly ($P < 0.05$) at 35 C and 30-40 C. Thermal effects on antibody titers at the other sampling days (day 0 and 10 after immunization) were not significant. The effect of feeding level and degree of acclimation on antibody titers was investigated within each temperature regimen. Restricted feeding at low or high temperatures increased antibody titers at day 5 after injection. Exchange of pullets, prior to immunization, to low or high temperatures decreased total antibody titers at day 5 after injection. With respect to the humoral immune response it can be concluded that, if regressive effects of environmental temperature are to be expected, a change in temperature at the moment of immunization may be more important than the absolute temperature itself.

More extreme low temperatures than the above mentioned, i.e. below 15 C, tended to reduce total antibody titers at day 5 after immunization in restricted-fed pullets. The investigated cold conditions were a constant temperature of 10 C and a temperature which fluctuated between 5-15 C. The results suggest that temperatures lower than the last mentioned may depress the immune response to SRBC if compared to the increase in immune response at moderate conditions.

In all experiments physiological data were gathered before as well as after immunization. At the moderate low temperatures feed conversion (g feed/g growth) was higher ($P < 0.05$) than at 25 C. Feed intake was increased by 12.9% (at 15 C)

and 10.5% (at 10-20 C) compared to intake at 25 C. Growth rate and protein gain were not significantly affected by low temperatures. High temperatures reduced ($P < 0.05$) feed intake (15.9% at 35 C and 14.9% at 30-40 C) and growth rate (12.3% at 35 C and 12.5% at 30-40 C) compared to 25 C. Protein gain and feed conversion were not significantly affected by high temperatures. The combined immunological and physiological data indicate that at moderate non-optimal, i.e. non-thermoneutral, conditions a positive relationship exists between immune responsiveness and metabolic rate. At extreme deviating temperatures this relationship may be reverted due to a prevalent non-specific, stress, response. The increased fat deposition found at such extreme temperatures may be an indication for the severity of the imposed conditions.

The results of this research indicate also that it is more important that appropriate minima and maxima are not trespassed than that the temperature is fixed. Such fluctuating temperatures have no reducing effect on performance characteristics and immune function if compared to constant temperatures. It remains to be investigated whether thermal effects on immune responsiveness are similar to thermal effects on disease resistance.

SAMENVATTING

Het effect van de omgevinstemperatuur op de immuunrespons en stofwisseling van kuikens werd onderzocht. Immuniseren werd uitgevoerd door intramusculair 0.5 ml packed SRBC (schape rode bloedcellen) in te spuiten in beide dijbenen van 32 dagen oude henkuikens (Warren SSL). De hoogte en het verloop van de immuunrespons werden beoordeeld op grond van de haemagglutinatie anti-SRBC antilichaam titers en het aantal plaque vormende miltcellen. De dieren waren gehuisvest in klimaatrespiratie-kamers. Hierdoor was het mogelijk de experimentele klimaatsomstandigheden nauwkeurig in te stellen en te handhaven. Op grond van warmteproduktie en balanskenmerken werd beoordeeld in hoeverre de gebruikte temperatuurregimes, van zowel vóór als na immunisatie, afweken van de optimale omstandigheden voor de dieren. De volgende drie aspecten werden bestudeerd: 1. Het effect van de immuunrespons tegen SRBC op groei en energie-aanzet; 2. Het effect van de omgevingstemperatuur op de immuunrespons tegen SRBC; 3. De relatie tussen temperatuurseffecten op de immuunrespons tegen SRBC en de energie stofwisseling.

Het effect van de immuunrespons tegen SRBC op groei, energie-aanzet en warmteproduktie kan slechts dan zuiver geschat worden als er een juiste controle behandeling tegenover staat. Omdat injectie op zich de warmteproduktie gedurende tenminste 2 à 4 uur na inspuiten bleek te verlagen, werden in dit onderzoek de controle dieren ingespoten met PBS (phosphate buffered saline). SRBC-immunisatie had geen significant effect op voeropname, lichaamsgewicht, groei en omzetbaarheid van de bruto energie, maar geïmmuniseerde dieren namen meer voer op terwijl ze minder hard groeiden dan met PBS ingespoten dieren. Immunisatie had wel effect op de hoogte en de samenstelling van de energie-aanzet. Geïmmuniseerde dieren hadden een significant hogere energie- en vet-aanzet ($P < 0.01$ resp. $P < 0.05$) en een significant ($P < 0.01$) lagere geschatte onderhoudsbehoefte aan omzetbare energie dan controle dieren gedurende dag 1 tot 5 na inspuiten. Gedurende dag 6 tot 10 na inspuiten hadden geïmmuniseerde dieren een significant ($P < 0.05$) lagere vet-aanzet dan controle dieren. De vraag of vaccinaties vergelijkbare effecten hebben op warmteproduktie en groeisamenstelling kan op dit moment niet beantwoord worden.

Het effect van de omgevingstemperatuur op de immuunrespons tegen SRBC kan alleen weergegeven worden in termen van afwijkingen van resultaten verkregen bij een standaard temperatuur. Dat een dergelijke standaard temperatuur meegenomen moet worden in al het onderzoek op dit gebied, wordt ondersteund door het feit dat er in alle gevallen significante verschillen waren tussen experimenten wat

de immunologische resultaten betreft van dieren gehouden bij eenzelfde temperatuur. In dit onderzoek werd een constante temperatuur van 25 C gebruikt als standaard controle.

Deze standaard temperatuur van 25 C ligt bij of op de onderste kritieke temperatuur (T_{cr}) van de in dit onderzoek gebruikte dieren. De T_{cr} was afhankelijk van voerniveau en tijdstip van de dag. Beperkt gevoerde (ongeveer 80% van de ad lib. opname) dieren hadden een T_{cr} van 27.9 C. Dat is ongeveer 2 C hoger dan de T_{cr} van ad lib. gevoerde dieren. Het verschil in thermoregulatoire warmteproduktie beneden en boven T_{cr} bij ad lib. opname was echter alleen significant als aktiviteitsvrije warmteproduktie gegevens werden gebruikt. De thermoregulatoire warmteproduktie beneden T_{cr} (ongeveer 0.345 $W.kg^{-1}.C^{-1}$) bleek onafhankelijk van het voerniveau te zijn. 's Nachts, met de lichten uit, was de T_{cr} ongeveer 3 C lager dan het gemiddelde over 24 uur. Overdag was de T_{cr} boven de 30 C en kon niet berekend worden.

Het effect van gematigde temperaturen (ongeveer 10 C afwijkend van T_{cr}) op de immuunrespons is onderzocht in 4 experimenten met gebruikmaking van 2 klimaatrespiratie-kamers. In elk experiment werd in één kamer de standaard temperatuur van constant 25 C aangehouden. De temperatuurregimes in de andere kamer waren als volgt: een constante temperatuur van 15 of 35 C, of een temperatuur die schommelde tussen de 10 en 20 C (10-20 C) of tussen de 30 en 40 C (30-40 C). De totaal titers op dag 5 na immunisatie, de dag met de hoogste titers, waren bij 10-20 C, 35 C en 30-40 C significant ($P < 0.01$) hoger dan de titers bij 25 C. de 2-mercaptoethanol resistente titers op dag 5 na immunisatie waren bij 35 C en 30-40 C significant ($P < 0.05$) verhoogd ten opzichte van die bij 25 C. Op de andere meetdagen (dag 0 en 10 na immunisatie) waren er geen significante temperatuurseffecten op de antilichaam titers. Het effect van voerniveau en mate van acclimatie op de antilichaam titers is onderzocht binnen elk temperatuurregime. Beperkt voeren bij lage of hoge temperaturen verhoogde de antilichaam titers op dag 5 na immunisatie. Overplaatsen van de dieren, kort voor immunisatie, naar lage of hoge temperaturen verlaagde de totaal titers op dag 5 na immunisatie. Met betrekking tot de humorale immuunrespons kan geconcludeerd worden dat negatieve temperatuurseffecten eerder te verwachten zijn bij een verandering van temperatuur op het moment van immuniseren dan bij een bepaalde hoogte van de temperatuur als zodanig.

Bij temperaturen beneden de 15 C waren de totaal titers, op dag 5 na immunisatie, van beperkt gevoerde dieren iets verlaagd. Dit bleek uit 2 experimenten waarin het effect van meer extreme koude (10 resp. 5-15 C) op de immuunrespons is onderzocht. Deze resultaten doen veronderstellen dat nog lagere temperaturen

de immuunrespons zullen verlagen in plaats van verhogen zoals bij gematigde temperaturen.

In alle experimenten werden zowel vóór als na immunisatie fysiologische gegevens verzameld. Bij de gematigd lage temperaturen was de voederconversie (g voer/g groei) hoger ($P < 0.05$) dan bij 25 C. De voeropname was verhoogd met 12.9 (bij 15 C) resp. 10.5% (bij 10-20 C) ten opzichte van de opname bij 25 C. Er was geen significant effect van de lage temperaturen op groei en eiwit-aanzet. Bij de hoge temperaturen namen de dieren minder ($P < 0.05$) voer op (-15.9% bij 35 C and -14.9% bij 30-40 C) en groeiden minder ($P < 0.05$) snel (-12.3% bij 35 C en -12.5% bij 30-40 C) dan die bij 25 C. De eiwit-aanzet en voederconversie waren niet significant beïnvloed bij de hoge temperaturen. De immunologische en fysiologische resultaten tezamen suggereren een positieve relatie tussen de immuunrespons en de warmteproduktie bij gematigde niet-optimale, i.e. niet-thermoneutrale, condities. Bij extreem afwijkende temperaturen zal deze relatie echter een negatieve zijn door een overwegend niet-specifieke, stress, reactie. De verhoogde vet-aanzet bij dergelijke extreme temperaturen kan een aanwijzing zijn voor de ernst van de temperatuursbelasting.

De resultaten van dit onderzoek tonen ook aan dat het belangrijker is dat bepaalde minima en maxima niet overschreden worden dan dat de temperatuur constant is. Een schommelende temperatuur heeft, vergeleken met een constante temperatuur, geen nadelig effect op produktiekenmerken en immuunrespons. De vraag of temperatuurseffecten op de immuunrespons vergelijkbaar zijn met die op het weerstandsvermogen tegen infectie-ziekten kan op dit moment niet beantwoord worden, maar verdient in de toekomst zeker aandacht.

Curriculum vitae

André Marthin Henken is op 1 januari 1953 te Utrecht geboren. Hij behaalde het diploma Gymnasium β aan het Revius Lyceum te Doorn in 1972. In datzelfde jaar begon hij zijn studie in de richting Zoötechniek aan de Landbouwhogeschool te Wageningen. In 1979 werd deze studie afgesloten met als hoofdvakken de Gezondheids- en Ziekteleer der Huisdieren en de Fysiologie der Huisdieren en als bijvak de Veeteelt. Na zijn afstuderen werd hij voor een periode van drie jaar aangesteld als promotie-assistent bij de vakgroep Veehouderij van de Landbouwhogeschool. Sinds 1 september 1982 is hij als wetenschappelijk medewerker verbonden aan de vakgroep Algemene Visteelt en Visserij van de Landbouwhogeschool.