Residual feed intake in young chickens:

Effects on energy partitioning and immunity

Promotoren:

Prof. dr. ir. B. Kemp

Hoogleraar Adaptatiefysiologie

Wageningen Universiteit

Prof. dr. ir. M. C. M. de Jong

Hoogleraar Kwantitatieve Veterinaire Epidemiologie

Wageningen Universiteit

Co-promotor:

Dr. ir. H. van den Brand

Universitair docent, leerstoelgroep Adaptatiefysiologie

Wageningen Universiteit

Promotiecommissie:

Prof. dr. J. Buyse

Universiteit van Leuven, België

Dr. P.-A. Géraert

Adisseo France SAS, Frankrijk

Prof. dr. R. F. Hoekstra

Wageningen Universiteit

Prof. dr. ir. M. W. A. Verstegen

Wageningen Universiteit

Dit onderzoek is uitgevoerd binnen de onderzoeksschool WIAS

(Wageningen Institute of Animal Sciences)

Residual feed intake in young chickens:

Effects on energy partitioning and immunity

Ellen van Eerden

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M. J. Kropff,
in het openbaar te verdedigen
op vrijdag 16 februari 2007
des namiddags om half twee in de Aula

Residual feed intake in young chickens: Effects on energy partitioning and immunity

Eerden, E. van, 2007
Ph.D. thesis, Wageningen University, The Netherlands.
With ref. – with summary in English and Dutch – 168 pp.
ISBN: 90-8504-593-2

Department of Animal Sciences, Adaptation Physiology Group,
Wageningen Institute of Animal Sciences, Wageningen University,
PO Box 338, 6700 AH Wageningen,
The Netherlands.

Current e-mail address: E.vanEerden@iras.uu.nl

ABSTRACT

The continuous selection in farm animals for efficient production and high production levels may have led to animals that are "programmed" to put a lot of resources in production processes, at the expense of resources for maintenance processes, among which the immune system. When efficiently and non-efficiently producing animals in a population are discriminated, it is hypothesized that non-efficient animals are better able to reallocate resources from production processes to maintenance processes than efficient animals. Non-efficient animals may, thus, be better off than efficient animals when maintenance processes are under pressure.

Residual feed intake is used as a trait to discriminate efficient and non-efficient animals. It is defined as the difference between observed feed intake and expected feed intake; in this thesis, expected feed intake is based on metabolic body weight and growth. Animals that eat more than expected have a high residual feed intake and are considered non-efficient, whereas animals that eat less than expected have a low residual feed intake and are considered efficient.

The research described in this thesis was carried out with pullets. Pullets are young, growing female chickens that do not produce eggs yet. Pullets were rated from high to low residual feed intake as a phenotypic trait; animals with the highest and lowest values for residual feed intake were selected for further research. Immune responses to non-replicating antigens and to live *Salmonella* Enteritidis bacteria were investigated.

The results showed that non-efficient pullets had a higher feed intake than efficient pullets, but body weight and growth were equal in efficient and non-efficient pullets. Energy partitioning trials showed that non-efficient pullets spent more energy on maintenance processes than efficient pullets. However, an infection with *Salmonella* Enteritidis did not lead to repartitioning of energy from production processes to maintenance processes. It was concluded that a *Salmonella* Enteritidis infection is not energetically costly. Efficient pullets had a lower immune status than non-efficient pullets in situations where the animals were not infected with *Salmonella* Enteritidis, whereas during a *Salmonella* Enteritidis infection the efficient pullets had higher immune responses than non-efficient pullets. It is suggested that efficient and non-efficient pullets, as measured by residual feed intake, may have different "immune coping styles".

Keywords: chicken, residual feed intake, resource allocation, immune response, *Salmonella* Enteritidis, energy partitioning.

Voor Ties en Maud

Laat nooit een dag voorbij gaan
zonder iets te leren

CONTENTS

CHAPTER 1

General Introduction

1.1 INTRODUCTION

Fitness is the relative contribution of a genotype to the next generation, and is expressed as the ability to survive and produce offspring that is "fit" enough to survive and reproduce. Fitness, as described by Beilharz et al. (1993), is composed of several fitness components that all need a certain amount of resources to contribute to total fitness. If resources are not limiting, fitness components can reach optimal values, which in combination will lead to maximal fitness. However, if resources are limiting, resources are divided in such a way that fitness components reach intermediate optimal values. In this situation, when resource demands for one component increase, resources for other components are cut down. Fitness components will then deviate from their intermediate optimal values, which will result in fitness that is lower than maximum (Beilharz et al., 1993).

Beilharz's description of fitness implicitly deals with natural selection; moreover, it also implies that reallocation of resources is flexible, and that all fitness components have equal priorities. However, artificial selection, as in farm animals, may drastically change his assumptions. When animals are selected for a particular (production) trait, priorities for this trait increase. Animals, thus, may be genetically "programmed" to put more resources in this trait, which results in fewer resources to other traits (Dunnington, 1990). This situation becomes apparent as negative correlations between traits.

Over the past decades, animals were selected for high production levels, such as high milk yield, high egg mass production, high growth rate, and high reproduction rate. Moreover, animals should not only reach high production levels, but they also had to be very efficient in reaching these levels. One may argue that the single focus from animal breeders and farmers on production traits has led to negligence of maintenance processes, among which the immune system as an important representative. Following this reasoning, animals selected for efficient production spend many resources on production processes and have few resources left for maintenance processes. Conversely, non-efficient animals spend relatively fewer resources on production processes and have relatively more resources available for maintenance processes. It is thus hypothesized that non-efficient animals are better capable of dealing with situations in which maintenance processes are affected.

To be able to address this issue, it was necessary to adopt a model, in which efficient and non-efficient animals can be discriminated. Moreover, it was necessary to have a selection trait with relatively high priority to the animal, in combination with a challenge that affects maintenance processes. In this situation, the animal would really have to choose how

to allocate its resources between the (production) trait it was selected for, and the maintenance processes that are under pressure. A selection trait that was suitable was residual feed intake (RFI). Selection for high or low residual feed intake (i.e. selection for non-efficient and efficient animals, respectively) uses feed intake, body weight and production traits in a direct selection. The residual feed intake trait is better than feed conversion ratio, because the latter merely uses feed intake and production traits, whereas RFI takes also maintenance costs into account, by including (metabolic) body weight. RFI is well known in poultry and (beef) cattle research. Considering a phenotypic selection for high and low-efficient animals from a large population, cattle would not be useful due to the large numbers of (expensive) animals necessary. Moreover, because the animals would have to be weighed frequently, it was necessary to have an animal that can be handled easily. Therefore, the study object was chosen to be the chicken, in particular the pullet. Pullets are young, growing, non-laying hens. Pullets were an optimum of having an animal that has priority for a production trait (i.e. growth), and practical considerations of avoiding a short production period, as in broilers.

Salmonella Enteritidis was chosen as a model to put a pressure on maintenance processes, in particular on the immune system. Infection with this bacterium should force chickens to choose between allocation of resources towards growth and allocation towards the immune system. After inoculation, *Salmonella* colonizes the gut and starts replicating. The subsequent shedding of *Salmonella* bacteria in the feces is a characteristic that is studied in this thesis, as well as humoral and cellular immune responses. *Salmonella* Enteritidis usually does not cause mortality in chickens older than two weeks. Therefore, S. Enteritidis is a suitable stressor, because for the purpose of studying allocation processes, mortality as a result of the immune challenge should be avoided. Energy partitioning experiments are carried out to study the effects of a bacterial infection on maintenance and production processes. The combined results are used to investigate the hypothesis that non-efficient animals have more resources available to be spent on maintenance processes, and will, thus, be better capable of dealing with an infectious challenge.

In the remaining part of this chapter differences in physiological properties of animals selected for high or low RFI are briefly reviewed (paragraph 1.2), as well as (immunological) characteristics of *Salmonella* in general and of *Salmonella* Enteritidis in poultry in particular (paragraph 1.3**).** A quick overview of differences between animals with a high or low RFI can be obtained from Table 1.2 in paragraph 1.2.8. The outline of the thesis is described in paragraph 1.4.

1.2 RESIDUAL FEED INTAKE

1.2.1 Selection

In the laying industry, efficient egg production, i.e. low feed costs and high egg output, was achieved by selecting for a high feed efficiency. High feed efficiency can be obtained indirectly by selecting chickens with a low adult body weight and a high egg mass output (Nordskog et al., 1972). A low body weight is related to a low maintenance need, which is expressed as the amount of feed needed to maintain 1 kg of metabolic body weight (body weight$^{0.75}$). Thus, low maintenance needs lead to lower feed costs. However, lighter chickens lay smaller eggs. Increasing body size results in increased egg size, but also in higher maintenance needs. Moreover, if maintenance costs increase with body weight$^{0.75}$, egg size must also increase with the same power to obtain equal efficiency. As egg size seems to increase only with body weight$^{0.15}$, higher egg production efficiency can be obtained by reducing body weight (Nordskog et al., 1972).

The concept of RFI was introduced by Koch et al. (1963) for cattle, and was defined as a residual portion of feed intake that was not accounted for by body weight and weight gain. In chickens, RFI became a trait of interest because it was found that there was variation in feed intake that was not attributable to differences in body weight and egg production (Bordas and Merat, 1974), and because of the possibility that selection limits for egg mass production or feed intake capacity would occur (Bentsen, 1983). RFI, thus, offered the possibility of discovering other sources of variation than differences in body weight and egg production (see also Nordskog et al., 1972). Moreover, feed efficiency is a ratio (feed intake/egg mass output), and using this ratio as a selection criterion may complicate the desired outcome, due to an antagonism between the desired responses in feed intake and egg mass production (Luiting, 1990). Therefore, RFI could be a new way of improving efficiency for egg production.

Basically, RFI is calculated as the difference between observed and predicted feed intake. There are several variables that predict feed intake, but feed intake is largely accounted for by body weight, changes in body weight and egg mass production (Bentsen, 1983; Bordas and Merat, 1981; Nordskog et al., 1972). Therefore, RFI is calculated as feed intake adjusted for body weight, body weight changes and egg mass production, using a multiple linear regression method (Bentsen, 1983; Bordas and Merat, 1981; Luiting and Urff, 1991; Nordskog et al., 1972). To get a linear relationship between body weight and feed intake, body weight should be transformed to metabolic body weight (Bentsen, 1983). The

residual error from the regression is calculated for each animal and represents RFI. RFI can have positive (R+) or negative (R-) values, for low-efficient or high-efficient animals, respectively. By definition, the average RFI in an experimental flock is equal to zero (Bentsen, 1983).

Some attempts were made to improve the model by adjusting body weight change for the direction of change (gain or loss) (Bentsen, 1983; Luiting and Urff, 1991), adjusting egg mass production for egg composition or age at first egg (Bentsen, 1983; Luiting and Urff, 1991), or heat production (Herremans et al., 1989), because it was assumed that feed intake would be affected by these variables. However, age at first egg and egg composition did not offer additional information about the total feed intake (Katle and Kolstad, 1991). Also adjusting for body weight gain or loss was shown not improve the fit of the model (Bentsen, 1983; Luiting and Urff, 1991). Therefore, in later studies these variables were no longer included in the model.

Adjusting for heat production had a slightly different approach. The traditional models for feed intake prediction are discussed in terms of grams, but feed intake can also be described in terms of energy (kJ): gross energy (GE) intake is partly lost as energy in feces and urine, and the remaining part is used as metabolizable energy (ME), which in turn is converted to product (net energy for body weight gain or eggs) and heat. When comparing energy intake and partitioning to the "traditional" RFI model in grams of feed as described above, it is shown that retained energy as product is covered by body weight change and egg mass production. However, heat production is covered solely by (metabolic) body weight, which does not take into account heat loss due to low environmental temperatures or poor insulation. It was shown that models for feed intake prediction improved when predicted heat production, calculated from an expression using environmental temperature and defeathering scores (Herremans et al., 1989) was included, but this was mainly true when environmental temperature was low or feathering was poor.

A lot of knowledge about RFI was obtained from experiments carried out with genetic selection lines that used RFI as a selection criterion. In France, divergent selection for RFI was started in 1976 in a Rhode Island Red population (Bordas and Merat, 1981). Similar selection lines were developed in White Leghorn populations in Norway in 1982 (Bentsen, 1983; Katle and Kolstad, 1991) and in The Netherlands in 1985 (Luiting et al., 1991b). The procedures by which the chickens were selected were basically the same. All groups used a feed intake prediction model with metabolic body weight, body weight change and egg mass production. The Norwegian group made a slight adjustment to RFI, and used percentage RFI

(PRFI), defined as RFI divided by expected feed intake (Katle and Kolstad, 1991). PRFI rather than RFI was used, because PRFI was thought to describe better the relative efficiency of the animal (Bentsen, 1983), without discriminating against animals with relatively low feed intake due to low BW or relatively high feed intake due to high egg mass output (Katle and Kolstad, 1991).

Continuous selection has led to substantial differences in feed intake and residual feed intake, which are summarized in Table 1.1.

TABLE 1.1 Mean feed intake (g/d) and residual feed intake (g/d) of chickens selected for low (R-) or high (R+) residual feed intake

Ref.	♀/♂	generation	Feed intake (g/d)				Residual feed intake (g/d)		Selection age
			R-	n	R+	n	R-	R+	
1.	♀	parent	93.5	30	107.3	32	-3.6	7.5	8-11 months
2.	♀	5	107.3	128	122.1	127	-3.6	3.6	33-37 weeks
	♀	10	94.7	161	119.1	102	-4.7	7.5	"
	♀	14	104.8	147	129.5	151	-10.5	10.2	"
	♂	5	87.0	37	96.8	32	-6.0	7.0	"
	♂	10	96.7	40	127.5	36	-15.9	17.7	"
	♂	14	88.7	39	133.1	37	-13.8	14.5	"
3.	♀ adult	10	102.7	156	125.4	161	-7.9	7.9	ns[c]
	♂ adult	10	86.0	40	118.3	40	-16.2	16.2	ns
	♂ young[a]	10	66.6	30	59.5	29	ns[c]	ns[c]	ns
4.	♀	17	102.2	12	141.0	12	-14.4	19.8	33-37 weeks
	♂	17	89.3	12	124.0	12	-18.2	16.7	"
5.	♀	17	ns[c]	8	ns	8	-10.0	10.2	32-36 weeks
	♀	17	ns	5	ns	5	-11.9	18.4	"
	♀	17	ns	5	ns	5	-10.1	9.1	"
6.	♀	18	91.4	49	119.7	50	-17.7	17.4	4-34 weeks
7.	♂	18	72.0	9	135.6	10	-26.6	31.1	ns
8.	♀control[b]	19	96.9	48	126.5	48	-10.7	12.1	31-35 weeks
	♀ heat[b]	19	83.1	48	105.1	48	-9.1	7.8	"
9.	♀	phenotypic	98.7	6	115.4	6	-5.7	9.4	56-60 weeks
	♀	phenotypic	84.5	5	109.2	5	-10.0	8.2	44-48 weeks

References:

1: Merat et al., 1980	4. El Kazzi et al., 1995	7. Morisson et al., 1997
2. Bordas et al., 1992	5. Gabarrou et al., 1998	8. Bordas and Minvielle, 1997
3. Tixier et al., 1988	6. Bordas and Minvielle, 1999	9. Luiting et al., 1991a

[a] Feed intake of these male chickens was measured from 35 until 61 days of age.

[b] These data from Bordas and Minvielle (1997) are derived from an experiment in which the chickens were kept at 21ºC (control group) or at 31ºC ('heat' group) from 18 until 48 weeks of age.

[c] ns: not specified.

Differences between R+ and R- may appear to increase with generations; however, it must be noted that these data mainly come from experiments, in which sometimes only a small number of animals was chosen from the population. Except for Bordas et al. (1992), the data in Table 1.1 do not represent the entire selection lines. Therefore, differences between R+ and R- must be interpreted carefully.

Table 1.1 shows that the age of selection was in most cases beyond 30 weeks of age. It follows that the animals had then reached an adult stage. Because male and female chickens were selected at the same age, it must be concluded that RFI as a selection criterion is different for males and females and relies upon a different biological basis. In males, feed intake (and RFI) is only related to body weight and body weight changes, thus representing mainly maintenance, whereas feed intake (and RFI) in females is also related to egg production (Tixier et al., 1988).

1.2.2 Metabolism and heat production

R+ chickens are characterized by a higher feed intake than R- chickens, expressed in grams (Gabarrou et al., 1998) or in terms of energy. R+ chickens, thus, have a higher gross energy (GE) intake (Geraert et al., 1991; Luiting et al., 1991a). Metabolizability (metabolizable energy/gross energy ratio), however, appeared to be similar for R+ and R- chickens (Gabarrou et al., 1998; Geraert et al., 1991; Luiting et al., 1991a; Luiting et al., 1991b). Therefore, R+ chickens have a higher metabolizable energy (ME) intake (Geraert et al., 1991; Luiting et al., 1991a), and differences in ME intake are proportional to differences in GE intake (Luiting et al., 1991a). As the production traits body weight gain and egg production are not significantly different between the lines, and assuming that efficiencies for production traits are not different between the lines**,** the difference in ME intake reflects a difference in maintenance expenditures: R+ chickens have a higher ME for maintenance (ME_m) (Geraert et al., 1991).

Because energy spent on maintenance is completely converted to heat, R+ and R- chickens will, thus, differ in heat production. Indeed it was shown that R+ chickens had a higher heat production (Katle, 1991), but this was only the case when the chickens were in a fed state (Gabarrou et al., 1998; Gabarrou et al., 1997; Luiting et al., 1991a). Heat production and basal metabolic rate in a fasted state or in the dark period were not significantly different between R+ and R- chickens (Gabarrou et al., 1998; Gabarrou et al., 1997; Geraert et al., 1991). It suggests that the difference in heat production is related to an increased diet-induced thermogenesis (DIT) in R+ chickens. R+ chickens were indeed shown to have a higher DIT (Gabarrou et al., 1998; Gabarrou et al., 1997; Geraert et al., 1991). DIT is heat production due to an increased basal metabolic rate after having eaten a meal. DIT is a result of sympathetic nervous stimulation of brown adipose tissue in mammals (Gabarrou et al., 1997), and can be divided in an obligatory component (due to digestion, absorption, processing and storage of nutrients) and a regulatory component (Gabarrou et al., 1997). This regulatory part is shown in mammals to be important in maintaining body weight after an increase in feed intake, and acts through β-adrenergic stimulation of brown adipose tissue (Bachman et al., 2002).

However, although birds have no brown adipose tissue (Saarela et al., 1991), treatment with DL-propranolol was shown to decrease heat production only in R+ birds, resulting in a similar heat production in fed R+ and R- chickens (Gabarrou et al., 1997). DL-propranolol is a β-antagonist that inhibits thermogenesis induced by β-adrenergic activity. The results from Gabarrou et al. (1997) show that R+ chickens lose more heat than required for obligatory thermogenesis, through a regulatory thermogenesis under sympathetic control, by which the excess energy that was taken in, is lost (Gabarrou et al., 1998).

1.2.3 Physical/physiological characteristics

In this section, the focus will be on differences in physical and physiological characteristics between genetically selected R+ and R- chickens. Divergent selection for RFI resulted in a number of direct (RFI, feed intake, body weight and feed efficiency) and indirect correlated selection responses (age at first egg, rectal and comb temperature, and shank – i.e. tarsometatarsus - and wattle length) (Bordas et al., 1992). Based on genetic correlations with RFI, the most important correlated responses were wattle and shank length, and feed intake (Tixier Boichard et al., 1995), and these will be discussed first.

Traits such as shank and wattle length, but also rectal and comb temperature, are clearly related to thermoregulatory mechanisms, which in turn are related to the established difference in heat production. Although Luiting et al. (1991b) found no differences in shank

surface, there is considerable agreement among several other reports that R+ chickens have larger appendages, i.e. combs and wattles, and larger unfeathered areas, such as shanks, that all help in getting rid of heat (Bordas and Minvielle, 1997; Bordas et al., 1992; Tixier et al., 1988). Conversely, R- chickens were better insulated due to a better plumage quality than R+ chickens (Braastad and Katle, 1989; Katle, 1991).

Although selection for RFI was performed in adult chickens (beyond 30 weeks of age), it appears that divergences of RFI and the correlated responses become already apparent at a younger age. Divergence for RFI was established at 14 and 12 weeks for males and females, respectively (Bordas and Minvielle, 1999). Also shank length in females diverged significantly from 14 weeks of age onwards, whereas in males divergence was not established until 22 weeks of age (Bordas and Minvielle, 1999). Divergence of wattle length in females started at 18 weeks of age (Bordas and Minvielle, 1999), whereas wattle length in males was similar until 20 weeks of age, before divergence started. It is hypothesized that divergence for RFI takes place in two steps, one at an early age, associated with divergence in shank length, and one around maturity, associated with divergence in feed intake and wattle length (Bordas and Minvielle, 1999). It was suggested that selection for RFI may have had a direct impact on morphological traits, and a secondary action on feed intake (Bordas and Minvielle, 1999).

Feed intake was similar for R+ and R- chickens until 16 weeks of age, but then abruptly diverged. In females, feed intake in R+ chickens continued to increase, probably due to the onset of laying eggs, whereas feed intake in R- chickens slightly decreased (Bordas and Minvielle, 1999). In males, feed intake decreased from 16 weeks of age onwards, but remained higher in R+ than in R- chickens (Bordas and Minvielle, 1999).

With respect to rectal temperature, Bordas et al. (1992) and Tixier et al. (1988) report higher values for R+ chickens, but Gabarrou et al. (1997), Katle (1991) and Luiting et al. (1991b) found that rectal temperatures were not different between R+ and R- chickens. Bordas et al. (1992) and Tixier et al. (1988) also report that comb temperatures are higher in R+ chickens; Gabarrou et al. (1997) found a similar effect only in fed R+ chickens and not in feed-deprived chickens. Katle (1991) found no significant differences in comb temperature. Therefore, effects on rectal and comb temperature are not conclusive.

Body weight was also affected as a result of selection for RFI. Although efficiency for egg production can be improved by selection for lower body weights, it was demonstrated that the efficient R- hens were significantly heavier than R+ hens after several generations of selection for RFI (Bordas and Minvielle, 1999; Braastad and Katle, 1989; Katle and Kolstad,

1991). Similar effects were found in cockerels (Katle and Kolstad, 1991; Tixier et al., 1988); although in Tixier et al. (1988) the opposite effect was found for adult females.

Carcass composition also differed between R+ and R- chickens: R+ chickens had heavier wings, bones, and legs, heavier leg muscles, and more blood, but there were no differences in feathers, viscera, and breast meat (El Kazzi et al., 1995; Zein-el-Dein et al., 1985). R+ chickens had also a heavier gastro-intestinal tract and heart (Zein-el-Dein et al., 1985). Proportional liver weight was significantly lower in R- cockerels (Tixier et al., 1988). The most striking difference in carcass composition is the difference in adiposity. R- chickens had more adipose tissue than R+ chickens (El Kazzi et al., 1995; Katle, 1991; Tixier et al., 1988; Zein-el-Dein et al., 1985), both as carcass lipid and as abdominal fat. Also plasma triglyceride levels (in males; Tixier et al. (1988)) and liver lipid (in females; El-Kazzi et al. (1995)) were higher in R- chickens. The difference in adiposity may be explained by an increased thermogenesis in R+ chickens, which leaves less energy for lipogenesis (El Kazzi et al., 1995). It is again important to note that divergence in adiposity was already observed around 9 weeks of age (Tixier et al., 1988), even though divergence of feed intake was not observed until around 18 weeks of age (Bordas and Minvielle, 1999). Therefore, lipogenesis is possibly directly affected by selection for RFI (El Kazzi et al., 1995), instead of indirectly affected through an increased thermogenesis.

Additionally, the lipid turnover in R+ chickens may be higher than in R- chickens (Gabarrou et al., 1998). From regressions of the respiratory quotient against time it was shown that R+ chickens had higher positive slopes – indicating lipid synthesis- right after (tube-) feeding and higher negative slopes – indicating lipid degradation- several hours after (tube-) feeding than R- chickens. Gabarrou et al. (1998), thus, suggests that part of the higher heat production in R+ chickens is a result of energy that is stored momentarily as lipids that are catabolized fast.

1.2.4 Behavior

It was shown that physical activity was higher in R+ birds with unlimited access to feed. However, when chickens were tube-fed, total activity expenditure was reduced, but even then it remained higher in R+ than in R- chickens (Gabarrou et al., 1997). In ad lib fed chickens, the time spent on eating was similar for R+ and R- chickens (Gabarrou et al., 1998; Gabarrou et al., 1997), but there appeared to be a difference between sexes for number of meals and meal size. R+ hens ate more frequently but shorter than R- hens (Gabarrou et al.,

1998), whereas R+ cockerels had a similar number of meals as R- cockerels, but meal size was higher than in R- cockerels (Gabarrou et al., 1997).

However, it is not only higher feeding activity due to higher feed intake that explains the higher total activity expenditure in R+ chickens; also differences in behavioral patterns play a role. Videograms of behavioral patterns showed that R+ chickens spent less time being inactive and that they were more agitated. Agitation was divided in walking, flight, and aggressive behavior; these types of behavior were all exhibited significantly longer in R+ chickens (Braastad and Katle, 1989). It is noteworthy that extreme pacing, as part of agitated behavior, was expressed only in R+ chickens, particularly during the pre-laying period. In an attempt to calculate the energy costs of activity, it was estimated that R+ chickens used 15-16% more energy for activity than R- chickens. It was suggested that a given activity bout in R+ chickens requires more energy than in R- chickens, possibly due to activity breaking the insulation layer, or a poorer plumage condition. Moreover, in this study only the time spent on behavioral activities was recorded, not the intensity of the activity (Braastad and Katle, 1989).

1.2.5 Egg production and reproduction

Correlated responses were also observed for egg production and reproduction. The age at first egg decreased in R+ as well as in R- chickens in the course of selection, but age at first egg remained higher in R+ chickens (Bordas et al., 1992; Katle and Kolstad, 1991 – compared to one of the R- lines). Egg mass production was not significantly different for R+ and R- hens, but was slightly higher in R- hens (Bordas and Minvielle, 1999). Moreover, egg mass production in R+ hens decreased during selection (Katle and Kolstad, 1991), although mean egg weight was slightly higher in R+ than in R- hens (Bordas et al., 1992; Katle, 1991 – in the third generation; Merat et al., 1980). RFI was slightly negatively associated with egg shell thickness and positively correlated with proportion cracked eggs (Bordas and Merat, 1981). Chemical composition of eggs was similar for R+ and R- hens (Gabarrou et al., 1998; Luiting et al., 1991a), although Katle (1991) reported higher yolk content in eggs from R+ hens. It seems that selection for RFI in general has a negative effect on egg production traits. However, in warm conditions R+ chickens were better able to sustain egg production, probably because R+ chickens are better capable of losing heat. In warm conditions, egg production was reduced in R+ as well as in R- chickens, but in R+ chickens to a lesser extent (Bordas and Minvielle, 1997).

As a correlated response of selection for RFI, reproduction was also affected. It was noticed that the R+ and R- line differed with respect to fertility and hatchability, already since

the third generation of selection (Bordas and Merat, 1993), with R+ chickens having a lower hatching rate (Bordas et al., 1992). The lower hatchability in R+ chickens was due to more unfertilized eggs and more early embryonic death, which was observed as death at day 5 of incubation (Bordas and Merat, 1993; Morisson et al., 1997). These results suggest that it is probably not only decreased egg quality, but also decreased semen quality that caused the lower hatchability in R+ chickens. Although ejaculate volume was similar in R+ and R- cocks, spermatozoa concentration and motility were lower in R+ cocks (Morisson et al., 1997). Moreover, the rate of dead spermatozoa was higher in R+ cocks (Morisson et al., 1997). Decreased spermatozoa motility may be related to mitochondria function. Although R+ and R- cocks had similar mitochondrial activity per mitochondrial inner membrane, mitochondrial content in spermatozoa was lower in R+ cocks (Morisson et al., 1997).

Bordas and Merat (1993) also found more late embryonic death (observed as death before day 18 of incubation) and dead in shell eggs (death between day 18 of incubation and hatch) in the R+ line, but these results were not confirmed by Morisson et al. (1997). In addition to increased embryonic mortality, the R+ line was also found to have an approximately 9 hours longer duration of incubation time compared to the R- line (Bordas and Merat, 1993). It was suggested that this was due to a difference in developmental rate, possibly related to a difference in thermogenesis, although the relation with embryonic mortality remains unclear.

1.2.6 Hormones

The association between metabolism and thermogenesis suggests that thyroid hormones may play a role in explaining differences between R+ and R- chickens. It is difficult to compare results, because there are effects of age, sex, feeding condition, and generation of selection. Plasma T_3 levels decreased from 4 until 17 weeks of age in R+ and R- hens, and were significantly higher in R+ hens at 17 weeks of age (Bordas and Minvielle, 1999). However, between 37 and 45 weeks of age, T_3 levels were not different between R+ and R- hens, regardless of feeding condition (fed or fasted) (Gabarrou et al., 1998), but it must be noted that this result was based on only five hens per line. After a fasting period of two days, adult R+ cockerels of the 17th generation had lower plasma T_3 levels than R- cockerels (Gabarrou et al., 1997), but after 16 hours of fasting, T_3 levels were not different between R+ and R- cockerels from the 21st generation (Gabarrou et al., 2000). T_3 levels were not different for fed R+ or R- cockerels from the 17th generation (Gabarrou et al., 1997), but T_3 levels were higher in R+ than in R- cockerels from the 21st generation (Gabarrou et al., 2000). Fasting

decreased plasma T_3 levels, both in R+ and in R- cockerels, but it increased T_4 levels (Gabarrou et al., 2000). In a fed state, T_4 levels were not different for R+ or R- chickens, regardless of sex and age (Bordas and Minvielle, 1999; Gabarrou et al., 1998; Gabarrou et al., 1997; Gabarrou et al., 2000). When fasted, T_4 levels were higher in 17th generation R+ females and 21st generation R+ males (Gabarrou et al., 1998; Gabarrou et al., 2000), but this result was not confirmed in 17th generation males (Gabarrou et al., 1997). Summarized, the effects of selection for RFI on thyroid hormones are not conclusive.

Another hormone that could be associated with metabolic differences between R+ and R- chickens is insulin. The fact that feed intake is higher and adiposity is lower in R+ chickens indicates that the glucose-insulin relationship may differ between R+ and R- chickens (Gabarrou et al., 2000). Glucose levels were similar for R+ and R- in a fed state, but were higher in R- in a fasted state. Insulin levels were higher in R- chickens, either in a fed or a fasted state, than in R+ chickens. After administration of a high dose of insulin, R+ chickens decreased glucose levels stronger than R- chickens. A glucose load resulted in consistently lower plasma insulin levels in R+ than in R- chickens until 120 minutes after loading, whereas plasma glucose showed a peak and then rapidly declined until baseline. In R- chickens the glucose peak was lower, but glucose levels remained relatively high until 120 minutes after loading, despite a high insulin level. Combined, these results suggest that R- chickens were in a state of insulin resistance, whereas R+ chickens were more sensitive to (exogenous) insulin, but may have lower β-cell sensitivity (Gabarrou et al., 2000). The lower T_3 levels and higher insulin levels in R- chickens may, thus, aid in explaining the lower heat production and higher adiposity in this line (Gabarrou et al., 2000).

From behavioral studies it was concluded that R+ chickens were more active, and it was hypothesized that a difference in stress susceptibility could play a role (Braastad and Katle, 1989). Indeed, young R+ chickens had higher corticosterone levels than R- chickens (Katle et al., 1988), although the corticosterone levels in both lines were very low. Adult R+ and R- chickens had similar basal levels of corticosterone (Luiting et al., 1994), but after intravenous (i.v.) injection of ACTH, R+ chickens had a higher peak, but also a stronger decline in corticosterone levels. This study showed that R- chickens may have a longer sustained stress response (Luiting et al., 1994). Therefore, the reduced activity level in R- as compared to R+ chickens should not be interpreted as being less susceptible to stress per se, because R- chickens may have less behavioral abilities to express stress than R+ chickens.

1.2.7 Residual feed intake and immune challenge

It was observed in the French selection lines that more R+ chickens survived after 18 weeks of age (Katle et al., 1988). Therefore, the selection lines were compared for responses after inoculation with *Eimeria acervulina*, an agent that causes morbidity, but does not cause mortality. Results showed that the lines did not clearly differ with respect to variations in body weight, feed intake, and lesion scores after *Eimeria* inoculation. Total serum protein was decreased due to *Eimeria*, but R+ birds had more total serum proteins, regardless of treatment. There were line × treatment interactions for caeruloplasmin, α2-globulins and transferrines. These substances belong to a group of proteins nowadays known as acute phase proteins (APP). APP levels were lower in control R- than in R+ chickens, but APP levels were higher in infected R- than in R+ chickens. Apart from these interactions between line and treatment, R+ and R- chickens, in general, do not appear to differ much in their response to *Eimeria* (Katle et al., 1988). T-cell activity and humoral immune responses were not taken into account in that study.

1.2.8 Concluding remarks

The previous paragraphs have shown that selection for RFI results in many correlated responses, which are summarized in Table 1.2. R+ and R- chickens, thus, appear to differ in many traits, although the physiological mechanisms behind those differences are largely unknown. In conclusion, R+ chickens may be characterized as spenders, whereas R- chickens may be characterized as savers. Still, from these studies it can not be concluded that R+ chickens are in some way “better off” as a result of having more maintenance resources than R- chickens. It must be noted, though, that the chickens were not explicitly challenged to investigate maintenance processes, except in the study from Katle et al. (1988). Considering the reduced fertility and the negative effects on egg characteristics in the R+ line, one may conclude that, instead of taking advantage of more maintenance resources, R+ chickens are at a disadvantage with respect to production traits.

TABLE 1.2 Overview of effects resulting from selection for high (R+) versus low (R-) residual feed intake

Eggs/reproduction	R+ vs R-	Body and energy	R+ vs R-	Blood parameters	R+ vs R-
Age at first egg	>	Body weight	=	T_3 (17 wk age)	>
Egg mass production	=	Feed intake	>	T_3 (older)	=
Mean egg weight	>	ME intake[1]	>	T_4 (fed)	=
Egg composition	=	Metabolizability	=	T_4 (fasted)	>
Shell thickness	<	Heat production (fed)	>	Glucose (fed)	=
Hatching rate	<	Heat production (fasted)	=	Glucose (fasted)	<
Spermatozoa motility	<	Activity	>	Insulin	<
% dead spermatozoa	>	Adiposity	<	APP[2] (uninfected)	>
Spermatozoa concentration	<	Diet-induced thermogenesis	>	APP (Eimeria infected)	<
Early embryonic death	>	Shank/wattle length	>	Corticosterone (young)	>
Unfertilized eggs	>	Proportional liver weight	>	Corticosterone (adult)	=

[1] ME = metabolizable energy

[2] APP = acute phase proteins

1.3 *SALMONELLA* ENTERITIDIS

In this thesis, *Salmonella* Enteritidis is used as a model antigen to investigate whether the immune status or the capacity to mount immune responses is different in pullets that were selected for a high or low residual feed intake. *Salmonella* characteristics are briefly reviewed.

1.3.1 General characteristics

Salmonellae are rod-shaped, non-spore-forming, gram-negative bacteria that usually possess flagella. *Salmonellae* that cause serious disease in humans are *Salmonella* Typhi and Paratyphi. *Salmonella* Typhi causes typhoid disease, characterized by bacteremia, persistent high fever, headache, enlarged liver and spleen, abdominal pain, and either recurrent diarrhea or constipation (WHO, 2000). In *Salmonella* Paratyphi the symptoms are usually milder. Typhoid *salmonellae* are mainly prevalent in developing countries, and can be fatal, if untreated. Other, non-typhoid *salmonellae*, e.g. *Salmonella* Typhimurium, cause gastro-enteritis, characterized by nausea, vomiting and diarrhea.

Salmonellae that cause serious disease in poultry are *Salmonella* Pullorum and Gallinarum. They are the causative agents of pullorum disease and fowl typhoid, respectively. Infections with these serovars result in typhoid-like disease, decreased egg production and

fertility, and increased mortality rates (Shivaprasad, 2000; Wigley et al., 2001); in chicks, infection results in severe disease with high mortality rates (Gast and Beard, 1990c). Pullorum disease and fowl typhoid were successfully eradicated from commercial poultry in North-America and Western Europe (Shivaprasad, 2000). Moreover, *Salmonella* Pullorum and Gallinarum are host-specific, and, thus, form no risk for public health. However, since the mid 1980s, a non-typhoid serovar, *Salmonella* Enteritidis, became increasingly important as the source of food poisoning in humans. Infections with *Salmonella* Enteritidis were associated with the consumption of contaminated eggs and poultry meat, although this serovar did not cause disease in chickens. In Europe, infections with *Salmonella* Enteritidis are mainly caused by phage type 4 strains and in the US mainly by phage type 8 and 13a strains (Usera et al., 1994).

Salmonella serovar Enteritidis belongs to the family *Enterobacteriaceae*, genus *Salmonella*, species *enterica*, subspecies *enterica* (Popoff et al., 2004). *Salmonellae* can be further serotyped based on somatic O, flagella H, and virulence Vi antigenic structures. *Salmonella* Enteritidis belongs to serogroup D1, as well as *Salmonella* Pullorum and Gallinarum; they share O 1, 9 and 12 antigens (Barrow, 1992; Timoney et al., 1990). However, there are some important differences. *Salmonella* Pullorum and *Salmonella* Gallinarum are host-adapted (to poultry), whereas *Salmonella* Enteritidis is non-host-specific. *Salmonella* Enteritidis is motile, whereas *Salmonella* Pullorum and *Salmonella* Gallinarum are not. This has implications for detection methods, because the latter 2 can not be detected in Modified Semisolid Rappaport Vassiliadis media. Non-typhoid *salmonellae*, such as *Salmonella* Enteritidis, do not characteristically cause systemic disease in chickens (Gast and Beard, 1990c) and mortality is rare in chickens older than 2 weeks (Cooper et al., 1989). However, younger chickens are very susceptible to infection (Gast and Beard, 1990c).

1.3.2 Route of infection

The route of infection is variable. Commonly, chickens become orally infected, but infection was also established after conjunctival challenge (Humphrey et al., 1992) and aerosolization of feces from infected hens can also cause infection of contact hens (Baskerville et al., 1992; Shivaprasad et al., 1990). In chickens, the susceptibility to *Salmonella* Enteritidis infection is age dependent (Okamura et al., 2004); *Salmonella* Enteritidis seldom causes mortality in birds older than 1 month (Suzuki, 1994), because resistance increases in proportion to age, probably due to development of the immune system and normal gut flora with ageing (Desmidt et al., 1997; Suzuki, 1994). *Salmonella* Enteritidis

colonizes the intestinal tract, invades tissues, provokes a specific antibody response and is deposited in eggs, but in older chickens, as opposed to young chicks, there is no clear evidence of clinical disease (Gast and Beard, 1993). Older chickens have a high level of resistance to invasive infection by *salmonella*e other than *Salmonella* Pullorum or Gallinarum. Even the more invasive isolates of *S*. Enteritidis were cleared from the tissues of most hens 28 to 42 days post inoculation (p.i.) (Shivaprasad et al., 1990).

Fecal shedding of *Salmonella* bacteria is intermittently (Barrow, 1992, 1994; Shivaprasad et al., 1990), and duration seems to depend on the route of infection, although there are some contradictory results. Most experimentally infected chickens, through oral inoculation, shed *S*. Enteritidis in the feces for 5 to 16 days; in one experiment shedding is prolonged in i.v. inoculated chickens compared to oral inoculation, however, the same authors reported reduced duration of shedding in i.v. inoculated chickens in another experiment (Shivaprasad et al., 1990). Chickens that were i.v. inoculated, showed clinical signs of disease (depression, reduced feed and water intake, reduced egg production, and some mortality), although the hens physiological condition improved within 1 week after inoculation (Withanage et al., 1999). Once *Salmonella* is introduced into a flock, it can perpetuate throughout a whole production period (Skov et al., 2002).

1.3.3 Organ colonization

Invasiveness of *Salmonella* appears to differ between strains, and may depend on the chickens' age. Presence of *Salmonella* in internal organs does not necessarily result in clinical signs (Desmidt et al., 1997; Shivaprasad et al., 1990). Depending on the invasiveness of the strain, *S*. Enteritidis bacteria are sometimes found in large numbers in spleen and caeca (Gast and Beard, 1990a; Sadeyen et al., 2004; Shivaprasad et al., 1990), in liver, ovary and oviduct (Gast and Beard, 1990a; Shivaprasad et al., 1990), and in heart, peritoneum, jejunum and colon (Shivaprasad et al., 1990). From *S*. Pullorum and Gallinarum it is known that they commonly localize the ovary and produce transovarian infection, but there are few reports of ovarian infection by other non-host adapted *salmonellae* (Shivaprasad et al., 1990); however, *S*. Enteritidis may also possess a tendency to localize in the ovary of chickens (Desmidt et al., 1997; Gast and Beard, 1990a). The caeca represent a reservoir of infection of *Salmonella* in poultry (Sadeyen et al., 2004), and there seems to be an inverse relationship between severity of caecal infection and colonization in systemic organs (Kramer et al., 2001; Sadeyen et al., 2004). Isolation of *S*. Enteritidis from internal organs started to decline from day 14 p.i.

onwards (Withanage et al., 1999) to complete clearance by day 30 after inoculation (Shivaprasad et al., 1990).

1.3.4 Immune responses and role of macrophages

Oral infection with *salmonellae* normally leads to the production of circulating antibody mainly of the IgG class (Barrow, 1992). The humoral immune response is often weak (Gast and Beard, 1990c), and peaks around 2 weeks after inoculation (Gast and Beard, 1990c; Gast et al., 2002), at which time number of *S*. Enteritidis (in liver and spleen) was reduced (Desmidt et al., 1998). Specific anti-*Salmonella* antibodies are found in the sera of both experimentally and naturally infected chickens (Gast and Holt, 2001).

It was observed that *S*. Enteritidis can survive in the oviduct, despite the presence of *S*. Enteritidis-specific antibodies (Withanage et al., 1999). It can be explained by the fact that *Salmonella* is a facultative intracellular bacterium (Barrow et al., 1987; Withanage et al., 1999) that mainly lives inside cells of liver and spleen (Desmidt et al., 1998). The main cell type is the macrophage (Barrow et al., 1987; Wigley et al., 2001). When *Salmonella* is intracellular, it can not be reached by antibodies (Desmidt et al., 1998). This means that a humoral immune response is important in clearing extracellular bacteria, but intracellular bacteria will survive (Wigley et al., 2001). Therefore, cellular immune responses are needed to lyse the infected cell (Desmidt et al., 1998), thereby releasing the bacteria from the macrophage. Complete protection from *S*. Enteritidis infection by antibodies alone is, thus, unlikely. Moreover, in bursectomized chickens, i.e. chickens that are not able to mount an antibody response, the number of *S*. Enteritidis in organs decreased during the first 4 wk p.i.. This also indicates that cellular immune responses must be involved (Desmidt et al., 1998). Moreover, when cellular immune responses are down-regulated, macrophage activation is inhibited, thereby preventing clearance of intracellular *salmonellae* (Wigley et al., 2001). Shortly after *Salmonella* inoculation, specific cellular responses appear to be suppressed, probably due to nitric oxide produced by infected macrophages. Specific cellular responses may be induced, when this innate response started to decrease (Okamura et al., 2004). Summarizing, protection from *Salmonella* requires both humoral and cellular mechanisms, as well as other non-specific immune responses (Withanage et al., 1999).

The fact that *S*. Enteritidis can invade and survive in macrophages in the host is one of its virulence factors (Suzuki, 1994). It was shown that *Salmonella* Pullorum persisted in low numbers in the mononuclear phagocyte system, reproductive tract and heart after recovery (Wigley et al., 2001), and can be released during times of stress.

1.3.5 Correlation of antibody responses to clearance

Salmonella-specific antibody titers have been shown to correlate with the incidence of fecal shedding and tissue and egg contamination (Gast and Beard, 1991). However, the magnitude of an antibody response does not consistently predict for an individual bird the likelihood of producing at least one contaminated egg. The magnitude of the antibody response also appears not to be indicative for the number of contaminated eggs produced. Thus, there is no overall pattern of increasing egg contamination frequencies being related to increasing levels of antibody titers (Gast and Holt, 2001).

The antibody response appears to parallel the clearance of organisms from tissues (Desmidt et al., 1997). Antibodies in bile, which are mainly of the IgA type, were shown to reach a peak at 2 wk p.i. and paralleled the reduction of fecal shedding. This result indicated that the peak of IgA in bile might play a role in the local clearance of *S*. Enteritidis from the gut (Desmidt et al., 1998).

1.3.6 Stress

Shedding of *Salmonella* bacteria can be reactivated by stressful conditions (Skov et al., 2002), such as an induced molt (Gast and Beard, 1993; Holt and Porter, 1993), or by the onset of lay. Molt is induced by feed withdrawal during several days, and is considered a period of stress. Hens that were challenged with *Salmonella* Enteritidis 3 weeks prior to feed withdrawal shed significantly more *S*. Enteritidis after feeding was resumed than unmolted hens (Holt and Porter, 1993). Also an increased transmissibility to contact hens was observed; this could be due to increased numbers of *S*. Enteritidis shed by the molted infected hens, or the observed increased susceptibility of molted hens to an *S*. Enteritidis infection (Holt and Porter, 1993), or both. This result may be explained by the fact that cellular immunity was significantly depressed by the molt procedure, whereas humoral immunity was unaffected (Holt and Porter, 1992).

One-day-old chickens that were inoculated with *S*. Typhimurium gradually decreased shedding, but shedding re-emerged when the chickens reached the onset of lay (Skov et al., 2002). Bacterial numbers in spleen and reproduction tract also increased at that time (Wigley et al., 2001). It is assumed that gonadal steroid hormones play a role here, through an influx of persistently infected macrophages to the reproductive tract (Wigley et al., 2001). Skov et al. (2002) found another stressful situation when chickens had to be caught and placed in boxes before being moved to another room, which also led to re-emerging of shedding.

1.3.7 Eggs

Egg production was greatly reduced for about 14 days in chickens that were i.v. inoculated with *S*. Enteritidis phage type 8 (Shivaprasad et al., 1990). Some hens laid malformed, thin-shelled eggs in the early phase of infection (Withanage et al., 1999). As was already shown for fecal shedding, egg contamination can also occur intermittently (Gast and Holt, 2001).

There is some discussion whether egg contamination occurs mainly on the shell or in the egg content. Egg shells can be contaminated with *salmonellae* either as a result of infection of the oviduct, by fecal contamination, or as a result of intestinal carriage (Humphrey, 1994). The shell gland or another part of the oviduct may be a site of infection, however, the numbers of *salmonellae* isolated from the shell were low (Humphrey, 1994). There is also a possibility that *S*. Enteritidis organisms present on egg shells can contaminate egg contents by migration through the shell and associated membranes (Humphrey, 1994). It appears, however, that contamination of egg content is more the result of infection of reproductive tissue (Humphrey, 1994). The main site of contamination in eggs seems to be either the albumen (Gast and Beard, 1993; Shivaprasad et al., 1990) or the outside of the vitelline membrane (Gast and Beard, 1990b). Yolk contents are rich in iron, which can be used by *salmonellae* for rapid growth. However, the vitelline membrane of fresh eggs prevents either the entrance of bacteria into yolk contents, or the release of iron into the albumen (Humphrey, 1994). During storage, the vitelline membrane breaks down and becomes permeable, causing *Salmonella* organisms to enter yolk contents. This could explain the contaminated yolks. Isolation of *S*. Enteritidis from ovaries led to belief that *S*. Enteritidis might be present in yolk. Indeed it has been isolated from yolks (Gast and Beard, 1993; Shivaprasad et al., 1990), but this may be associated with the high inoculation dose used (Humphrey, 1994). Yolks that were artificially inoculated, even with a small inoculum, showed rapid growth of *salmonellae*. If yolks were a frequent site of contamination, the majority of contaminated eggs would contain a high level of *S*. Enteritidis organisms, even in fresh eggs, but this is not the case (Humphrey, 1994). In conclusion, high levels of contamination will only be achieved when yolk contents are invaded (Humphrey, 1994).

1.4 OUTLINE

Continuous genetic selection for RFI in experimental lines resulted in many correlated responses, but effects on the immune system are hardly taken into account. This thesis aims to clarify processes in chickens regarding feed intake, feed efficiency and the immune system and their inter-relationships. Phenotypic selection for RFI in pullets (i.e. young, growing, non-laying hens) appears to be an unexplored area. Moreover, it is also not known whether differences in RFI are correlated with differences in immune responses. The first step is to deal with the procedure for RFI selection in pullets, which is described in Chapter 2. Humoral immune responses after immunization with non-replicating antigens are also described.

In Chapter 3, a challenge with an infectious agent, *Salmonella* Enteritidis, is introduced. This chapter describes humoral immune responses in R+ and R- pullets after infection with a replicating antigen (live bacteria) and immunization with heat-killed *Salmonella*, and it also describes effects on organ weights.

Metabolism is known to differ in adult R+ and R- chickens, but it remained to be established whether this difference was also present in R+ and R- pullets. Moreover, metabolism is thought to be affected by a challenge with an infectious agent. Chapter 4 describes the effects of a *Salmonella* Enteritidis infection on metabolism and thyroid hormone levels in R+ and R- pullets.

Chapter 5 investigates local effects of *Salmonella* infection on *Salmonella*-specific and natural anti-α-Gal antibody responses in bile.

Closely related to Chapter 5 is Chapter 6, which describes differences between R+ and R- pullets after *S*. Enteritidis infection with respect to immune parameters and shedding characteristics.

In the General Discussion, chapter 7, the results from all experiments are summarized and discussed.

1.5 REFERENCES

Bachman, E. S., H. Dhillon, C. Y. Zhang, S. Cinti, A. C. Bianco, B. K. Kobilka, et al. 2002. betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science, 297:843-845.

Barrow, P. A. 1992. ELISAs and the serological analysis of salmonella infections in poultry: a review. Epidemiology and Infection, 109:361-369.

Barrow, P. A. 1994. Serological diagnosis of Salmonella serotype enteritidis infections in poultry by ELISA and other tests. International Journal of Food Microbiology, 21:1-2.

Barrow, P. A., M. B. Huggins, M. A. Lovell, and J. M. Simpson. 1987. Observations on the pathogenesis of experimental Salmonella typhimurium infection in chickens. Res Vet Sci, 42:194-199.

Baskerville, A., T. J. Humphrey, R. B. Fitzgeorge, R. W. Cook, H. Chart, B. Rowe, et al. 1992. Airborne infection of laying hens with Salmonella enteritidis phage type 4. Vet Rec, 130:395-398.

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? Journal of Animal Breeding and Genetics, 110:161-170.

Bentsen, H. B. 1983. Genetic variation in feed efficiency of laying hens at constant body weight and egg production 1. efficiency measured as a deviation between observed and expected feed consumption. Acta Agriculturae Scandinavica., 1983:289-304.

Bordas, A., and P. Merat. 1974. Genetic variation in laying hens and phenotypic correlations of feed consumption corrected for body weight and egg production. Annales de Genetique et de Selection Animale, 6:369-379.

Bordas, A., and P. Merat. 1981. Genetic variation and phenotypic correlations of food consumption of laying hens corrected for body weight and production. British Poultry Science, 22:25-33.

Bordas, A., and P. Merat. 1993. Durée d'incubation et effet du stockage des oeufs sur le taux d'eclosion dans des lignées de poules sélectionnées sur la consommation alimentaire résiduelle. Genetics Selection Evolution, 25:397-402.

Bordas, A., and F. Minvielle. 1997. Effects of temperature on egg laying hens from divergent lines selected on residual feed consumption. Genetics Selection Evolution Paris, 29:279-290.

Bordas, A., and F. Minvielle. 1999. Patterns of growth and feed intake in divergent lines of laying domestic fowl selected for residual feed consumption. Poultry Science. March, 78:317-323.

Bordas, A., M. Tixier Boichard, and P. Merat. 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode Island red laying hens. British poultry science, 33:741-754.

Braastad, B. O., and J. Katle. 1989. Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. British Poultry Science, 30:533-544.

Cooper, G. L., R. A. Nicholas, and C. D. Bracewell. 1989. Serological and bacteriological investigations of chickens from flocks naturally infected with Salmonella enteritidis. Veterinary Record, 125:567-572.

Desmidt, M., R. Ducatelle, and F. Haesebrouck. 1997. Pathogenesis of Salmonella enteritidis phage type four after experimental infection of young chickens. Veterinary Microbiology, 56:99-109.

Desmidt, M., R. Ducatelle, J. Mast, B. M. Goddeeris, B. Kaspers, and F. Haesebrouck. 1998. Role of the humoral immune system in Salmonella enteritidis phage type four infection in chickens. Veterinary Immunology and Immunopathology, 63:355-367.

Dunnington, E. A. 1990. Selection and homeostasis. Pages pp 5-12 in Proceedings of the 4th World Congress on genetics applied to livestock production. Edinburgh; UK.

El Kazzi, M., A. Bordas, G. Gandemer, and F. Minvielle. 1995. Divergent selection for residual food intake in Rhode Island red egg-laying lines: Gross carcase composition, carcase adiposity and lipid contents of tissues. British Poultry Science, 36:719-728.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. British Poultry Science, 39:79-89.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. Journal of Nutrition, 127:2371-2376.

Gabarrou, J. F., P. A. Geraert, J. Williams, L. Ruffier, and N. Rideau. 2000. Glucose-insulin relationships and thyroid status of cockerels selected for high or low residual food consumption. British journal of nutrition, 83:645-651.

Gast, R. K., and C. W. Beard. 1990a. Isolation of Salmonella enteritidis from internal organs of experimentally infected hens. Avian Diseases, 34:991-993.

Gast, R. K., and C. W. Beard. 1990b. Production of Salmonella enteritidis-contaminated eggs by experimentally infected hens. Avian Dis, 34:438-446.

Gast, R. K., and C. W. Beard. 1990c. Serological detection of experimental Salmonella enteritidis infections in laying hens. Avian Diseases, 34:721-728.

Gast, R. K., and C. W. Beard. 1991. Detection of Salmonella serogroup D-specific antibodies in the yolks of eggs laid by hens infected with Salmonella enteritidis. Poult Sci, 70:1273-1276.

Gast, R. K., and C. W. Beard. 1993. Research to understand and control Salmonella enteritidis in chickens and eggs. Poultry Science, 72:1157-1163.

Gast, R. K., and P. S. Holt. 2001. The relationship between the magnitude of the specific antibody response to experimental salmonella enteritidis infection in laying hens and their production of contaminated eggs. Avian Dis, 45:425-431.

Gast, R. K., M. S. Nasir, M. E. Jolley, P. S. Holt, and H. D. Stone. 2002. Serological detection of experimental Salmonella enteritidis infections in laying hens by fluorescence polarization and enzyme immunoassay. Avian Diseases, 46:137-142.

Geraert, P. A., S. Guillaumin, A. Bordas, and P. Merat. 1991. Evidence of a genetic control of diet-induced thermogenesis in poultry. Pages 380-383 in 12th Symposium on Energy Metabolism in Farm Animals. EAAP, Kartause Ittingen.

Herremans, M., E. Decuypere, and O. Siau. 1989. Effects of feather wear and temperature on prediction of food intake and residual food consumption. British poultry science, 30:15-22.

Holt, P. S., and R. E. Porter, Jr. 1992. Microbiological and histopathological effects of an induced-molt fasting procedure on a Salmonella enteritidis infection in chickens. Avian Dis, 36:610-618.

Holt, P. S., and R. E. Porter, Jr. 1993. Effect of induced molting on the recurrence of a previous Salmonella enteritidis infection. Poultry Science, 72:2069-2078.

Humphrey, T. J. 1994. Contamination of egg shell and contents with Salmonella enteritidis: a review. International Journal of Food Microbiology, 21:31-40.

Humphrey, T. J., A. Baskerville, H. Chart, B. Rowe, and A. Whitehead. 1992. Infection of laying hens with Salmonella enteritidis PT4 by conjunctival challenge. Veterinary Record, 131:386-388.

Katle, J. 1991. Selection for efficiency of food utilization in laying hens: Causal factors for variation in residual food consumption. British poultry science, 32:955-970.

Katle, J., N. Hamet, L. Durand, P. Rombauts, and P. Merat. 1988. Lignées divergentes pour la consommation alimentaire "résiduelle" des pondeuses: réponse des poussins à une inoculation par *Eimeria acervulina* et comparaison de paramètres biologiques Génét. Sél. Evol., 20:387-396.

Katle, J., and N. Kolstad. 1991. Selection for efficiency of food utilisation in laying hens: Direct response in residual food consumption and correlated responses in weight gain, egg production and body weight. British poultry science, 32:939-954.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of Animal Science, 22:`486-494.

Kramer, J., A. H. Visscher, J. A. Wagenaar, A. G. Boonstra Blom, and S. H. M. Jeurissen. 2001. Characterization of the innate and adaptive immunity to Salmonella enteritidis PT1 infection in four broiler lines. Veterinary Immunology and Immunopathology, 79:3-4.

Luiting, P. 1990. Genetic variation of energy partitioning in laying hens: causes of variation in residual feed consumption. World's Poultry Science Journal, 46:133-152.

Luiting, P., P. N. De Groot, E. Decuypere, and J. Buyse. 1994. Selection for feed efficiency and consequences for stress susceptibility. Proceedings of the 45th annual meeting of the European Association for animal production, Edinburgh (abstract):7.

Luiting, P., J. W. Schrama, W. v. d. Hel, and E. M. Urff. 1991a. Metabolic differences between White Leghorns selected for high and low residual food consumption. British Poultry Science, 32:763-782.

Luiting, P., J. W. Schrama, W. Van Der Hel, E. M. Urff, P. G. J. J. Van Boekholt, E. M. W. Van Den Elsen, et al. 1991b. Metabolic differences between White Leghorns selected for high and low residual feed consumption. Pages 384-387 in 12th Symposium on Energy Metabolism in Farm Animals. EAAP, Kartause Ittingen, Switzerland.

Luiting, P., and E. M. Urff. 1991. Optimization of a model to estimate residual feed consumption in the laying hen. Livestock Production Science, 27:321-338.

Merat, P., A. Bordas, and F. H. Ricard. 1980. Composition anatomique, production d'oeufs et effficacité alimentaire de poules pondeuses. Corrélations phénotypiques. Ann. Génét. Sél. anim., 12:191-200.

Morisson, M., A. Bordas, J. M. Petit, C. Jayat Vignoles, R. Julien, and F. Minvielle. 1997. Associated effects of divergent selection for residual feed consumption on reproduction, sperm characteristics, and mitochondria of spermatozoa. Poultry Science, 76:425-431.

Nordskog, A. W., H. L. French, Jr., C. R. Arboleda, and D. W. Casey. 1972. Breeding for efficiency of egg production. World's Poultry Science Journal, 28:175-188.

Okamura, M., H. S. Lillehoj, R. B. Raybourne, U. S. Babu, and R. A. Heckert. 2004. Cell-mediated immune responses to a killed Salmonella enteritidis vaccine: lymphocyte proliferation, T-cell changes and interleukin-6 (IL-6), IL-1, IL-2, and IFN-gamma production. Comp Immunol Microbiol Infect Dis, 27:255-272.

Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling. 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol, 155:568-570.

Saarela, S., J. S. Keith, E. Hohtola, and P. Trayhurn. 1991. Is the "mammalian" brown fat-specific mitochondrial uncoupling protein present in adipose tissues of birds? Comp Biochem Physiol B, 100:45-49.

Sadeyen, J. R., J. Trotereau, P. Velge, J. Marly, C. Beaumont, P. A. Barrow, et al. 2004. Salmonella carrier state in chicken: comparison of expression of immune response genes between susceptible and resistant animals. Microbes and infection, 6:1278-1286.

Shivaprasad, H. L. 2000. Fowl typhoid and pullorum disease. Rev Sci Tech, 19:405-424.

Shivaprasad, H. L., J. F. Timoney, S. Morales, B. Lucio, and R. C. Baker. 1990. Pathogenesis of Salmonella enteritidis infection in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. Avian Diseases, 34:548-557.

Skov, M. N., N. C. Feld, B. Carstensen, and M. Madsen. 2002. The serologic response to Salmonella enteritidis and Salmonella typhimurium in experimentally infected chickens, followed by an indirect lipopolysaccharide enzyme-linked immunosorbent assay and bacteriologic examinations through a one-year period. Avian Diseases, 46:265-273.

Suzuki, S. 1994. Pathogenicity of Salmonella enteritidis in poultry. Int J Food Microbiol, 21:89-105.

Timoney, J. F., N. Sikora, H. L. Shivaprasad, and M. Opitz. 1990. Detection of antibody to Salmonella enteritidis by a gm flagellin-based ELISA. Veterinary Record, 127:168-169.

Tixier Boichard, M., D. Boichard, E. Groeneveld, and A. Bordas. 1995. Restricted maximum likelihood estimates of genetic parameters of adult male and female Rhode Island Red chickens divergently selected for residual feed consumption. Poultry Science, 74:1245-1252.

Tixier, M., A. Bordas, and P. Merat. 1988. Divergent selectionfor residual feed intake in laying hens: effects on growth and fatness. Pages 129-132 in Leanness in domestic birds: Genetic, metabolic and hormonal aspects. B. Leclercq and C. C. Whitehead eds. INRA and Buttersworth, London, U.K.

Usera, M. A., T. Popovic, C. A. Bopp, and N. A. Strockbine. 1994. Molecular subtyping of Salmonella enteritidis phage type 8 strains from the United States. J Clin Microbiol, 32:194-198.

Wigley, P., A. Berchieri, Jr., K. L. Page, A. L. Smith, and P. A. Barrow. 2001. Salmonella enterica serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infection and Immunity. [print] December, 2001;, 69:7873-7879.

Withanage, G. S. K., K. Sasai, T. Fukata, T. Miyamoto, and E. Baba. 1999. Secretion of Salmonella-specific antibodies in the oviducts of hens experimentally infected with Salmonella enteritidis. Veterinary Immunology and Immunopathology, 67:185-193.

World Health Organization (WHO). 2000. Typhoid vaccines. Pages 257-264 in Weekly epidemiological record.

Zein-el-Dein, A., A. Bordas, and P. Merat. 1985. Selection divergente pour la composante "residuelle" de la consommation alimentaire des poules pondeuses: effets sur la composition corporelle. Archiv für Geflügelkunde, 49:158-160.

CHAPTER 2

Phenotypic selection for residual feed intake and its effect on humoral immune responses in pullets

E. Van Eerden, H. Van den Brand, H. K. Parmentier, M. C. M. De Jong, B. Kemp

Published in Poultry Science (2004) 83:1602-1609

ABSTRACT

According to the resource allocation theory, animals have to make a trade-off between resource demanding life traits to obtain maximal fitness. Artificial selection towards efficient producing farm animals, however, may have created animals that have an impaired ability to divert resources to maintenance processes, such as responding to immune challenges. Residual Feed Intake (RFI), defined as the difference between observed feed intake and expected feed intake based on metabolic body weight (BW) and growth, was used as a measure for feed efficiency. Individual BW and feed intake (FI) of 352 pullets were weekly recorded from 4 until 14 wk of age, in order to estimate RFI. The top 50 efficient R– and the top 50 non-efficient R+ animals were selected. BW and BW gain in both groups were similar. FI and RFI, however, were significantly higher in R+ animals.

Thirty animals out of every group were randomly allocated to one of three treatments: immunization with Keyhole Limpet Hemocyanin (KLH), *Mycobacterium butyricum*, or heat-inactivated *Salmonella* Enteritidis bacteria. Antibody titers against KLH, *M. butyricum*, or *Salmonella* lipopolysaccharide did not differ between R+ and R– animals. Antibody titer against *Salmonella* protein was higher in R+ animals.

We conclude that a population of chickens from a commercial breed shows considerable variation in RFI. Specific antibody production against KLH, *M. butyricum*, and *S.* Enteritidis lipopolysaccharide, however, is not influenced by efficiency in terms of RFI. R+ animals may have a higher level of non-antigen specific antibodies, indicated by the higher antibody response to *Salmonella* protein.

Abbreviation Key: AFE = age at first egg; BW = body weight; EM = egg mass; FI = feed intake; KLH = Keyhole Limpet Hemocyanin; LPS = lipopolysaccharide; RFI = residual feed intake

2.1 INTRODUCTION

The aim of modern breeding programs for farm animals is efficient production of safe and wholesome milk, meat, or eggs at low maintenance costs. The basic concept behind this aim is that energy used for maintenance processes is not available for production processes. Lowering costs of maintenance would thus result in more energy remaining, potentially to be expended on production. The resource allocation theory (Beilharz et al., 1993) provides a theoretical background for this concept. In brief, this theory states that, in an environmentally limiting situation, animals have a package of finite resources, meaning that resources used by one function are no longer available for other functions. Thus, animals have to make a trade-off between allocation of resource-demanding life traits to obtain maximal fitness. The theory applies to natural selection and assumes that animals are flexible in the way they allocate their resources.

A different situation arises in artificial selection. For decades animal breeding has focused on maximizing production traits. It is not unusual to suppose that farm animals are genetically programmed to allocate a large portion of their resources to a particular production trait, reducing their ability to respond to other demands (Dunnington, 1990). Breeding programs for laying hens aim for efficient egg mass production (EM). Deducing from Dunnington (1990), laying hens can be considered as being genetically programmed for efficient egg mass production. There is, however, a lot of variation in efficiency between individuals within a population, as shown in variation in feed consumption that can not be explained by metabolic BW, BW gain and EM. This variation in feed consumption is reflected in differences in residual feed intake (RFI) (Bentsen, 1983; Bordas and Merat, 1974; Luiting and Urff, 1991). RFI is defined as the difference between observed FI and FI predicted from metabolic BW, representing maintenance, and BW gain and EM, both representing production. RFI is thus a measure for feed efficiency: chickens with low RFI (R–) need less feed to reach the same BW and production level and are, therefore, more efficient producers than chickens with high RFI (R+).

The question arises whether selection for RFI affects an animal's ability to adequately respond to environmental stressors. Responding to stress, i.e. adapting to a changing situation in order to maintain homeostasis, may be an energy demanding process. Many stressors that animals encounter are immunological challenges. Provided that having and activating an immune system is energetically costly (Deerenberg et al., 1997; Demas et al., 1997; Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996; Verhulst et al., 1999),

animals would have to make a trade-off between mounting an immune response, and maintaining their production level. Being genetically programmed to efficient production, modern farm animals might have an impaired ability to make this trade-off, meaning that they are less capable to cope with an immune-related stress. Therefore, it can be hypothesized that selecting animals for RFI within a population as a measure for efficiency results in animals with different immune competence, with efficient (R–) animals possibly responding less adequate than non-efficient (R+) animals.

The current experiment was carried out to investigate the possibility to phenotypically select a population of pullets based on residual feed intake. Whether selected groups of animals with high or low RFI differ in their capability to mount specific antibody responses, was investigated subsequently.

2.2 MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Wageningen University approved all experimental protocols.

Animals

A population of 400 one-day-old Lohmann Brown chickens was reared in floor pens until four weeks of age. A starter diet (ME 2,600 kcal/kg, CP 200 g/kg) and water were available ad libitum. At 3 weeks of age all chickens had their beaks trimmed, and the starter diet was gradually replaced by a grower diet (ME 2,600 kcal/kg, CP 175 g/kg). The grower diet was fed throughout the experimental period.

The experiment started when the chickens were 4 weeks of age. A group of 352 chickens was selected on BW (chickens with BW < 190 g were not used), and was individually housed in wire bottom battery cages. Individual feeding troughs were placed in front of the cages and were designed to prevent spilling. Feed and water were available ad libitum.

Once a week, BW and feed intake of all animals were recorded individually. Every week the troughs were refilled with fresh feed. Data were collected during 72 days from 4 until 14 weeks of age. The interval of 72 days was divided in 10 weeks: week 2 contained 9 days; all other weeks contained 7 days.

The chickens were routinely vaccinated according to the schedule recommended by the Dutch Animal Health Service. To prevent interference in the subsequent immunization experiment they were not vaccinated after 11 weeks of age. The chickens received 9 hours of light: 15 hours dark, with the light period from 8.00 a.m. until 5.00 p.m. The average room temperature was adjusted to 21°C.

Calculation of Residual Feed Intake

Data of BW, BW gain, and feed intake from the overall selection interval of 72 d were analyzed to calculate RFI, using the model:

$$FI = b1 \times BW^{b2} + b3 \times \Delta W + e,$$

where FI = feed intake (g per wk), BW = average body weight between two recordings (g), ΔW = body weight gain (g per wk), e = error, and b1, b2, b3 = partial regression coefficients. The error term is considered to be RFI (g per week). The PROC NLIN Procedure of SAS® (SAS Institute, 1999) was used to analyze data per week and for the overall selection interval of 72 days.

Selection procedure

After week 10 overall RFI was calculated. The top 50 efficient (R–) chickens with low RFI and the top 50 non-efficient (R+) chickens with high RFI were selected. The remaining 250 chickens were removed from the experiment. Each RFI-group was sorted from high to low values of RFI, and divided in 10 clusters of five animals with comparable RFI. The five chickens within each cluster were randomly allocated to one of four immunization treatments, or an inoculation treatment. The treatments were immunization with either Keyhole Limpet Hemocyanin (K; one group), *Mycobacterium butyricum* (M; one group), or heat-inactivated *Salmonella* Enteritidis bacteria (S–; two groups), or inoculation with live *Salmonella* Enteritidis bacteria (S+; one group). Each treatment group consisted of 20 animals: 10 R– and 10 R+ chickens.

One group of chickens receiving S– treatment and the group receiving S+ inoculation were taken out of the experiment, and were housed separately in one of two identical climate respiration chambers (Verstegen et al., 1987) for further research as described by Van Eerden et al. (2004).

Immunization experiment

Housing and management of the group of 60 chickens receiving either K, M, or S– treatment was identical to the RFI-selection period (animals were individually housed and fed; feed and water were available ad lib).

At 16 weeks of age the chickens were immunized subcutaneously with 1 mL PBS containing either 1 mg Keyhole Limpet Hemocyanin (KLH, ICN Biomedicals), 1 mg *Mycobacterium butyricum* protein (Difco Laboratories), or 4×10^7 colony forming units (CFU) heat-inactivated *Salmonella* Enteritidis bacteria (provided by J. Wagenaar, ASG Lelystad). The *Salmonella* heat-inactivating procedure is described later. A booster immunization with the same kind and amount of antigen was given on day 23 post immunization (p.i.). KLH was used because of its supposed T_H2 activating qualities in rodents (Bradley et al., 1995; Bliss et al., 1996), and is, therefore, associated with humoral immune responses. *Mycobacterium*, on the other hand, was used because of its supposed T_H1 activating qualities (Mosmann and Sad, 1996), and is, therefore, associated with cell-mediated immune responses. Heat-inactivated *Salmonella* was used to investigate its immunogenic properties, without arousing the whole range of inflammatory effects due to a bacterial infection. Immunization doses were based on results of previous pilot experiments (unpublished results).

A blood sample (0.5 mL) of each animal was taken on day 0 before primary immunization, and on day 9, 16, 23 and 29 p.i. Fresh blood samples were centrifuged and plasma was stored at -20°C until further processing.

Body weight and feed intake were recorded weekly until day 30 p.i., when the animals had reached the age of 20 wk. At this point the immunization experiment ended. Age at first egg (AFE), total egg number and total egg weight per chicken were continued to be recorded until day 46 p.i., when the animals were 22 weeks of age.

Salmonella* Enteritidis *preparation

Salmonella Enteritidis was grown in buffered pepton water overnight at 37°C. The bacteria were centrifuged at 3,000 × g for 15 minutes and washed twice in sterile PBS. The cell pellets were diluted to a final concentration of 4×10^7 CFU/mL. The suspension was poured into a bottle and placed in a water bath at 65°C for one hour. The final suspension of heat-inactivated bacteria was determined to be sterile, as culture in Brain Heart Infusion was found to be negative.

ELISA procedure

Total immunoglobulin titers to KLH, *M. butyricum*, *Salmonella* Enteritidis lipopolysaccharide (LPS, Sigma Aldrich Chemie), and *Salmonella* Enteritidis protein were determined by indirect ELISA in plasma of all chickens. In short, 96-well plates were coated with either 1μg/mL KLH, 4 μg/mL *M. butyricum*, 4 μg/mL *Salmonella* Enteritidis LPS, or 1.5 × 10^7 CFU/mL heat-inactivated *Salmonella* Enteritidis bacteria; all were diluted in carbonate buffer. The plates were incubated for one hour at room temperature and then washed with PBS and 0.05% Tween. Two-step serial dilutions of plasma in PBS, 0.05% Tween, and 1% fetal calf serum were added, starting with a dilution of 1/80 for KLH, 1/20 for *M. butyricum*, 1/80 for *Salmonella* LPS, and 1/40 for *Salmonella* protein. Plates were incubated for one hour at room temperature. After washing, binding of antibodies was detected using 1/20,000 diluted Rabbit-anti-chicken coupled to peroxidase (RACh/IgG_{H+L}/PO, Nordic). After washing, tetramethylbenzidine and 0.05% H_2O_2 was added. The reaction was stopped after 10 minutes with 2.5 N H_2SO_4. Extinctions were measured using a Multiskan® reader (Labsystems) at a wavelength of 450 nm. Titers were expressed as the ^{2}log values of the highest dilution giving a positive reaction.

Statistical analysis

The six treatment combinations were arranged as a 3 x 2 factorial model: each of treatment K, M, and S– at two levels (R– and R+). Data of BW and FI, and egg laying characteristics were analyzed using the model:

$$Y = \mu + R + T + R \times T + e,$$

where Y = dependent variable, μ = overall mean, R = RFI class (R– or R+), T = treatment (K, M, or S–), R × T = interaction between RFI class and treatment, and e = residual error. Egg laying characteristics were analyzed only for chickens with at least one non-defective egg laid. Antibody titers were analyzed using a model including repeated measurements:

$$Y = \mu + R + T + R \times T + \text{Bird}(R \times T) + \text{time} + \text{time} \times R + \text{time} \times T + \text{time} \times R \times T + e,$$

where Y = dependent variable, μ = overall mean, R = RFI class (R– or R+), T = treatment (K, M, or S–), R × T = interaction between RFI class and treatment, Bird (R × T) = animal nested for RFI class and treatment, time = day of blood sampling (0, 9, 16, 23, or 29), time × R = interaction between time and RFI class, time × T = interaction between time and treatment, time × R × T = interaction between time, RFI class, and treatment, and e = residual error. The significance of R and T was tested with Bird (R × T) as error term, whereas time and its

interactions were tested against the residual error. Statistical analysis was carried out in two parts: one for the primary immune response (including day 0, 9, 16, and 23) and another one for the secondary immune response (including day 23 and 29).

Unless otherwise stated, all levels of significance were based on testing at the $P < 0.05$ level.

2.3 RESULTS

Selection for Residual Feed Intake

During the 72 days of selection for RFI, one chicken was euthanized because of leg problems. One chicken had some missing feed intake data, which made calculation of RFI over the whole selection period impossible for this animal. Calculation and statistical analysis were, therefore, carried out for 350 animals. The final model was defined as:

$$FI = 19.39 \times BW^{0.75} + 1.50 \times \Delta G + RFI.$$

RFI had a normal distribution. Retrospectively BW, BW gain, FI, and RFI of both groups of 50 animals, selected for high or low RFI, were calculated per week. Values within a week were always transformed to values per day. BW of both R– and R+ group was equal during the whole period. BW between both lines differed only in the first three weeks, with BW of R– animals being slightly higher than BW of R+ animals. BW gain curves of both R– and R+ animals did not differ, except for week 6 and 7 (10 and 11 weeks of age, respectively): BW gain of R+ vs. R– animals was increased in week 6, whereas it was decreased in week 7. FI in R+ animals was higher than in R– animals, from week 2 throughout the selection period, as shown in Figure 2.1.

Figure 2.2 shows the course of RFI. Soon after the experiment started, both lines diverged. The population mean was zero. In week 10, at 14 weeks of age, the values for R– and R+ were -28.1 g and +17.9 g respectively, which means a difference in RFI of about 6.6 g/d. Mean FI in this period calculated for the whole population, is 77 g/d. The realized difference in RFI, therefore, is 8.6 % of the mean FI.

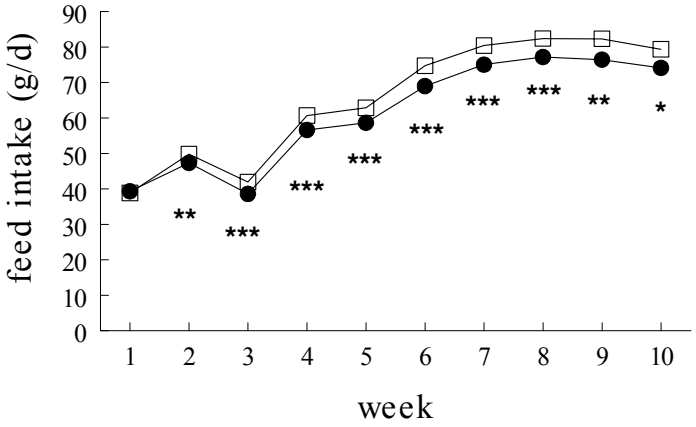


FIGURE 2.1 Retrospective mean feed intake of the top 50 efficient R– chickens (–●–) with low residual feed intake and the top 50 non-efficient R+ chickens (–□–) with high residual feed intake. Phenotypic selection for residual feed intake was made after a 10-wk selection period. Asterisks indicate level of significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

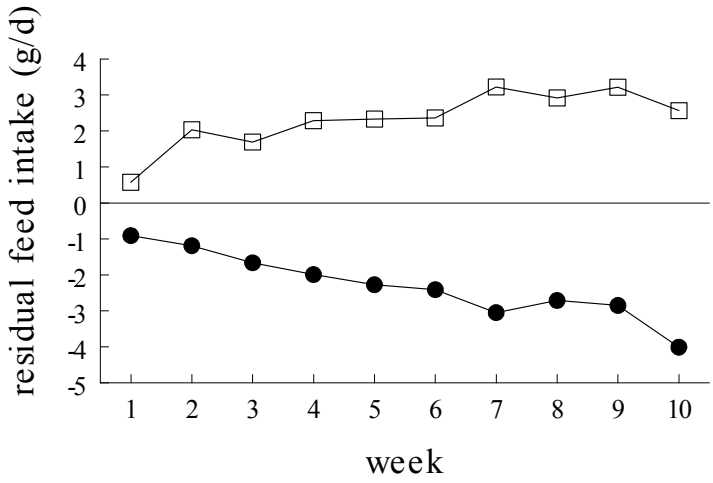


FIGURE 2.2 Retrospective mean residual feed intake of the top 50 efficient R– chickens (–●–) with low residual feed intake and the top 50 non-efficient R+ chickens (–□–) with high residual feed intake. Phenotypic selection for residual feed intake was made after a 10-wk selection period. Differences between R– and R+ were significant throughout the selection period ($P < 0.001$).

General Characteristics of Selected R− and R+ Animals

Both groups of 30 animals had equal BW during the remaining part of the experiment from week 12 onwards. BW of R+ animals was slightly lower than BW of R− animals in week 10 and 11. There were no treatment effects. Differences in FI gradually decreased, starting in week 9 before selection, to almost zero in week 16. Egg laying characteristics were analyzed only for birds with at least one non-defective egg laid. There were no differences between R− and R+ chickens for AFE, total egg number, or total egg weight. Mean egg weight in R− animals was higher than in R+ animals. Results of egg laying characteristics are shown in Table 2.1.

TABLE 2.1. Egg laying characteristics of chickens with low (R−) or high (R+) residual feed intake, immunized with three different antigens

treatment	RFI class[4]	number of egg laying chickens	age at first egg (d)	total number of eggs laid	total egg weight (g)	mean egg weight (g)
K[1]	R-	7	147.1	8.31	439.40	52.81
	R+	7	150.0	9.51	476.60	49.89
M[2]	R-	6	149.1	7.86	443.80	55.12
	R+	10	148.2	7.80	408.39	52.50
S−[3]	R-	9	151.1	7.26	379.58	52.82
	R+	7	148.4	8.24	420.13	51.40
SEM		-	1.5	1.24	64.90	1.39
				P values		
R[4]		-	0.84	0.49	0.79	0.05
T[5]		-	0.69	0.59	0.67	0.21
R × T		-	0.21	0.86	0.80	0.85

[1] Keyhole Limpet Hemocyanin

[2] *Mycobacterium butyricum*

[3] Heat-inactivated *Salmonella enteritidis* bacteria

[4] Class of residual feed intake

[5] Treatment

Immunological Characteristics of Selected R- and R+ Animals

Results of statistical analysis of antibody titers against KLH, *M. butyricum*, *Salmonella* Enteritidis LPS, and *Salmonella* Enteritidis protein are shown in Table 2.2 for the primary response and in Table 2.3 for the secondary response.

TABLE 2.2 Mean total antibody titer during primary immune response of chickens with low (R–) or high (R+) residual feed intake, immunized with one of three different antigens*

treatment	RFI[4] class	anti-KLH-antibodies[1]	anti-*M. butyricum*-antibodies[2]	anti-*S. enteritidis*[3] LPS[6]-antibodies	anti-*S. enteritidis*[3] protein-antibodies
K[1]	R−	7.80	4.77	5.13	4.13
	R+	7.61	3.45	5.00	4.73
M[2]	R−	2.60	4.61	5.18	4.08
	R+	2.63	4.87	5.28	4.75
S–[3]	R−	2.52	4.28	6.88	5.38
	R+	2.30	4.54	6.94	6.10
SEM		0.21	0.60	0.32	0.41
		P values			
R[4]		0.48	0.59	0.98	0.05
T[5]		< 0.001	0.58	< 0.001	0.002
R × T		0.82	0.32	0.93	0.99
time		< 0.001	< 0.001	< 0.001	< 0.001
time × R		0.69	0.28	0.90	0.51
time × T		< 0.001	0.06	< 0.001	0.002
time × R × T		0.55	0.97	0.40	0.96

*Differences are within columns

[1]Keyhole Limpet Hemocyanin

[2]*Mycobacterium butyricum*

[3]Heat-inactivated *Salmonella enteritidis* bacteria

[4]Class of residual feed intake

[5]Treatment (immunization with one of three different antigens)

[6]Lipopolysaccharide

Anti-KLH-antibodies. There was no interaction between RFI class and immunization treatment in the primary antibody response, indicating that R– and R+ chickens did not differ in their antibody response, regardless of treatment. Significantly higher titers were found in both R- and R+ chickens immunized with KLH compared to the animals immunized with *M. butyricum* or heat-inactivated *S.* Enteritidis ($P < 0.001$). Anti-KLH-antibody titers of animals immunized with *M. butyricum* or heat-inactivated *Salmonella* bacteria were similar.

Anti-M. butyricum-antibodies. There were no interactions between RFI class and treatment. There were no significant differences between RFI classes or between treatments, neither in the primary response, nor in the secondary response. Apparent differences in levels of antibody production at day 0 were not significant. Antibody production to *M. butyricum* was slowly increasing in all treatment groups. After the booster immunization on day 23, the rate of antibody production was increased.

Anti-Salmonella LPS-antibodies. There were no interactions between main effects, but there was a significant treatment effect: both R– and R+ chickens that were immunized with heat-inactivated *Salmonella* reached higher titers compared to birds immunized with KLH or *M. butyricum*. There were no differences between R– and R+ birds, neither in the primary response, nor in the secondary response.

Anti-Salmonella protein-antibodies. No interaction between RFI class and treatment was found. In both primary and secondary response there was a significant RFI class effect, with R+ animals reaching higher titers than R– animals. There was also a significant treatment effect: *S.* Enteritidis-immunized animals reached higher titers than KLH or *M. butyricum* immunized animals.

TABLE 2.3 Mean total antibody titer during secondary immune response of chickens with low (R–) or high (R+) residual feed intake, immunized with one of three different antigens*

treatment	RFI[4] class	anti-KLH-antibodies[1]	anti-*M. butyricum*-antibodies[2]	anti-*S. enteritidis*[3] LPS[6]-antibodies	anti-*S. enteritidis*[3] protein-antibodies
K[1]	R−	10.22	5.94	5.77	4.78
	R+	10.84	4.92	5.78	5.20
M[2]	R−	2.63	6.11	6.03	4.65
	R+	2.61	7.06	5.97	5.63
S–[3]	R−	2.78	5.56	9.15	7.58
	R+	2.42	6.08	10.66	9.23
SEM		0.26	0.56	0.43	0.50
			P values		
R[4]		0.72	0.74	0.17	0.02
T[5]		< 0.001	0.12	< 0.001	< 0.001
R × T		0.17	0.19	0.13	0.49
time		< 0.001	< 0.001	< 0.001	< 0.001
time × R		0.59	0.88	0.10	0.21
time × T		< 0.001	0.41	< 0.001	< 0.001
time × R × T		0.63	0.80	0.29	0.14

* Differences are within columns

[1] Keyhole Limpet Hemocyanin

[2] *Mycobacterium butyricum*

[3] Heat-inactivated *Salmonella enteritidis* bacteria

[4] Class of residual feed intake

[5] Treatment (immunization with one of three different antigens)

[6] Lipopolysaccharide

2.4 DISCUSSION

Residual feed intake

Our first goal was phenotypic selection of pullets on RFI. For three reasons it was doubtful, however, whether pullets could be phenotypically selected on RFI. Firstly, because previous research on RFI involved mostly laying hens and in some cases cocks (Gabarrou et al., 1997; Morisson et al., 1997; Gabarrou et al., 2000); and secondly, because in previous research the experiments started when the animals had already reached maturity (Bordas and Merat, 1981; Braastad and Katle, 1989; Luiting et al., 1991). Finally, because in a lot of research on RFI selection lines were used, in which animals were divergently selected for RFI for several generations (Bordas et al., 1992; Gabarrou et al., 1998; Bordas and Minvielle, 1999). Instead, we used chickens of a common available, commercial breed, and phenotypically selected them for RFI from 4 until 14 weeks of age. Pullets instead of mature animals were used, because our goal was to characterize young chickens on growth efficiency. Furthermore, younger animals are more suitable to obtain information on immune competence, because immune responses may decline with advancing age (Tizard, 2000), making it more difficult to observe potential differences in antibody response between efficient and non-efficient animals.

For calculation of RFI we used a model derived from Luiting and Urff (1991). They concluded that there are two alternative models to relate FI to maintenance, both equally superior to a model with a zero intercept and an exponent of 3/4 ($BW^{3/4}$): an approximation by inclusion of an intercept into the model, or a non-linear approximation with the exponent to be estimated. The metabolic BW exponent varies with age, as reviewed by Van Kampen (1987) and as established by Luiting and Urff (1991). We used, therefore, the non-linear approximation in our experiment, and found a value of 0.747 for the metabolic BW exponent. So, both alternative models would have been useful.

We found no differences in BW between R– and R+ animals. Therefore, R– and R+ animals could not be distinguished based on their BW during the experiment. Differences in FI were considerable, and were already present early in life. FI in the third week was decreased in both groups, which might have been caused by an effect of vaccination against fowl pox. A more probable explanation, however, is that after week 2 the residues in the feeding troughs were not replaced by fresh feed, but again put in the feeders after weighing. Obviously the "old" feed was less attractive. From this moment on, leftovers of one week were always replaced by fresh feed. Despite a reduced FI, BW still increased in this particular

week, but at a slower rate. In general, differences in FI are pronounced, with R+ animals eating significantly more than R– animals. The large decrease in BW gain for both groups in week 3 can be attributed to the decreased FI in this week. It is unclear why BW gain in R+ animals was higher in week 6 and lower in week 7 compared to BW gain of R– animals.

It can be concluded that R– animals need less feed than R+ animals to reach the same BW. Differences in RFI between both lines can be attributed to a high FI in R+ animals and a low FI in R– animals. As a consequence, R– chickens are considered as more efficient growing animals than R+ chickens. BW and BW gain curves are similar in both R– and R+ groups. It can be concluded, therefore, that extra FI in R+ animals is not invested in extra BW gain, but obviously is used for maintenance processes. Differences in RFI between chickens at 14 weeks of age, calculated over a 72-day period, are already present early in life and gradually increasing. This is in accordance with observations from Bordas and Minvielle (1999). We found in our experiment a difference in RFI of about 7 g/d in week 10, when the animals were 14 weeks of age. Differences in RFI found in White Leghorn laying hens were approximately 15 g/d at about 50 weeks of age (Luiting et al., 1991), 7 g/d in 37-week old Rhode Island Red laying hens after five years of selection, gradually increasing to about 21 g/d in the 14^{th} generation (Bordas et al., 1992) and 34 g/d in the 17^{th} generation (El Kazzi et al., 1995) of RFI-selected lines. It is obvious that our results represent small but significant differences between R– and R+ chickens.

In conclusion, our experiment demonstrated that differences in efficiency within a commercial outbred flock are considerable, and that it is possible to select young hens on RFI. Because no information on egg laying characteristics was included in the model, it does not have predictive value for efficiency in future egg production.

Immunization experiment

The next stage of the experiment was to investigate whether selected populations of 30 R– and 30 R+ animals differ in their capability to mount an antibody response against various antigens. KLH immunization leads to a strong antibody response to KLH, with a typical peak on d 9 after primary immunization, and a memory response after booster immunization. The same situation is applicable for antibody production against *S.* Enteritidis LPS and *S.* Enteritidis protein, which also show a peak after primary immunization, and a memory response after booster immunization. Yet, the antibody response against *M. butyricum* does not show a typical peak in the primary immune response, but is slowly increasing throughout

the immunization experiment. This is in agreement with results from Sijben et al. (2000), who furthermore concluded that KLH and *M. butyricum* could serve as controls for each other when studying antibody responses. The current experiment, however, shows that *M. butyricum* serves as a good control for KLH, but not vice versa.

Previously, it was shown that mounting antibody responses may not be an energy demanding process (Klasing, 1998), but may be connected with reallocation of fat resources (Parmentier et al., 2002). In the former study birds were not selected for RFI. In the present study we used animals that differ in efficiency, but we detected no differences in antibody production to KLH, *M. butyricum*, nor *S.* Enteritidis LPS between R– and R+ animals within each treatment group. Involvement of fat reallocation was not determined. *S. enteritidis* protein, however, gave rise to significantly higher antibody titers in R+ animals than R– animals within every treatment group. The reason for this observation is not clear. *S.* Enteritidis protein used in the ELISA originated from a batch of heat-inactivated *S.* Enteritidis bacteria. It is suggested that heat-treatment may have released a mix of various bacterial components. The results of the current experiment show that R+ animals in all treatment groups are better responders to this mix of *S.* Enteritidis components than R– animals, which suggests a higher level of non-antigen specific antibodies or natural antibody level to *Salmonella* protein in R+ animals. From an immunological point of view it is not efficient to put a lot of energy in mounting a specific immune response to an excess of antigens. Yet, it would be much more profitable to be able to respond in a non-specific manner. This might be a careful indication that inefficient (in terms of RFI) R+ animals have more energy left to maintain their "thermostat" of non-antigen specific immune reactivity at a higher level than efficient R– animals.

The antigens used in this experiment were non-replicating, and were therefore relatively harmless. Furthermore, the chickens were housed under thermoneutral conditions, and they were fed ad lib. These circumstances may have contributed to a situation in which animals could easily deal with the model antigens we used. Concluding from this experiment, immunoglobulin production in chickens between 16 and 20 weeks of age is probably not influenced by efficiency in terms of RFI. It remains to be established, whether immune responses have similar fitness priorities when a heavier pressure is put on the immune system by simultaneously occurring stress conditions, for instance due to an energy-demanding infection.

2.5 ACKNOWLEDGEMENTS

We thank Ger de Vries Reilingh, Wageningen University, for technical assistance during the experiment.

2.6 REFERENCES

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? J. Anim. Breed. Gen. 110:161-170.

Bentsen, H. B. 1983. Genetic variation in feed efficiency of laying hens at constant body weight and egg production 1. efficiency measured as a deviation between observed and expected feed consumption. Acta Agric. Scand. 33:289-304.

Bliss, J., V. Van Cleave, K. Murray, A. Wiencis, M. Ketchum, R. Maylor, T. Haire, C. Resmini, A. K. Abbas, S. F. Wolf. 1996. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. J. Immunol. 156:887-894.

Bordas, A., and P. Merat. 1974. Genetic variation in laying hens and phenotypic correlations of feed consumption corrected for body weight and egg production. Ann. Genet. Sel. Anim. 6:369-379.

Bordas, A., and P. Merat. 1981. Genetic variation and phenotypic correlations of food consumption of laying hens corrected for body weight and production. Br. Poult. Sci. 22:25-33.

Bordas, A., and F. Minvielle. 1999. Patterns of growth and feed intake in divergent lines of laying domestic fowl selected for residual feed consumption. Poult. Sci. 78:317-323.

Bordas, A., M. Tixier Boichard, and P. Merat. 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode Island red laying hens. Br. Poult. Sci. 33:741-754.

Braastad, B. O., and J. Katle. 1989. Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. Br. Poult. Sci. 30:533-544.

Bradley, L. M., K. Yoshimoto, and S. L. Swain. 1995. The cytokines IL-4, IFN-gamma, and IL-12 regulate the development of subsets of memory effector helper T cells in vitro. J. Immunol. 155:1713-1724.

Deerenberg, C., V. Arpanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proc. R. Soc. Lond. B. 264:1021-1029.

Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. Am. J. of Physiol. 42:R1631-R1637.

Dunnington, E. A. 1990. Selection and homeostasis. Pages 5-12 in Proceedings of the 4th World Congress on genetics applied to livestock production. Edinburgh, Scotland. UK.

El Kazzi, M., A. Bordas, G. Gandemer, and F. Minvielle. 1995. Divergent selection for residual food intake in Rhode Island red egg-laying lines: Gross carcase composition, carcase adiposity and lipid contents of tissues. Br. Poult. Sci. 36:719-728.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39:79-89.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127:2371-2376.

Gabarrou, J. F., P. A. Geraert, J. Williams, L. Ruffier, and N. Rideau. 2000. Glucose-insulin relationships and thyroid status of cockerels selected for high or low residual food consumption. Br. J. Nutr. 83:645-651.

Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77:1119-1125.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos. 88:87-98.

Luiting, P., J. W. Schrama, W. v. d. Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. Br. Poult. Sci. 32:763-782.

Luiting, P., and E. M. Urff. 1991. Optimization of a model to estimate residual feed consumption in the laying hen. Livest. Prod. Sci. 27:321-338.

Morisson, M., A. Bordas, J. M. Petit, C. Jayat Vignoles, R. Julien, and F. Minvielle. 1997. Associated effects of divergent selection for residual feed consumption on reproduction, sperm characteristics, and mitochondria of spermatozoa. Poult. Sci. 76:425-431.

Mosmann, T. R., and S. Sad. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol. Today, 17:138-146.

Parmentier, H. K., S. Bronkhorst, M. G. B. Nieuwland, G. De Vries Reilingh, J. M. Van Der Linden, M. J. W. Heetkamp, B. Kemp, J. W. Schrama, M. W. A. Verstegen, H. Van Den Brand. 2002. Increased fat deposition after repeated immunizations in growing chickens. Poult. Sci. 81:1308-1316.

SAS Institute. 1999. Version 8. SAS Institute Inc., Cary, NC, USA

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. TREE 11:317-321.

Sijben, J. W. C., H. de Groot, M. G. B. Nieuwland, J. W. Schrama, and H. K. Parmentier. 2000. Dietary linoleic acid divergently affects immune responsiveness of growing layer hens. Poult. Sci. 79:1106-1115.

Tizard, I. R. 2000. Veterinary Immunology: an introduction. Sixth edition. W. B. Saunders Company, Philadelphia.

Van Eerden, E., H. Van den Brand, G. De Vries Reilingh, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004. Residual feed intake and its effect on Salmonella enteritidis infection in growing layer hens. Poult. Sci. 83:1904-1910.

Van Kampen, M. 1987. Climatic conditions and energy metabolism of laying hens. Pages 199-216 in Energy metabolism in farm animals: Effects of housing, stress and disease. M. W. A. Verstegen and A. M. Henken ed. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Verhulst, S., S. J. Dieleman, and H. K. Parmentier. 1999. A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. Proc. Natl. Acad. Sci. USA. 96:4478-4481.

Verstegen, M. W. A., W. v. d. Hel, H. A. Brandsma, A. M. Henken, and A. M. Bransen. 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. Pages 21-48 in Energy metabolism in farm animals: Effects of housing, stress and disease. M. W. A. Verstegen and A. M. Henken ed. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

CHAPTER 3

Residual Feed Intake and its effect on *Salmonella* Enteritidis infections in pullets

E. Van Eerden, H. Van Den Brand, G. De Vries Reilingh, H. K. Parmentier,
M. C. M. De Jong, B. Kemp

Published in Poultry Science (2004) 83:1904-1910

ABSTRACT

Previous phenotypic selection on residual feed intake (RFI) identified 20 efficient R– chickens and 20 non-efficient R+ chickens. RFI is defined as the difference between observed feed intake (FI) and expected FI based on metabolic body weight (BW) and BW gain, and was used as a measure for feed efficiency. BW and BW gain were similar for both groups. FI and RFI were significantly higher in R+ animals. It is hypothesized that non-efficient R+ animals are more flexible to divert resources from production processes towards maintenance processes, thus being better capable of handling a bacterial challenge. Chickens of both groups were randomly allocated to immunization with heat-inactivated *Salmonella* Enteritidis bacteria, or inoculation with live *Salmonella* bacteria.

Transportation to the isolation units caused a decrease in FI in R+ animals. This may reflect a particular way of coping with stress in R+ animals.

More R+ animals stopped bacterial shedding considering a non-shedding interval of 10 days or 11 days (P = 0.041). Non-antigen-specific antibody responses against Keyhole Limpet Hemocyanin were higher in R– animals.

We conclude that R+ animals are able to keep their metabolism at a higher level, indicated by higher heart and liver weights, and that *Salmonella* infection leads to reduced heart, liver, and gizzard weights. Oviduct weight and number of small yellow follicles were reduced in infected animals. Antigen-specific antibody responses were not different between the groups, indicating high priority for this parameter as a life trait. Possible differences in stress susceptibility between efficient and non-efficient chickens need further examination.

Abbreviation Key: AFE = age at first egg; BGA = Brilliant Green Agar; BPW = buffered pepton water; BW = body weight; EM = egg mass production; FI = feed intake; KLH = Keyhole Limpet Hemocyanin; LPS = lipopolysaccharide; MSRV = Modified Semisolid Rappaport Vassiliadis; RFI = residual feed intake

3.1 INTRODUCTION

Efficient production at low costs is the most important goal in poultry husbandry. Therefore, laying hens are selected for generations towards highly efficient egg mass production at low maintenance costs. There is, however, a lot of variation in feed intake (FI) between chickens with the same BW and the same production level, which is reflected in variation in residual feed intake (RFI). RFI is the difference between observed feed intake and feed intake as predicted from metabolic BW, growth, and egg mass production (Bentsen, 1983; Bordas and Merat, 1974; Luiting and Urff, 1991). Chickens with low RFI (R–) eat less than predicted, and are, therefore, considered as more efficient producing animals than chickens with high RFI (R+). It is hypothesized that the extra energy intake of R+ animals, which is not put into production processes, is available for other resource demanding functions or life traits, as can be deduced from the resource allocation theory (Beilharz et al., 1993). This theory states that, in an environmentally limiting situation, animals have a package of finite resources. Resources used by one function are no longer available for other functions, meaning that animals have to make a trade-off between allocation of resources towards life traits to obtain maximal fitness. The theory hypothesizes that, under natural selection, animals are flexible in the way they allocate their resources. Continuous artificial selection for efficient production may, however, have lead to animals that are genetically programmed to put a lot of resources in production processes (Dunnington, 1990), which may result in an impaired ability to divert resources to maintenance processes. In a situation where animals would have to make a trade-off between vital life traits, R– animals would be less flexible to put resources into maintenance processes, whereas R+ would have resources remaining.

In previous experiments we selected a population of efficient R– chickens and a population of non-efficient R+ chickens, phenotypically and divergently selected for RFI (Van Eerden et al., 2004). Although non-specific antibody responses against *Salmonella* Enteritidis components were higher in R+ animals, no differences in specific antibody responses between efficient and non-efficient animals were found after immunizations with relatively harmless, non-replicating antigens. A challenge that would put a heavier pressure on the immune system, such as a bacterial infection with an accompanying energy demanding acute phase response, however, is more likely to force animals to make a trade-off between vital life traits. Klasing (1998) stated that the specific immune system only needs a low

amount of resource substrates relative to needs for growth or egg production. Yet, an acute phase response (production of acute phase proteins, fever) is supposed to need more nutrients during an infectious challenge than the immune system itself (Klasing, 1998), especially because infection may be accompanied by a state of anorexia. In the current experiment we investigated the effect of phenotypic selection for RFI on the ability of chickens to cope with a bacterial challenge. We used *Salmonella* Enteritidis as an infection model. Immunization with the same strain, but heat-inactivated *Salmonella* Enteritidis was included in the experiment to activate the immune system without arousing effects of bacterial colonization.

3.2 MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Wageningen University approved all experimental protocols.

Animals

Chickens used in the current experiment were phenotypically selected for Residual Feed Intake (RFI) as described in Van Eerden et al. (2004). In short, individual BW, BW gain (ΔG), and FI of 352 Lohmann Brown chickens were recorded weekly from 4 weeks until 14 weeks of age (week 1 until week 10 in the experiment). RFI is the part of FI that can not be explained by mean metabolic BW and BW gain in this period, and is, therefore, the error term in the model (Van Eerden et al., 2004)

$$FI = 19.39 \times BW^{0.75} + 1.50 \times \Delta G + e.$$

At the end of week 10, at 14 weeks of age, 50 efficient R– chickens and 50 non-efficient R+ chickens were selected, based on RFI. Twenty chickens from both groups were used in the current experiment. In week 11 the animals were transported from the stable to two identical, large open circuit respiration chambers (Verstegen et al., 1987), which were used as isolation units. Each chamber contained 10 R– animals as well as 10 R+ animals. Temperature was maintained at 21°C, and humidity at 60 % in both chambers. The chickens were individually house in wire bottom battery cages, and received 9 hours light: 15 hours dark, with the light period from 8.00 a.m. until 5.00 p.m.

All animals were fed a grower diet (ME 2,600 kcal/kg, CP 175 g/kg) in individual feeding troughs. Feed and water were available ad libitum. FI was not recorded in week 11.

BW and FI were recorded weekly, restarting the first day of week 12. When applicable, age at first egg (AFE), total egg number and total egg weight per chicken were recorded until the end of the experiment, when the chickens were 20 weeks of age. Week 2 during RFI selection contained nine days, and week 11 contained 5 days; all other weeks contained 7 days.

Treatment

Treatment started when the animals were 16 weeks of age: the animals in chamber 1 were orally inoculated with 0.5 mL of a bacterial suspension containing 1.5×10^8 colony forming units (CFU) *Salmonella* Enteritidis phage type 4 (treatment S+). The animals in chamber 2 were immunized subcutaneously (s.c.) with 1 mL PBS containing 4×10^7 CFU heat-inactivated *Salmonella* Enteritidis bacteria (treatment S–). Immunization and inoculation doses were based on previous pilot experiments (unpublished results). In chamber 1 one cage was left empty between every two chickens to avoid bacterial transmission through physical contact. A strict hygiene protocol was applied to prevent *Salmonella* transmission from chamber 1 to chamber 2.

A blood sample of each animal was taken on day 0 before treatment, and on day 9, 16, 23, and 29, post inoculation/immunization (p.i.). Fresh blood samples were centrifuged and plasma was stored at –20°C until further processing. On day 23 p.i. the chickens in chamber 2 received a booster immunization s.c. with the same amount of antigen as on day 0.

Starting on day 0, feces samples from each of the inoculated chickens in chamber 1 were taken daily through day 29 p.i. to examine bacterial shedding. Immunization of the animals in chamber 2 was not expected to result in bacterial shedding. Yet, mixed feces samples were taken occasionally from the animals in chamber 2, as a control. The experiment ended at day 31 p.i., when the animals were euthanized.

Salmonella* Enteritidis *preparation

The inoculation dose was prepared by growing *Salmonella* Enteritidis (provided by J. Wagenaar, ASG Lelystad) in buffered pepton water (BPW) overnight at 37°C. The bacteria were centrifuged at 3000 × g for 15 minutes and washed twice in sterile PBS. The final concentration was 3×10^8 CFU/mL.

The immunization dose was prepared by diluting the bacteria to a concentration of 4×10^7 CFU/mL. The suspension was poured into a bottle and placed in a water bath at 65°C for

one hour. The final suspension of heat-inactivated bacteria was determined to be sterile, as culture in Brain Heart Infusion was found to be negative.

Feces samples and bacteriological examination

For qualitative *Salmonella* determination, a fresh feces sample from each animal was homogenized and 1 g was suspended in 10 mL buffered pepton water (BPW). The samples were kept overnight at 37°C. The tubes were shaken briefly and 1 mL of fluid was stored in 15% glycerol at −70°C until further processing. The frozen samples were thawed at 37°C and a 0.1 mL drop of each sample was put on a Modified Semisolid Rappaport Vassiliadis (MSRV) plate. Glycerol was proven not to be disturbing. The plates were incubated at 42°C during 24 hours. Negative plates were incubated another 24 hours. Positive plates were randomly verified by culture on Brilliant Green Agar (BGA), and serotyped with *Salmonella* agglutination sera. The 0.9 mL leftover of each sample was grown in BPW. When a sample was negative on the MSRV plate, the culture in BPW was plated onto BGA, incubated at 37°C for 24 hours, and evaluated for typical *Salmonella* colonies.

Post mortem examination

All 40 chickens were killed on d 31 by injecting T61® (Hoechst Roussel Vet GmbH) in the wing vein. Body weight was recorded as well as organ weights: heart, liver, spleen, gizzard plus proventriculus, bursa, oviduct, ovary, and stroma; stroma being defined as ovary without large yellow follicles. Total intestinal length was measured. Large yellow follicles (> 10 mm) were counted and individually weighed; small yellow follicles (5-10 mm) were counted.

Samples of liver, spleen, and ovary of each of the inoculated chickens were cut into small pieces and suspended in 10 mL BPW for qualitative *Salmonella* determination. One gram of caecal content of each of the inoculated chickens as well as each of the immunized chickens was suspended in 10 mL BPW. All samples in BPW were kept overnight at 37°C. The tubes were briefly shaken, and 1 mL of fluid of each tube was stored in 15% glycerol at −70°C until further processing. Bacteriological examination of organ samples and caecal samples was carried out as described for the feces samples in the previous section.

ELISA procedure

Natural antibody titers to Keyhole Limpet Hemocyanin (KLH, ICN Biomedicals) and *Mycobacterium butyricum* protein (Difco Laboratories), and specific antibody titers to *Salmonella* Enteritidis lipopolysaccharide (LPS, Sigma Aldrich Chemie) and *Salmonella* Enteritidis protein were determined by indirect ELISA in plasma of all 40 chickens. The procedure is described in detail in Van Eerden et al., 2004

Statistical analysis

The four treatment combinations were arranged as a 2 x 2 factorial model: each of treatment S– and S+ at two levels (R– and R+). BW, FI, data collected during post mortem examination, and egg laying characteristics were analyzed using the model:

$$Y= \mu + R + T + R \times T + e,$$

where Y = dependent variable, μ = overall mean, R = RFI class (R– or R+), T = treatment (S+ or S–), R × T = interaction between RFI class and treatment, and e = residual error. Number of large and small yellow follicles were analyzed only for animals with ovarian development; ovarian development being defined as presence of at least one small yellow follicle on the ovary. Egg laying characteristics were analyzed only for chickens with at least one, non-defective egg laid.

Antibody titers were analyzed using a model including repeated measurements:

$$Y = \mu + R + T + R \times T + \text{Bird } (R \times T) + \text{time} + \text{time} \times R + \text{time} \times T + \text{time} \times R \times T + e,$$

where Y = dependent variable, μ = overall mean, R = RFI class (R– or R+), T = treatment (S+ or S–), R × T = interaction between RFI class and treatment, Bird (R × T) = animal nested for RFI class and treatment, time = day of blood sampling (0, 9, 16, 23, and 29), time × R = interaction between time and RFI class, time × T = interaction between time and treatment, time × R × T = interaction between time, RFI class and treatment, and e = residual error. The significance of R and T was tested with Bird (R × T) as error term, whereas time and its interactions were tested against the residual error.

The course of FI during treatment was also analyzed with a model including repeated measurements:

$$Y = \mu + \text{Group} + \text{Bird (Group)} + \text{period} + \text{Group} \times \text{period} + e,$$

where Y is the difference in FI between two time points, μ = overall mean, Group = treatment combination (R+S+, R+S–, R–S+, or R–S–), Bird (Group) = animal nested for treatment combination, period = interval between two subsequent FI recordings (from week 10 until

week 16), Group × period = interaction between treatment combination and period, and e = residual error.

Bacterial shedding was determined qualitatively, meaning that at a particular day an animal was positive (shedding) or negative (not shedding). To overcome problems with statistical analysis concerning intermittent shedding, a definition was used: animals were considered to be negative, only if they were negative for at least an interval of d before getting positive again; positive being defined as shedding for at least three consecutive d. Considering the bacterial status "negative" as failure, we calculated survival functions for both groups of R+ and R– animals, using Kaplan-Meier estimates. Analysis was carried out stepwise for non-shedding intervals of three d up to 12 days of non-shedding. All observations were censored at day 29 p.i.

Unless otherwise stated, all levels of significance were based on testing at the $P < 0.05$ level.

3.3 RESULTS

Body weight and feed intake

Figure 3.1 shows FI including the pre-selection period (week 1 till 10). In the pre-selection period FI of R+ animals was higher than FI of R– animals, starting from week 2 until week 10. FI was not recorded in week 11. The course of FI between week 10 and week 16 was analyzed in pairs, i.e. differences in FI were compared between week 10 and 12, between week 12 and 13, et cetera. Based on the presence or absence of interaction ($P < 0.05$) between treatment combination and time interval, it was decided whether the change in FI was significant or not. FI of all R+ animals was significantly decreased in week 12, before treatment was started. FI of R– animals remained almost unchanged. After treatment the immunized R+ animals showed a large increase in FI compared to the inoculated R+ animals. The immunized R– animals also increased their FI, but at a slower rate. After the initial period of increase, FI in both immunized groups decreased after week 15.

FI in both groups of inoculated animals was depressed until week 14, but increased afterwards, though R– animals at a slower rate than R+ animals. In week 14 FI of inoculated R+ animals was even below FI of immunized R– animals.

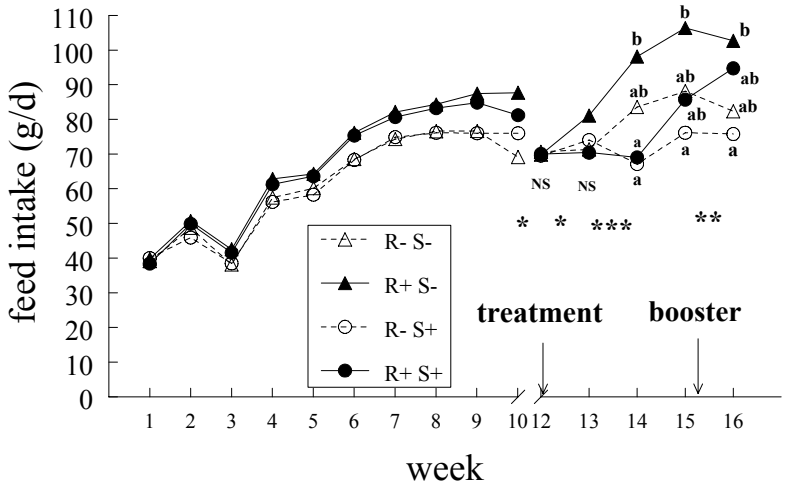


FIGURE 3.1 Mean feed intake of efficient R– and non-efficient R+ chickens, immunized with heat-inactivated *Salmonella* Enteritidis bacteria (S–), or inoculated with live *Salmonella* Enteritidis bacteria (S+). Values within a week lacking a common superscript differ ($P < 0.05$). NS = not significant. Interactions between treatment group and period between two recordings, indicating a significant change in FI, are indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Interaction in FI between RFI class and treatment was significant only in week 13. There was a significant treatment effect in week 14, with S– treated animals having higher FI than S+ treated animals. In week 15 both RFI class effects and treatment effects were significant: FI was higher in R+ animals than in R– animals, while FI in S– treated animals was higher than in S+ treated animals. Week 16 showed a significant RFI class effect, with R+ animals having higher FI than R– animals.

There were significant treatment effects in BW at day 98 and day 105 (first and last day of week 15 respectively), with S– treated animals reaching higher BW than S+ treated animals.

Post-mortem examination

The results of statistical analysis of post-mortem examination are shown in Table 3.1. There were no interactions between main effects. There were no differences in BW at necropsy. R+ chickens had higher heart and liver weights, longer intestines, and higher oviduct and ovary weights than R– chickens. Also stroma weight was higher in R+ animals.

TABLE 3.1 Body and reproduction characteristics of chickens with high (R+) or low (R–) residual feed intake, inoculated with live *Salmonella* Enteritidis bacteria (S+) or immunized with heat-inactivated *Salmonella* Enteritidis bacteria (S–)

	S+		S-			P values		
	R+	R-	R+	R-	SEM	R	S	R x S
BW (g)	1842.3	1811.2	1922.1	1808.8	53.47	0.19	0.47	0.45
Heart (g)	6.0	5.4	7.1	5.9	0.28	0.002	0.006	0.29
Liver (g)	51.4	39.6	58.6	48.0	3.49	0.03	0.03	0.86
Spleen (g)	3.1	3.4	3.2	2.8	0.24	0.76	0.36	0.18
Intestines (cm)	150.1	140.4	150.4	141.2	4.35	0.04	0.90	0.96
Gizzard (g)	57.7	57.7	52.9	50.9	1.86	0.58	0.004	0.60
Bursa (g)	1.8	1.3	1.1	1.9	0.38	0.70	0.92	0.08
Oviduct (g)	33.6	17.9	57.5	33.5	8.29	0.02	0.02	0.62
Ovary (g)	23.8	7.4	37.0	21.1	7.47	0.04	0.08	0.98
Stroma (g)	3.5	1.7	5.2	3.4	0.69	0.01	0.02	1.00
Chickens with ovarian development (#)[1]	7	5	9	6	-	-	-	-
Large yellow follicles (#)	9.2	4.7	8.3	8.8	1.25	0.12	0.21	0.06
Small yellow follicles (#)	6.9	1.8	10.6	7.8	2.29	0.10	0.05	0.62

[1] number

S+ treated chickens had lower heart and liver weights, lower number of small yellow follicles, and lower oviduct and stroma weights than S− treated animals. Total weight of gizzard and proventriculus was higher in S+ treated chickens than in S− treated chickens.

There were neither differences in spleen weight, nor differences in number of large yellow follicles between groups.

Egg laying characteristics

The results of statistical analysis of egg laying characteristics are shown in Table 3.2. Analysis was carried out only for birds with at least one non-defective egg laid. AFE did not differ between the groups. Also total number of eggs laid, total egg weight, as well as mean egg weight were not different.

TABLE 3.2 Egg laying characteristics of chickens with high (R+) or low (R−) residual feed intake, inoculated with live *Salmonella* Enteritidis bacteria (S+) or immunized with heat-inactivated *Salmonella* Enteritidis bacteria (S−)

	S+		S−			P values		
	R+	R−	R+	R−	SEM	R	S	R × S
Egg laying chickens (#)[1]	4	1	8	5	-	-	-	-
Age at first egg (d)	135.0	133.0	133.5	133.4	1.83	0.59	0.77	0.61
Eggs laid (#)	7.3	9.0	6.8	7.2	2.12	0.61	0.60	0.76
Total egg mass (g)	352.1	438.5	327.6	374.9	109.5	0.55	0.69	0.86
Mean egg weight (g)	48.4	48.7	48.0	50.6	3.17	0.65	0.81	0.72

[1] number

Humoral immune responses

Results of statistical analysis of the primary antibody response against KLH, *M. butyricum*, *Salmonella* LPS, and *Salmonella* protein are shown in Table 3.3; results of the secondary antibody response are shown in Table 3.4.

KLH. There were no interactions between RFI class and treatment. After primary and secondary immunization with *S.* Enteritidis there was a significant RFI class effect, with R– animals reaching higher titers to KLH than R+ animals. In the secondary response there was a treatment effect as well: S– treated animals had higher titers to KLH than S+ treated animals.

M. butyricum. There were no interactions between RFI class and treatment. Main effects were not significant after primary immunization with *S.* Enteritidis. There was a treatment effect after secondary immunization, with S– animals reaching higher titers to *M. butyricum* than S+ animals.

TABLE 3.3 Mean total antibody titer during primary immune response of chickens with low (R–) or high (R+) residual feed intake, inoculated with live *Salmonella* Enteritidis bacteria (S+), or immunized with heat-inactivated *Salmonella* Enteritidis bacteria (S–)

treatment	RFI[1] class	anti-KLH[3]-antibodies	anti-*M. butyricum*[4]-antibodies	anti-*S.* Enteritidis[5] LPS[6]-antibodies	anti-*S.* Enteritidis protein-antibodies
S–	R–	2.61	5.49	7.34	6.20
	R+	2.38	5.49	7.36	6.68
S+	R–	2.41	5.61	11.53	10.64
	R+	1.96	4.42	11.36	10.22
SEM		0.16	0.54	0.39	0.42
		P values			
R[1]		0.04	0.28	0.85	0.94
S[2]		0.06	0.38	< 0.001	< 0.001
R × S		0.48	0.28	0.81	0.29
time		0.002	< 0.001	< 0.001	< 0.001
time × R		0.30	0.94	0.19	0.42
time × S		0.24	0.96	< 0.001	< 0.001
time × R × S		0.13	0.17	0.83	0.29

[1] residual feed intake
[2] treatment (S+ or S–)
[3] Keyhole Limpet Hemocyanin
[4] *Mycobacterium butyricum*
[5] *Salmonella* Enteritidis
[6] lipopolysaccharide

Salmonella* Enteritidis *LPS. S+ treated animals reached much higher titers to *S.* Enteritidis LPS than S– treated animals, both in primary and in secondary antibody response. From day 23 p.i. onwards, an increase in the rate of antibody production in S– treated animals was shown. There was no significant RFI class effect, nor interaction between main effects.

Salmonella* Enteritidis *protein. S+ treated chickens had higher antibody titers to *S.* Enteritidis protein than S– treated chickens. RFI class did not lead to differences between treatment groups. After day 23 p.i., S– treated chickens increased their rate of antibody production.

TABLE 3.4 Mean total antibody titer during secondary immune response of chickens with low (R–) or high (R+) residual feed intake, inoculated with live *Salmonella* Enteritidis bacteria (S+), or immunized with heat-inactivated *Salmonella* Enteritidis bacteria (S–)

treatment	RFI[1] class	anti-KLH[3]-antibodies	anti-*M. butyricum*[4]-antibodies	anti-*S.* Enteritidis[5] LPS[6]-antibodies	anti-*S.* Enteritidis protein-antibodies
S–	R–	3.02	6.84	10.01	8.26
	R+	2.29	6.66	9.40	8.22
S+	R–	2.43	6.07	14.12	13.08
	R+	2.09	5.48	13.93	12.50
SEM		0.17	0.46	0.59	0.60
			P values		
R[1]		0.004	0.42	0.51	0.61
S[2]		0.03	0.04	< 0.001	< 0.001
R × S		0.28	0.66	0.72	0.65
time		0.51	< 0.001	< 0.001	< 0.001
time × R		0.56	0.93	0.40	0.30
time × S		< 0.001	0.03	< 0.001	< 0.001
time × R × S		0.42	0.82	0.28	0.16

[1] residual feed intake

[2] treatment (S+ or S–)

[3] Keyhole Limpet Hemocyanin

[4] *Mycobacterium butyricum*

[5] *Salmonella* Enteritidis

[6] lipopolysaccharide

Bacteriological examination

All animals were found to be negative before inoculation. All animals were continuously positive from day 2 through day 8 p.i.. The first signs of intermittent shedding became visible on day 9 p.i.. Kaplan-Meier survival functions for non-shedding intervals of three days up to 12 days showed that in all cases numerically more R+ animals than R– animals became negative. Results, however, were not significant, except for non-shedding intervals of 10 days and 11 days ($P = 0.041$). In both RFI groups only one animal was positive for caecal content. All other organs were negative. Feces samples and caecal content, which were taken as a control from the immunized animals in chamber 2, were confirmed to be negative.

3.4 DISCUSSION

The animals in this experiment originated from subpopulations of 50 R– and 50 R+ animals, selected for RFI in a population of 350 animals, from 4 until 14 weeks of age. It was concluded that both RFI groups could not be distinguished on BW. FI, however, was significantly higher in R+ animals (Van Eerden et al., 2004). Continuation of FI measurements after selection for RFI showed that FI of R+ animals sharply decreased after week 10. The drop in FI coincided with the moment that the chickens were moved to their new environment in the climate respiration chambers. FI of R– animals was less affected. The fact that R+ animals respond to transportation through a drop in FI might point to a particular way of coping with stress. A possible relation between RFI and stress susceptibility was previously noted by Braastad and Katle (1989). They suggested that selection for higher efficiency of food utilization in laying hens may result in animals which are less frustrated prior to laying, i.e. act less stressfully. Assuming that reduction in FI is an indicator of stress, then the observation that FI of our non-efficient R+ animals decreased considerably after transportation, whereas FI of R– animals remained almost unaffected, may agree with the conclusion of Braastad and Katle (1989). Luiting et al. (1994), however, showed that efficient laying hens had a lower maximum ACTH induced corticosterone response, but this maximum response was longer sustained than in non-efficient hens. It remains to be established how selection for RFI is related to stress susceptibility.

After treatment the immunized (S–) chickens in both RFI classes adapted rapidly to the new situation and their FI highly increased to a level that even exceeded pre-selection level. Previous research (Van Eerden et al., 2004) showed that immunization with heat-inactivated *Salmonella* did not affect FI. The increase in FI in the current experiment must, therefore, be attributed partly to changed housing conditions. Light intensity, for instance, was higher than in the stable where the animals were housed previously, and was probably the major cause for increased FI. The climate respiration chambers had a very constant climate as well, in which ambient temperature and relative humidity were maintained at constant levels day and night. Part of the increased FI in immunized R+ animals can probably be attributed to compensation for the drop in FI they had experienced after transportation. In general, while S– treated animals took advantage of improved housing conditions, *Salmonella* infection in S+ treated animals outweighed those benefits. After treatment the inoculated (S+) animals show depressed FI for a prolonged period of time, which may be due to an anorexic response to infection. FI was equally low in both R– and R+ animals. Two weeks after inoculation FI started to increase. FI in R+ animals increased at a faster rate than in R– animals, possibly due to a higher need for compensation. Eventually, differences in FI between R– and R+ chickens receiving S+ treatment are re-established from week 15 onwards.

Despite changed housing conditions and a bacterial challenge, FI of S+ treated R– animals almost unchangingly stays at the same low level, with only a small decrease in week 14. FI of S+ treated R– animals does not rise above pre-selection level. Yet, their FI seems to balance at a level that can be considered as a minimum requirement for survival.

The pre-selection period ended at day 72, the last day of week 10. BW did not differ between the groups at that time. In week 15, however, S– treated animals had higher BW than S+ treated animals. This can be explained by the fact that FI in the S– treated animals was higher than FI of S+ treated animals until week 15. FI in both groups of S– treated animals was slightly reduced in week 16, whereas FI of S+ treated animals with high RFI was still increasing at that point. Despite the fact that FI of S+ treated animals with low RFI was at a constant low level during the experiment, even their BW increased. Eventually there were no differences in BW between the treatment combinations at post mortem-examination.

Post-mortem examination revealed that R+ chickens have a higher heart weight, liver weight, and intestinal length within each treatment group, indicating that they are able to keep their metabolism at a higher level than R– animals. R+ chickens had higher oviduct, ovary, and stroma weights than R– chickens. Number of large yellow follicles was not different

between groups. Although not significant, more R+ chickens were laying eggs within each treatment group. Our results suggest that R+ animals put more energy in organ development and reproductive body characteristics than R– animals.

S+ treated chickens had a lower heart weight and liver weight than immunized animals within each RFI class. They also had slightly less large yellow follicles and lighter ovary (although both not significant), significantly less small yellow follicles, and a lighter oviduct and stroma weight than immunized chickens within each RFI class. These data, and the observation that the S+ group had a lower number of egg laying chickens, suggest that *Salmonella* infection may have a temporarily postponing effect on organ development and reproductive development. The primary cause might be an anorexic response due to infection, and not necessarily the infection itself, because decreased nutrient intake is known to result in delayed onset of egg production (Bruggeman et al., 1999; Leeson and Summers, 1983; Renema et al., 1999). Once the chickens had started laying eggs, there were no longer differences in egg laying characteristics.

Bacteriological examination did not lead to clear results. Statistical analysis depended heavily on the definitions of "positive" and "negative" being used. The general picture, however, is that, irrespective of the chosen interval of non-shedding, numerically more R+ animals became negative than R–, probably caused by the fact that more R– animals than R+ animals show a pattern of intermittent shedding. The criterion of becoming negative for at least an interval of several days is, therefore, more difficult to meet in R– animals. Only non-shedding intervals of 10 days and 11 days led to significant differences between R+ and R– animals.

Specific antibody responses against *Salmonella* LPS and *Salmonella* protein showed marked differences between treatment groups, with S+ animals reaching much higher titers than S– animals. Booster immunization led to a strong memory response, indicated by the increased rate of antibody production from day 23 p.i. onwards. Yet, not even booster immunization could raise antibody production to the level that was seen in inoculated animals. Inoculation of living bacteria, leading to a persistent high level of antibody titers, is concluded to result in a stronger activation of the immune system than plain immunization. There were no differences between RFI groups. However, the difference in non-specific antibody responses between R+ and R– animals, as measured to KLH, suggests that there may be a trade-off between tissue development and innate immunity. Current research is in

progress to study the effect of RFI on other components of the immune system (cell-mediated immunity) in relation to infection.

We conclude from this experiment that there are indications that non-efficient R+ animals are more susceptible to stressful conditions, although they seem to be able to compensate rapidly. Whether this susceptibility to stress is related to flexibility towards trade-offs between life processes, remains to be established. Efficient and non-efficient chickens, however, do not cope differently with stress related to specific immunity, as far as antibody responses are concerned. This fact might point to the high priority that is given to immune responsiveness as a life trait.

3.5 REFERENCES

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? J. Anim. Breed. Gen. 110:161-170.

Bentsen, H. B. 1983. Genetic variation in feed efficiency of laying hens at constant body weight and egg production 1. efficiency measured as a deviation between observed and expected feed consumption. Acta Agric. Scand. 1983:289-304.

Bordas, A., and P. Merat. 1974. Genetic variation in laying hens and phenotypic correlations of feed consumption corrected for body weight and egg production. Ann. Genet. Sel. Anim. 6:369-379.

Braastad, B. O., and J. Katle. 1989. Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. Br. Poult. Sci. 30:533-544.

Bruggeman, V., O. Onagbesan, D. H. E, N. Buys, M. Safi, D. Vanmontfort, et al. 1999. Effects of timing and duration of feed restriction during rearing on reproductive characteristics in broiler breeder females. Poult. Sci. 78:1424-1434.

Dunnington, E. A. 1990. Selection and homeostasis. Pages 5-12 in Proceedings of the 4th World Congress on genetics applied to livestock production. Edinburgh, Scotland. UK.

Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77:1119-1125.

Leeson, S., and J. D. Summers. 1983. Consequence of increased feed allowance for growing broiler breeder pullets as a means of stimulating early maturity. Poult. Sci. 62:6-11.

Luiting, P., P. N. De Groot, E. Decuypere, and J. Buyse. 1994. Selection for feed efficiency and consequences for stress susceptibility (abstract). pp 104 in Proceedings of the 45th annual meeting of the European Association for animal production, Edinburgh, Scotland. UK.

Luiting, P., and E. M. Urff. 1991. Optimization of a model to estimate residual feed consumption in the laying hen. Livest. Prod. Sci. 27:321-338.

Renema, R. A., F. E. Robinson, J. A. Proudman, M. Newcombe, and R. I. McKay. 1999. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poult. Sci. 78:629-639.

SAS Institute. 1999. Version 8. SAS Institute Inc., Cary, NC, USA

Van Eerden, E., H. Van Den Brand, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004. Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. Poult. Sci. 83:1602-1609.

Verstegen, M. W. A., W. v. d. Hel, H. A. Brandsma, A. M. Henken, and A. M. Bransen. 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. Pages 21-48 in Energy metabolism in farm animals: Effects of housing, stress and disease. M. W. A. Verstegen and A. M. Henken, ed. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

CHAPTER 4

Energy partitioning and thyroid hormone levels during *Salmonella* Enteritidis infections in pullets with high or low residual feed intake

E. Van Eerden, H. Van Den Brand, M. J. W. Heetkamp, E. Decuypere, B. Kemp

Published in Poultry Science (2006) 85:1775-1783

ABSTRACT

This experiment was conducted to investigate whether feed efficiency, as measured by residual feed intake as a phenotypic trait, affects energy partitioning in pullets that have received *Salmonella* inoculation as an immune challenge. In each of 8 trials, energy partitioning was measured during 5 weeks in 15-week-old efficient R- and non-efficient R+ pullets, which were housed per efficiency group in 2 identical climate respiration chambers. After 1 week of adaptation, the pullets in 4 trials were orally inoculated with 10^8 CFU *Salmonella* Enteritidis; pullets in the remaining trials were not inoculated and served as controls. Heat production was calculated from continuous recordings of O_2 consumption and CO_2 production. Energy and nitrogen partitioning were recorded on a weekly basis. Blood samples for analyses on thyroid hormones were taken at 16, 17 and 19 weeks of age. There were no interactions between efficiency type and *Salmonella* treatment, or *Salmonella* treatment effects in energy partitioning, except for a short-term increase in heat production in inoculated pullets. R+ pullets had higher gross energy (GE) and ME intake, higher estimated ME for maintenance, lower ME/GE ratio, and higher total heat production and non-activity-related heat production, compared to R- pullets. T_3 levels in R+ pullets were higher at 16 and 17 weeks, but were lower at 19 weeks of age compared to R- pullets. T_4 levels were higher in R- at 16 weeks, and showed interactions between efficiency type and *Salmonella* treatment at 17 and 19 weeks of age. Body weights and spleen weights did not differ between efficiency groups. R+ pullets had higher heart-, liver-, and ovary weights and more large yellow follicles than R- pullets. There were no *Salmonella* effects on body and organ weights.

We conclude that R+ pullets have a faster running energy metabolism, and that they put more resources into organ development than R- pullets. Inoculation with *Salmonella* has a short-term effect on non-activity-related heat production, but does not affect energy partitioning, regardless of efficiency type.

Abbreviation key: BW = body weight; FI = feed intake; GE = gross energy; HP = heat production; ME_m = metabolizable energy for maintenance; NE_{tot} = total net energy for body weight gain and egg production; NE_{bwg} = net energy for body weight gain; NE_{bwgP} = net energy for body weight gain as protein; NE_{bwgF} = net energy for body weight gain as fat; NE_{egg} = net energy for egg production; NE_{eggP} = net energy for egg production as protein; NE_{eggF} = net energy for egg production as fat; RFI = residual feed intake

4.1 INTRODUCTION

Residual feed intake (RFI) is a parameter that can be used to differentiate chickens as feed efficient or non-efficient. RFI is defined as the difference between observed feed intake (FI) and expected FI based on metabolic body weight (BW), growth, and, in laying hens, egg production as well (Bentsen, 1983; Bordas and Merat, 1974; Luiting et al., 1991). Energy balance studies have shown that efficient R- and non-efficient R+ chickens differ in a number of physiological characteristics. The most obvious is the difference in FI: R+ chickens eat more than R- chickens. R+ chickens also have a higher heat production (Gabarrou et al., 1998; Luiting et al., 1991), higher ME for maintenance (Katle, 1991) and they are more active (Braastad and Katle, 1989). These effects have been extensively described for adult hens, which have priorities for laying eggs, but it is not known whether the physiological differences found in mature hens are also present in pullets, because of their differences in developmental priorities.

Previously, we demonstrated that BW of phenotypically selected R- and R+ pullets did not differ, but that R+ pullets had significantly heavier organ weights at 20 weeks of age (Van Eerden et al., 2004a). It was concluded that R+ pullets put more resources into organ development than R- pullets. Adult R- hens from genetically selected RFI lines were shown to have more carcass lipid and abdominal fat than R+ hens (El Kazzi et al., 1995; Gabarrou et al., 1998; Tixier et al., 1988; Zein-el-Dein et al., 1985). However, carcass composition and the amount of abdominal fat were not recorded in our previous experiment with phenotypically selected pullets (Van Eerden et al., 2004a). Therefore, it remained to be established whether energy allocated to growth would be different for R- and R+ pullets, in particular with regard to differences in protein and fat deposition.

If such a difference in protein and fat deposition exists, it can be questioned whether this partitioning is affected by energy-demanding processes, such as an activated immune system. Furthermore, it can be questioned whether in that case energy is reallocated from production processes, such as growth or egg production, towards maintenance processes.

The period during which antibodies were formed, was shown to cause a shift in metabolism in favor of fat deposition (Henken and Brandsma, 1982; Parmentier et al., 2002), although ME for maintenance was not increased in these studies. This latter observation could be due to the fact that the energy-demanding part of the immune response seems to be restricted to infections with an acute phase response (Klasing, 1998), because of the occurrence of fever and the release of cytokines. Both events interfere with metabolism, as

fever involves a change in body temperature set-point, and because cytokines stimulate hepatocytes to synthesize and secrete acute phase proteins. Therefore, it is hypothesized that an infection with an acute phase response will cause a change in heat production (HP) and a shift in energy partitioning between maintenance and production processes, with more energy being spent on maintenance and less on growth or egg production.

The objective of the current experiment was to investigate whether there are differences between young efficient and non-efficient pullets in energy partitioning towards maintenance processes instead of growth or egg production, and whether this partitioning is affected by a bacterial infection, in particular with regard to protein and fat deposition. We used *Salmonella* Enteritidis as a model pathogen. *Salmonella* Enteritidis is a non-host specific pathogen. It is able to invade and colonize the chicken intestines (Suzuki, 1994), and elicits both humoral and cellular immune responses, including an acute phase response (Holt and Gast, 2002).

4.2 MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Wageningen University approved all experimental protocols.

Animals, selection and housing

This experiment was carried out in 8 consecutive trials. For every trial 176 Lohmann Brown egg-type pullets were housed individually in battery cages from 4 until 14 weeks of age. Feed (2,600 kcal of ME/kg, 175 g/kg CP) and water were available ad libitum. Individual BW and feed intake were recorded once a week. These data were used to calculate residual feed intake as a measure for efficiency (procedure described in Van Eerden et al., 2004b). At 14 wk of age, 10 most efficient (R-) and 10 least efficient (R+) hens were selected; all other hens were removed from the experiment. At 15 weeks of age 2 hens from each selected group were killed through i.v. administration of Euthasan® (Anisane) and dissected to determine heart weight, liver weight, spleen weight and intestinal length, in order to examine potential differences in organ weights prior to *Salmonella* treatment. The remaining 16 hens were individually housed in 2 identical, open circuit climate respiration chambers (Verstegen et al., 1987) during 5 weeks. Each chamber contained 8 R- or 8 R+ hens. Temperature was maintained at 21°C and RH at 60% in both chambers. The light schedule was 9L: 15D (onset of light 7.00 h) during the whole experiment. Total number of animals used was 160; 32 for

evaluation of organ weights at 15 weeks of age (16 R- and 16 R+) and 128 for further examination of energy partitioning (64 R- and 64 R+).

Treatment

After entering the chambers, the first week was for acclimation to the chambers. At the first day of week 16, the pullets in 4 trials were orally inoculated with 10^8 colony forming units (CFU) *Salmonella* Enteritidis; this treatment was designated S+. In the other 4 trials the hens were not inoculated; these trials served as controls and were designated S-. *Salmonella* and control treatments were applied alternately.

A blood sample of each pullet was taken in heparinized tubes at 16, 17 and 19 weeks of age, i.e. on day 0 before inoculation and on day 7 and 21 after inoculation in S+ trials. The blood samples were centrifuged and plasma was stored at –20°C until further processing.

Energy and nitrogen partitioning

In each trial, energy and nitrogen partitioning were determined per chamber during 5 consecutive balance periods of 1 week each. After each balance period the pullets were weighed and individual FI was recorded. Feces (including urinary compounds), feathers, dust, and, if applicable, eggs were collected quantitatively per chamber and sampled for energy and nitrogen analysis, using bomb calorimetry and Kjeldahl analysis, respectively.

Respiration measurements were conducted throughout the balance period in 9-min intervals. HP per chamber was calculated from the O_2 consumed and CO_2 produced, according to the formula of Romijn and Lokhorst (1961). Physical activity was measured using Doppler radar equipment.

ME intake was calculated as the difference between gross energy (GE) intake and energy content of feces including urinary components. Total net energy for body weight gain and egg production (NE_{tot}) was calculated as ME minus total HP. Net energy for body weight gain (NE_{bwg}) was calculated as NE_{tot} minus net energy in eggs (NE_{egg}).

Nitrogen retention in protein for body weight gain (NE_{bwgP}) was calculated as nitrogen in feed minus nitrogen in feces (including urinary components), feathers, dust, eggs (NE_{eggP}), aerial NH_3 and NH_4^+ in water that condensed on the heat exchanger. For aerial NH_3 measurements, samples of the total outgoing air flow were directed through a wash bottle filled with a 25% H_2SO_4 solution. Samples from the wash bottle content were collected after each balance period and analyzed for nitrogen content. Fat retention for body weight gain

(NE_{bwgF}) was calculated as NE_{bwg} minus NE_{bwgP}. Fat retention in eggs (NE_{eggF}) was calculated as NE_{egg} minus NE_{eggP}.

ME for maintenance (ME_m) was calculated as total ME minus ME for body weight gain (ME_{bwg}) and ME for egg production (ME_{egg}). ME_{bwg} was calculated as $NE_{bwgP}/0.59$ plus $NE_{bwgF}/0.83$ (partial efficiencies derived from Chwalibog and Thorbek, 1980). ME_{egg} was calculated as $NE_{eggP}/0.50$ plus $NE_{eggF}/0.79$ (partial efficiencies derived from Chwalibog, 1985). Additional to body weight gain in terms of energy (in kJ per $kg^{0.75}$), we calculated growth in terms of weight (in grams per animal).

S. Enteritidis *preparation*

A nalidixic acid resistant strain of *Salmonella* Enteritidis was used in this experiment. A sample of the bacterial stock, which was stored in glycerol at –80°C, was first grown overnight at 37°C on a Brilliant Green Agar plate containing 0.01% nalidixic acid. A few colonies were transferred into 0.5 L of buffered pepton water and grown overnight. The bacterial suspension was centrifuged at 3,000 × g for 15 min. The bacteria were washed twice in sterile PBS. The final concentration was 2×10^8 CFU/mL. The inoculation dose was 0.5 mL.

Hormone assays

Triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured in all plasma samples by radioimmunoassays as described by Darras et al. (1990) for T_3 and by Darras et al. (1991) for T_4. All samples were analyzed per hormone in the same assay.

Post mortem examination

After the 5-week balance period, the now 20-week-old hens were killed through i.v. administration of Euthasan®. Body weight, heart-, liver-, and spleen weight, ovary- and stroma (defined as ovary without large yellow follicles) weight were recorded, and total intestinal length was measured. Large yellow follicles (> 10 mm) and small yellow follicles (5-10 mm) were counted.

Statistical Analysis

The PROC GLM procedure of SAS was used for statistical analysis (SAS Institute, 2004).

The pullets that were killed immediately after selection had not been subjected to *Salmonella* treatment. Therefore, differences in organ weights were tested only for efficiency type effects, using

$$Y = \mu + R + e$$

as a model, with Y = dependant variable; μ = overall mean; R = efficiency type (R- or R+), and e = residual error.

The energy balance experiment consisted of 16 balance groups: 8 trials × 2 respiration chambers, each containing R- or R+ selected pullets. There were 4 treatment combinations, arranged as a 2 × 2 factorial model: each of treatment S- and S+ at 2 levels (R- and R+). *Salmonella* exposure and control trials were applied alternately. Therefore, trial was nested within treatment. We used a model with repeated measurements:

$$Y = \mu + S + trial(S) + R + R\times S + group(R\times S\times trial) + time + interactions + e,$$

where Y = dependant variable; μ = overall mean; S = *Salmonella* treatment (S- or S+); trial(S) = trial nested within treatment; R = efficiency type (R- or R+); R×S = interaction between efficiency type and treatment; group(R×S×trial) = balance group nested within efficiency type, treatment, and trial; time = week of sampling after a balance period (week 16, 17, 18, 19); and e = residual error. Treatment was tested with trial(S) as error term, efficiency type and its interaction were tested with group(R×S×trial) as error term, and time and its interactions were tested against the residual error. Results from the first wk in the chambers were omitted from analysis, because this wk was for adaptation only. In view of the obtained results we performed a more detailed analysis by hour on HP (total, activity-related, and non-activity-related) for the first wk after *Salmonella* inoculation.

Thyroid hormone levels and post mortem data from the pullets after the 5-week balance period were analyzed using

$$Y = \mu + S + trial(S) + R + R\times S + e$$

as a model, where Y = dependant variable; μ = overall mean; S = *Salmonella* treatment (S- or S+); trial(S) = trial nested within treatment; R = efficiency type (R- or R+); R×S = interaction between efficiency type and treatment; and e = residual error. *Salmonella* treatment was tested with trial(S) as error term, whereas efficiency type and its interaction were tested against the residual error. Numbers of large and small yellow follicles were analyzed only for pullets with ovarian development, which is defined as presence of at least 1 large or small yellow

follicle on the ovary. Number of pullets with ovarian development was analyzed with multivariate logistic regression (PROC LOGISTIC from SAS). Thyroid hormone levels were not analyzed with repeated measurements in order to account for the onset of laying eggs.

Unless otherwise stated, all levels of significance were tested at the $P < 0.05$ level.

4.3 RESULTS

Five pullets were removed from the experiment after the acclimation week in the respiration chambers, because their feed intake in this period was extremely low (less than 50 g). All pullets were removed before *Salmonella* inoculation had taken place. After inoculation the chickens showed no clinical signs of illness.

Energy Partitioning

The results of energy partitioning measurements are shown in Table 4.1. There were no interactions between efficiency type and *Salmonella* treatment, or *Salmonella* treatment effects.

GE, ME, and NE. R+ pullets had higher GE intake (865.2 vs. 774.9 kJ/kg$^{0.75}$, d), higher ME intake (611.6 vs. 560.4 kJ/kg$^{0.75}$, d), and higher ME_m (388.6 vs. 362.9 kJ/kg$^{0.75}$, d), but a lower ME/GE ratio (70.8 vs. 72.3) than R- pullets. There were time×R interactions for ME_m, NE_{total}, NE_{bwg}, NE_{bwgP}, NE_{bwgF}., NE_{egg}, NE_{eggP}, and NE_{eggF}. Therefore, net energy partitioning was analyzed by week, and it was shown that the interactions were caused in particular by changes during the last balance week. From week 16 through 18, NE_{bwg} gradually increased both in R- and R+ pullets, but in week 19 NE_{bwg} in R+ pullets sharply decreased, whereas NE_{bwg} in R- pullets remained unchanged. NE_{egg} was zero in week 15 and 16, but from week 17 onwards R+ pullets started laying eggs, whereas R- pullets started in week 18. The rate of increase in NE_{egg} was higher for R+ pullets than for R- pullets. Only in the last balance week differences in NE_{egg} between R- and R+ pullets were significant: R+ pullets had higher NE_{egg} (34.2 vs. 11.9 kJ/kg$^{0.75}$, d; $P = 0.04$), higher NE_{eggP} (17.2 vs. 6.0 kJ/kg$^{0.75}$, d; $P = 0.03$) and NE_{eggF} (17.0 vs. 5.9 kJ/kg$^{0.75}$, d; $P = 0.05$) than R- pullets. This was accompanied by higher NE_{bwgP} (59.2 vs. 40.1 kJ/kg$^{0.75}$, d; $P = 0.02$) for R- pullets (Figure 4.1).

TABLE 4.1 Energy partitioning in efficient R- and non-efficient R+ pullets from 16 to 20 weeks of age, during a *Salmonella* Enteritidis infection (S+) or in a control situation (S-). Variables are expressed in $kJ/kg^{0.75}$ per d, unless otherwise stated in brackets

	R-		R+			P values					
	S-	S+	S-	S+	SEM	R	S	R×S	wk	wk×R	wk×S
n	31	29	31	32							
initial BW (g)[1]	1470	1450	1457	1486	20	0.56	-	-	-	-	-
GE intake	776.0	773.9	887.3	843.1	22.4	0.007	0.52	0.38	0.005	0.24	0.40
ME intake	556.6	564.1	619.4	603.8	13.9	0.01	0.84	0.44	0.007	0.11	0.23
ME/GE	71.8	72.9	70.0	71.6	0.4	0.009	0.49	0.60	0.03	0.27	0.26
ME_m	359.1	366.7	390.1	387.1	5.3	0.003	0.90	0.36	0.0007	0.03	0.95
HP_{total}	419.5	429.3	460.2	452.9	6.4	0.002	0.94	0.23	0.12	0.12	0.66
HP_{act}	53.2	55.8	59.8	60.4	5.2	0.32	0.70	0.86	0.0003	0.78	0.92
$HP_{non\text{-}act}$	366.3	373.3	402.3	392.5	4.5	0.0008	0.92	0.11	0.003	0.13	0.58
NE_{total}	137.0	134.9	159.2	150.9	10.0	0.10	0.80	0.77	< 0.0001	0.04	0.37
NE_{bwg}	134.6	128.6	145.8	142.5	11.4	0.31	0.84	0.91	0.0005	0.009	0.67
NE_{bwgP}	64.0	65.7	64.7	63.8	1.9	0.77	0.96	0.51	< 0.0001	0.002	0.24
NE_{bwgF}	70.7	62.9	81.1	78.6	10.1	0.24	0.78	0.80	< 0.0001	0.04	0.81
NE_{egg}	2.4	6.3	13.4	8.4	3.2	0.09	0.87	0.21	< 0.0001	0.001	0.52
NE_{eggP}	1.2	3.2	6.7	4.2	1.6	0.08	0.89	0.20	< 0.0001	0.001	0.54
NE_{eggF}	1.3	3.1	6.7	4.2	1.6	0.09	0.85	0.23	< 0.0001	0.002	0.52

[1] Initial body weight measured at 15 weeks of age; GE = gross energy intake; ME = metabolizable energy intake; ME/GE = % of GE intake used for ME; ME_m = metabolizable energy for maintenance; HP_{total} = total heat production; HP_{act} = activity-related heat production; HP_{nonact} = non-activity-related heat production; NE_{tot} = total net energy for body weight gain and egg production; NE_{bwg} = net energy for body weight gain; NE_{bwgP} = net energy for body weight gain as protein; NE_{bwgF} = net energy for body weight gain as fat; NE_{egg} = net energy for egg production; NE_{eggP} = net energy for egg production as protein; NE_{eggF} = net energy for egg production as fat

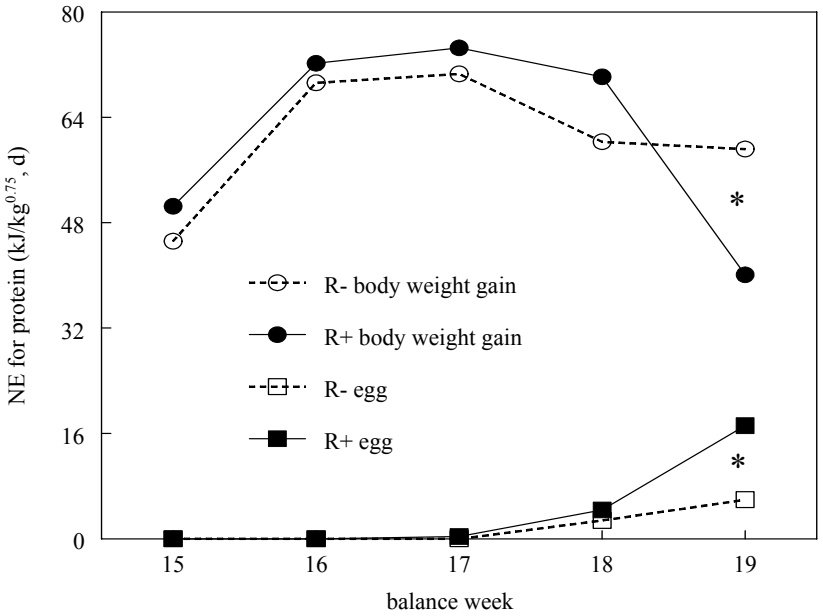


FIGURE 4.1 Net energy for protein deposition as body weight gain (circles) or eggs (squares) for efficient R- pullets (open symbols) and non-efficient R+ pullets (closed symbols) during a 5-week balance trial. Balance weeks correspond with week of age. Asterisks indicate significant differences between R+ and R- pullets within a trait ($P < 0.05$).

Heat Production. R+ pullets had a higher total HP (456.5 vs. 424.4 kJ/kg$^{0.75}$, d) and higher non-activity-related HP (396.6 vs. 369.8 kJ/kg$^{0.75}$, d). Analysis by hour for the first week after inoculation showed that, of the 168 hours tested, there were hardly interactions between efficiency type and *Salmonella* treatment (at only 2 time points for total-, 2 times for activity-related-, and 5 times for non-activity-related HP). *Salmonella* treatment effects were only present at 1 time point for total -, 11 times for activity-related-, and 6 times for non-activity-related HP. There were many efficiency type effects for total-, and non-activity-related HP (76 times and 30 times, respectively), but only a few for activity-related HP (15 times). *Salmonella* treatment effects by hour for activity-related and non-activity-related HP were too few to lead to significant effects in the analysis by week, but they appeared per variable rather closely in time. *Salmonella* effects for activity-related HP were significant at 5 time points from 69 h until 75 h after inoculation, and they are presented in Figure 4.2. Inoculated pullets in this period have a higher activity-related HP than control pullets. *Salmonella* effects for non-activity-related HP were significant at 4 time points from 21 h until 27 h after inoculation, and they are presented in Figure 4.3. Inoculated pullets in this period have a higher non-activity-related HP than control pullets.

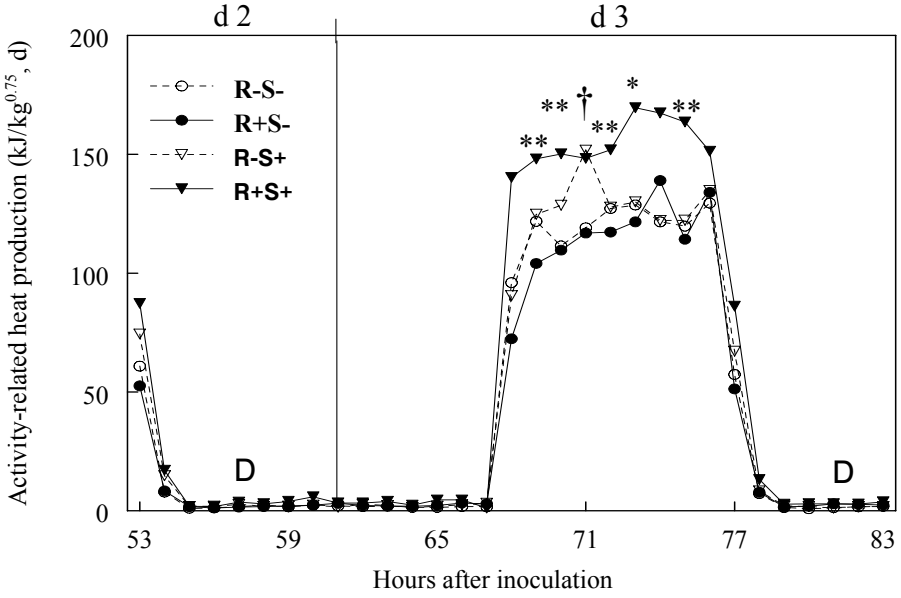


FIGURE 4.2 Activity-related heat production in efficient R- and non-efficient R+ pullets during a *Salmonella* infection (S+) or in a control situation (S-). The symbols at the x-axis where activity-related heat production is zero, represent the dark period (indicated with D). Levels of significant *Salmonella* effects are indicated by † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

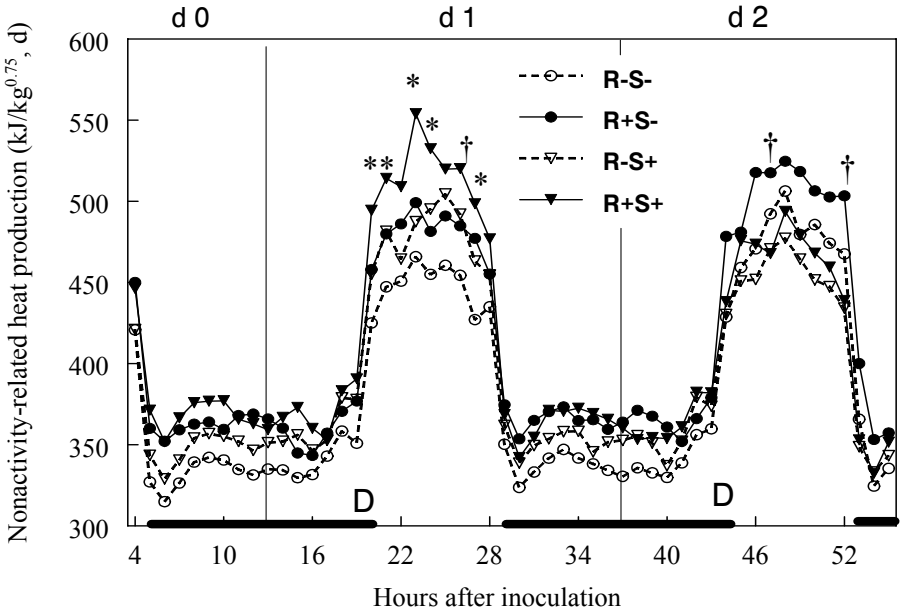


FIGURE 4.3 Non-activity related heat production in efficient R- and non-efficient R+ pullets during a *Salmonella* infection (S+) or in a control situation (S-). The dark lines at the bottom indicated with D, represent the dark period. Levels of significant *Salmonella* effects are indicated by † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

Growth. Growth in terms of weight is not included in Table 4.1, because this variable is calculated and expressed differently (in g per animal). There was no interaction between efficiency type and *Salmonella* treatment, and there were no significant main effects, but there was a significant time×R interaction (P = 0.01). Therefore, growth was analyzed by week for R- and R+ pullets, and the results are presented in Figure 4.4. It shows that growth in week 15 was low in R+ pullets and even negative in R- pullets. Results from this first balance week were not further elaborated, because this week was for adaptation only, but the low or negative level of growth can be explained by the strong reduction in FI in both efficiency groups during week 15, probably as a result of stress due to transportation, or a new environment, or both. Growth rate in R- pullets increased from week 16 onwards until the end of the experiment, whereas R+ pullets showed an increased growth rate until week 17 and a decreased growth rate in week 18 and 19.

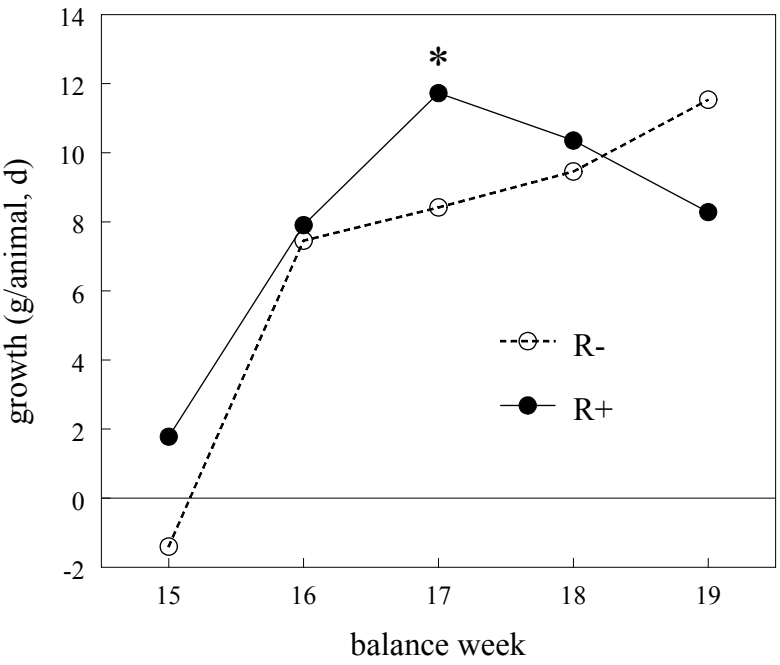


FIGURE 4.4 Growth of efficient R- pullets and non-efficient R+ pullets during a 5-week balance trial. The asterisk indicates a significant difference (P < 0.05). Balance weeks correspond with week of age

Thyroid Hormones

Intra-assay coefficients of variation for T_3 and T_4 assays were 4.5 and 5.4, respectively. The results of T_3 and T_4 hormone assays are shown in Table 4.2. There were no *Salmonella* treatment effects. Levels of T_3 in R+ pullets were higher than in R- pullets at 16 and 17 weeks of age (1.52 vs. 1.39 ng/mL and 1.53 vs. 1.37 ng/mL for 16 and 17 wk, respectively), but lower at 19 weeks of age (0.9 vs. 1.0 ng/mL; P = 0.06). At 16 weeks of age levels of T_4 were higher in R- than in R+ pullets (9.7 vs. 7.8 ng/mL). There were significant interactions between efficiency type and *Salmonella* treatment in T_4 levels at 17 and 19 weeks of age, with R+ pullets having lower levels of T_4 in control situations, but having higher levels in infection trials compared to R- pullets.

TABLE 4.2 Mean T_3 and T_4 hormone levels (in ng/mL) in efficient R- and non-efficient R+ pullets during a *Salmonella* Enteritidis infection (S+) or in a control situation (S-), measured at 16, 17, and 19 weeks of age

	R-		R+			P values		
	S-	S+	S-	S+	SEM	R	S	R×S
n	31	29	31	32				
T_3; 16 wk[1]	1.43	1.35	1.51	1.48	0.06	0.04	0.53	-
T_3; 17 wk	1.39	1.35	1.65	1.41	0.08	0.05	0.66	0.22
T_3; 19 wk	0.95	1.14	0.89	0.95	0.07	0.06	0.38	0.31
T_4; 16 wk[1]	10.14	9.31	8.03	7.56	0.55	0.0006	0.64	-
T_4; 17 wk	8.61	7.35	7.67	8.22	0.40	0.92	0.82	0.03
T_4; 19 wk	8.96	6.01	7.35	8.81	0.48	0.22	0.61	< 0.0001

[1]At this age the animals were not infected yet. Therefore, interactions are not indicated.

Post mortem Examination

Organ weights of R- and R+ pullets that were killed at 15 weeks of age, were not different (data not shown).

The results of post mortem examination of the pullets that were killed after the balance trials are shown in Table 4.3. There were no interactions between efficiency type and *Salmonella* treatment, except for intestinal length. Body weights, spleen weights, and number of small yellow follicles did not differ between efficiency types. R+ pullets had a heavier heart (5.91 vs. 5.67 g; P = 0.06), heavier liver (41.37 vs. 37.89 g; P = 0.08), heavier ovary (18.62 vs. 8.49 g) and stroma (2.78 vs. 2.12 g), and they had more large yellow follicles (5.23

vs. 2.65 g) than R- pullets, but there were no *Salmonella* effects. Prevalence of ovarian development was 74.2% for non-infected pullets, and 52.4 for infected pullets (odds ratio = 2.6; 95% confidence interval = [1.2 - 5.6]; P = 0.01). Ovarian development did not differ between efficiency types.

TABLE 4.3 Mean organ weights and follicle numbers of efficient R- and non-efficient R+ pullets after a *Salmonella* Enteritidis infection (S+) or in a control situation (S-), recorded at 20 wk of age

	R-		R+			P values		
	S-	S+	S-	S+	SEM	R	S	R×S
n	31	29	31	32				
BW (g)	1737.1	1709.5	1734.9	1772.2	30.0	0.31	0.90	0.28
heart (g)	5.29	6.05	5.68	6.13	0.13	0.06	0.29	0.24
liver (g)	39.51	36.28	42.46	40.28	1.99	0.08	0.53	0.79
spleen (g)	3.29	3.44	3.08	3.29	0.14	0.18	0.44	0.86
intestines (cm)	133.3	142.4	141.8	141.4	2.6	0.16	0.53	0.07
ovary (g)	8.99	8.00	20.71	16.53	3.58	0.002	0.62	0.62
stroma (g)[1]	2.14	2.10	3.14	2.42	0.32	0.04	0.44	0.29
pullets (#)[2] with developed ovary	22	13	24	19	-	-	-	-
LYF (#)[3]	2.78	2.53	5.34	5.12	0.80	0.002	0.87	0.99
SYF (#)[4]	3.95	8.62	6.50	6.29	1.19	0.93	0.20	0.05

[1] stroma is defined as ovary without large yellow follicles

[2] numbers; ovarian development is defined as presence of at least 1 large or small yellow follicle on the ovary

[3] number of large yellow follicles on the ovary (> 10 mm)

[4] number of small yellow follicles on the ovary (5-10 mm)

4.4 DISCUSSION

Our goal was to investigate whether there are differences between phenotypically selected R- and R+ pullets in the way they allocate their energy towards maintenance and production processes, and whether this allocation is affected by an energy-demanding immune response. Allocation towards production processes did not differ between R+ and R- pullets, because there were no effects of efficiency type on protein and fat deposition for body

weight gain or egg production. The amount of energy deposited as egg protein or fat was higher in R+ pullets only in the last balance week, and coincided with higher NE_{bwgP} in R- pullets. This result can be explained by the fact that R+ pullets had their first oviposition earlier than R- pullets, and it is thus indicative of a difference in developmental priorities between efficiency types in this stage of life.

Although allocation towards production processes was not different, there were significant differences between R+ and R- pullets on the level of energy intake and utilization. R+ pullets had a higher GE intake, and despite the lower ME/GE ratio, they still had a higher ME intake. As there were no differences between efficiency types in allocation towards production processes, the higher ME in R+ pullets was spent on maintenance processes. Higher ME_m will result in higher HP - which indeed was the case- because energy spent on maintenance is completely converted to heat. Gabarrou et al. (1998) found a higher HP in genetically selected R+ hens, and related the higher HP to the "excessive" energy intake through involvement of an increased diet-induced thermogenesis (Gabarrou et al., 1997). As an approximation of diet-induced thermogenesis, we calculated for both efficiency types the difference between non-activity-related HP during the light period (representing the fed state) and the dark period (representing the fasted state) as a percentage of ME intake, but there were no efficiency type effects (data not shown). Therefore, it is likely that the investment in higher heart- and liver weights, organs which are metabolically highly active, may partly explain the higher ME_m and thus the higher HP in our pullets.

From behavioral studies it was concluded that R+ hens from a genetically selected RFI line were physically more active (Braastad and Katle, 1989) than R- hens, but this conclusion was not reflected in activity-related HP in phenotypically selected hens in metabolic studies (Luiting et al., 1991) or in this study. Our results showed that total HP in R+ pullets was higher than in R- pullets; however, this was attributable to higher non-activity-related HP. This result indicates that R+ pullets produce more heat due to a higher metabolic activity, and that differences in physical activity play only a minor role.

The higher metabolic rate in R+ pullets is linked with higher levels of the metabolically active T_3. Bordas and Minvielle (1999) found that T_3 levels were higher in R+ pullets from genetic RFI lines at 17 weeks of age, which is in agreement with our findings that T_3 levels were higher in R+ pullets at 16 and 17 weeks of age. Gabarrou et al. (1998) found that 18-week-old R+ chickens from genetically selected RFI lines had higher levels of T_3 only after a fasting period of 2 days, but it must be noted that their experiment contained only 5 chickens per efficiency group. However, at 19 weeks of age, T_3 levels were lower in our phenotypic R+

pullets compared to R- pullets. It is likely that a developmental issue played a role here. During the onset of lay plasma T_3 levels are decreased, as described for turkeys by Lien and Siopes (1993), moreover, levels of T_3 seem to be positively correlated with relative growth (Kühn et al., 1982). Around 18 weeks of age some pullets in our experiment, mostly R+ pullets, started laying eggs. At necropsy it was also shown that reproductive development in R+ pullets was ahead of R- pullets, as indicated by the larger number of large yellow follicles on the ovary. Together with the fact that growth in R+ pullets was decreasing from 18 weeks of age onwards, whereas growth in R- pullets was increasing through the end of the experiment, it is likely that at 19 weeks of age R- pullets had still priorities for growth, while R+ pullets had already set priorities for laying eggs, resulting in a more pronounced decreased T_3 level in the latter.

The *Salmonella* challenge did not cause a reallocation of energy towards maintenance and production processes; furthermore, not a single energy balance parameter was affected by the *Salmonella* challenge, neither in the overall analysis for 4 weeks, nor in the analysis by week. It is unlikely that this result was caused by a too low inoculation dose, because shedding parameters showed that only 2 out of 61 chickens remained completely negative during the entire infection experiment (Van Eerden, unpublished data). In contrast to a previous experiment (Van Eerden et al., 2004a), there were also no effects of *Salmonella* on organ weights, except for ovarian development, which was delayed in inoculated pullets. There may be 2 causes to explain why the *Salmonella* challenge did not result in a shift in energy partitioning between maintenance and growth, firstly, because the pullets were relatively old when they were inoculated, and secondly, because the interval between 2 balances may have been too long to measure short-term effects. It is known that the response to a *Salmonella* infection is strongly age-dependent: chicks older than 2 weeks are much more resistant to intestinal colonization than younger chicks, and chicks older than 1 month generally do not show clinical signs of disease or mortality (Desmidt et al., 1997). Nevertheless, *Salmonella* treatment elicited a strong humoral immune response in 16 to 20 week-old pullets (Van Eerden et al., 2004a) and an acute phase response in chickens older than 50 weeks (Holt and Gast, 2002). The responses in the present study, however, were obviously not energetically costly enough to be detected in the energy balance. Moreover, the interval between 2 balances was 1 week. It is likely that there have been some effects, but those effects were probably mild, and compensated within the same balance period, resulting in a zero net effect. There is some evidence for this hypothesis, when we take a closer look at HP within a balance period. HP was the only parameter that was measured continuously

during the entire experiment, in contrast to all other parameters, which were determined only once a week. Further analysis by hour for this parameter revealed that there indeed was a *Salmonella* effect: non-activity-related HP was higher in S+ pullets than in S- pullets on day 1 after inoculation, but this effect was reversed on day 2, although S+ and S- did not differ significantly at that moment. From day 3 post inoculation onwards, there were no longer *Salmonella* effects for non-activity-related HP. Instead, there were *Salmonella* effects for activity-related HP on this day, which may have been attributable to a compensatory feeding activity. These results support our hypothesis that possible differences in energy balance parameters between treatment groups were averaged out within 1 balance period.

As an overall conclusion, we state that, despite differences in age-dependent developmental priorities and differences in the basis for RFI selection, both young pullets and mature hens with high RFI have higher FI, GE and ME intake, higher ME_m and higher HP, as compared to young pullets and mature hens with low RFI. *Salmonella* Enteritidis infection caused in our phenotypically selected pullets only a short-term increase in non-activity-related HP and did not result in a change in energy partitioning between maintenance and production processes, regardless of efficiency type.

4.5 ACKNOWLEDGMENTS

We gratefully acknowledge Koos Van Der Linden and Ger De Vries Reilingh for their excellent technical assistance during the experiment.

4.6 REFERENCES

Bentsen, H. B. 1983. Genetic variation in feed efficiency of laying hens at constant body weight and egg production 1. efficiency measured as a deviation between observed and expected feed consumption. Acta Agric. Scand. 33:289-304.

Bordas, A., and P. Merat. 1974. Genetic variation in laying hens and phenotypic correlations of feed consumption corrected for body weight and egg production. Ann. Genet. Sel. Anim. 6:369-379.

Bordas, A., and F. Minvielle. 1999. Patterns of growth and feed intake in divergent lines of laying domestic fowl selected for residual feed consumption. Poult. Sci. 78:317-323.

Braastad, B. O., and J. Katle. 1989. Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. Br. Poult. Sci. 30:533-544.

Chwalibog, A. 1985. Studies on energy metabolism in laying hens. Statens Husdyrbrugsforsøg, Copenhagen, Denmark, 139 pp.

Chwalibog, A., and G. Thorbek. 1980. Nitrogen retention and energy cost of protein retention in chickens kept at different temperatures. Pages 318-328 in Protein metabolism and nutrition. H. J. Oslage and K. Rohr eds. European Association of Animal Production, Braunschweig.

Darras, V. M., L. M. Huybrechts, L. Berghman, E. R. Kuhn, and E. Decuypere. 1990. Ontogeny of the effect of purified chicken growth hormone on the liver 5' monodeiodination activity in the chicken: reversal of the activity after hatching. Gen. Comp. Endocrinol. 77:212-220.

Darras, V. M., A. Vanderpooten, L. M. Huybrechts, L. R. Berghman, E. Dewil, E. Decuypere, et al. 1991. Food intake after hatching inhibits the growth hormone induced stimulation of the thyroxine to triiodothyronine conversion in the chicken. Horm. Metab. Res. 23:469-472.

Desmidt, M., R. Ducatelle, and F. Haesebrouck. 1997. Pathogenesis of Salmonella enteritidis phage type four after experimental infection of young chickens. Vet. Microbiol. 56:99-109.

El Kazzi, M., A. Bordas, G. Gandemer, and F. Minvielle. 1995. Divergent selection for residual food intake in Rhode Island red egg-laying lines: Gross carcase composition, carcase adiposity and lipid contents of tissues. Br. Poult. Sci. 36:719-728.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127:2371-2376.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. Br. Poult. Sci. 39:79-89.

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

Holt, P. S., and R. K. Gast. 2002. Comparison of the effects of infection with Salmonella enteritidis, in combination with an induced molt, on serum levels of the acute phase protein, alpha1 acid glycoprotein, in hens. Poult. Sci. 81:1295-1300.

Katle, J. 1991. Selection for efficiency of food utilization in laying hens: Causal factors for variation in residual food consumption. Br. Poult. Sci. 32:955-970.

Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77:1119-1125.

Kühn, E. R., E. Decuypere, L. M. Colen, and H. Michels. 1982. Posthatch growth and development of a circadian rhythm for thyroid hormones in chicks incubated at different temperatures. Poult. Sci. 61:540-549.

Lien, R. J., and T. D. Siopes. 1993. The relationship of plasma thyroid hormone and prolactin concentrations to egg laying, incubation behavior, and molting by female turkeys exposed to a one-year natural daylength cycle. Gen. Comp. Endocrinol. 90:205-213.

Luiting, P., J. W. Schrama, W. v. d. Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. Br. Poult. Sci. 32:763-782.

Parmentier, H. K., S. Bronkhorst, M. G. B. Nieuwland, G. D. Reilingh, J. M. van der Linden, M. J. W. Heetkamp, et al. 2002. Increased fat deposition after repeated immunization in growing chickens. Poult. Sci. 81:1308-1316.

Romijn, C., and W. Lokhorst. 1961. Some aspects of energy metabolism in birds. Pages 49-59 in 2nd Symposium on Energy Metabolism in Farm Animals. EAAP publication (10), Wageningen, The Netherlands.

SAS Institute. 2004. SAS/STAT® 9.1 *User's Guide* SAS Institute Inc., Cary, NC.

Suzuki, S. 1994. Pathogenicity of Salmonella enteritidis in poultry. Int. J. Food Microbiol. 21:89-105.

Tixier, M., A. Bordas, and P. Merat. 1988. Divergent selection for residual feed intake in laying hens: efects on growth and fatness. Pages 129-132 in Leanness in domestic birds: Genetic, metabolic and hormonal aspects. B. Leclercq and C. C. Whitehead eds. INRA and Buttersworth, London, U.K.

Van Eerden, E., H. Van den Brand, G. De Vries Reilingh, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004a. Residual feed intake and its effect on Salmonella enteritidis infection in growing layer hens. Poult. Sci. 83:1904-1910.

Van Eerden, E., H. Van Den Brand, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004b. Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. Poult. Sci. 83:1602-1609.

Verstegen, M. W. A., W. v. d. Hel, H. A. Brandsma, A. M. Henken, and A. M. Bransen. 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. Pages pp 21-48 in Energy metabolism in farm animals: Effects of housing, stress and disease. M. W. A. Verstegen and A. M. Henken eds. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Zein-el-Dein, A., A. Bordas, and P. Merat. 1985. Selection divergente pour la composante "residuelle" de la consommation alimentaire des poules pondeuses: effets sur la composition corporelle. Arch. Geflügelkd. 49:158-160.

CHAPTER 5

Natural "anti-Gal" and *Salmonella*-specific antibodies in bile and plasma of hens differing in diet efficiency

P. F. Cotter and E. Van Eerden

Published in Poultry Science (2006) 85:435-440

ABSTRACT

Specific anti-*Salmonella* Enteritidis (SE) and natural anti-α-gal epitope (Galα1-3Galβ-1-4GlcNAc-R; anti-Gal) antibodies were measured in plasma sample pools and individual bile specimens obtained from hens differing in diet efficiency. More SE somatic (O) and flagella (H) antibodies were found in plasma pools from efficient hens (R-) compared with non-efficient hens (R+) after oral challenge with live SE. Mean titers of somatic agglutinins in bile were 2.3 in R- hens and 1.9 in R+ hens (P = 0.06) following live challenge. SE antibodies were also found in bile of non-challenged hens of both types but their levels were not significantly different. Flagella (H) agglutinin scores were higher in SE challenged hens compared with non-challenged hens (3.1 vs. 2.1, $P \leq 0.004$) but efficiency types did not differ. Bile also contained high titers of the anti-Gal antibody indicated by the agglutination of glutaraldehyde stabilized rabbit erythrocytes. The average titer of all non-SE exposed hens was 9.0 corresponding to 1:5120 when corrected for the initial dilution and expressed in conventional terms. SE exposure was associated with higher anti-Gal titers. The average anti-Gal titer for all SE exposed hens was 10.0, corresponding to 1:10240 in conventional terms and this difference was significant ($P \leq 0.016$). Diet efficiency type associated differences in anti-Gal titers were not significant.

Collectively our data indicate that diet efficiency status is not associated with compromised salmonella specific immune responses. Rather it appears that the immune responses of diet efficient hens (R-) are also more efficient. This is because R- hens produced higher levels of O and H type antibody only as the result of direct exposure to living SE. On the contrary, R+ hens produced H type antibody as a result of challenge with heat killed SE a circumstance that will not result in disease. Moreover the hen type difference does not seem to occur at the expense of innate immunity as measured by anti-Gal antibody levels.

Abbreviation Key: anti-Gal = antibodies reactive with Galα1-3Galβ-1-4GlcNAc-R, the α-gal epitope; H = flagella antigen; KLH = keyhole limpet hemocyanin; LPS = lipopolysaccharide; O = somatic antigen; R- = efficient hens; R + = non-efficient hens; SE = *Salmonella* Enteritidis; S- = hens challenged by heat-killed SE, or not SE challenged; S+ = hens challenged by live SE

5.1 INTRODUCTION

Hens may be differentiated as efficient or non-efficient based on food consumption. That portion of phenotypic variation not explained by metabolic BW, BW gain, and egg mass is defined as the residual feed intake. Hens with low residual feed intake (R−) need less feed to reach the same BW and production level and are, therefore, more diet efficient than hens with high residual feed intake (R+), (Van Eerden et al., 2004a).

Questions concerning possible negative consequences of efficiency status have arisen from these observations. Do R- hens differ from R+ hens with respect to how they allocate metabolic resources to maintain immunity? Perhaps dietary efficiency is attained at the expense of other physiological processes. If this is the case with respect to immunity then the course of infectious disease, for example, might be altered in efficient hens.

Previously the antibody responses of R- and R+ hens were examined using KLH, *M. butyricum*, *Salmonella* LPS, and *Salmonella* total protein as test antigens. These results have been described in detail earlier. Briefly, titers of efficiency types immunized with KLH, *M, butyricum,* or *Salmonella* LPS did not differ, but R+ hens had higher titers to *Salmonella* total protein antigen (Van Eerden et al., 2004a). Conversely R- hens inoculated with live *Salmonella* produced higher natural KLH antibodies than did R+ hens (Van Eerden et al., 2004b). If these observations represent an altered immune status in R- hens is unknown. They have prompted the present experiments concerning the composition of bile due to its importance as a source of gastrointestinal IgA. Additionally samples of pooled plasma obtained from the original study were re-examined for *Salmonella* Enteritidis surface (O) and flagella (H) antibody using an adaptation of the classic "Widal" test originally designed for the diagnosis of typhoid (Widal, 1896). This was based in part on the earlier observation of higher total SE protein titers in R+ hens when measured by an ELISA procedure (Van Eerden et al., 2004a).

The relation between efficiency status and IgA has not yet been reported. This isotype is likely an important means of defence against *Salmonella* (Sheela, et al. 2003) and high quantities of IgA are found in bile (Cotter, 2000). Since half of the hens of each R type were exposed to *S.* Enteritidis by oral challenge, we have compared their bile O and H antibody levels. As an indicator of innate immunity we measured titers to the naturally occurring rabbit hemagglutinin "anti-Gal". This represents an important innate antibody (Cotter et al., 2005) and it has not yet been reported in bile.

5.2 MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Wageningen University approved all experimental protocols.

Hens and Husbandry

The hens of the current experiment and those used earlier were the Lohmann Brown Egg type. They were reared on floor pens through 4 weeks. Phenotypic selection to determine efficiency status was based on energy consumption calculations made at 14 weeks after 10 weeks of data collection. The mean residual feed intake difference for the 2 dietary types and the 4 trials was 4 SD. Hens were fed a starter diet (ME 2,600 kcal/kg; CP, 200 g/kg) and water ad libitum. This was gradually replaced by a grower diet (ME 2,600 kcal/kg; CP, 175g/kg) fed throughout the remainder of the experimental period. Climate respiration chambers receiving 9L: 15D with the average temperature adjusted to 21°C served as isolation housing. The details of these procedures have been given earlier (Van Eerden et al., 2004b).

Salmonella Exposure

Four batches of 16 hens each were alternately exposed or not exposed to 1 x 10^8 colony forming units (CFU) of a nalidixic acid resistant strain of *Salmonella* Enteritidis (SE) by oral challenge given at 16 weeks (Van Eerden et al., 2004b).

Collection of Bile and Plasma

Bile samples (N = 59) were obtained at necropsy at 20 weeks by puncturing the gall bladder with a 21G needle attached to a 1 mL tuberculin syringe. A few gall bladders were found empty but typically a volume of bile ranging from 0.25 to 1.5 mL was recovered. Bile samples were stored at -20^o until the time of testing.

Plasma pools were made by combining individual specimens from the original experiment obtained on post- exposure day 0, 9, 16, 23, and 29 (Van Eerden et al., 2004b). Pools representing each day post-exposure contained approximately 20uL from each of 10 hens representing the four treatment groups. Those hens challenged with live *Salmonella* are designated S+, those challenged with heat killed *Salmonella* antigen by S-. Hens of the original experiment exposed to heat-killed SE (S-) were boosted with 4 × 10^7 CFU of the same antigen on day 23 by subcutaneous (s.c.) injection. Thus with respect to the plasma

study the treatment combinations were: R-S-, R-S+, R+S-, and R+S+; where R (+ or –) indicates efficiency status, and S (+ or –) indicates live or heat-killed *Salmonella* exposure. With respect to the bile study, S+ and S- refer to hens live SE challenged or not challenged.

Salmonella O and H Antigen Preparation

Both O and H antigens were prepared by standard procedures (Campbell et al., 1964).The same isolate of *S.* Enteritidis (SE) used for challenges was recovered from frozen (-80^{o} C) storage and grown out overnight at 36^{o} C on Brilliant Green agar plates (BGA, Oxoid) containing 0.01% nalidixic acid (Van Eerden et al., 2004b).

Somatic (O) antigen was prepared by streaking a few typical colonies from the BGA plate onto Brain Heart Infusion (BHI) agar plates using a sterile cotton swab. Plates were incubated overnight at 38^{o} C after which plate surfaces were completely covered by a dense bacterial lawn. Sterile PBS was used to harvest the lawns and a bottle containing 250 mL bacterial suspension was immersed in a boiling water bath for 3.5 h. It was left to stand at room temperature overnight and again immersed in boiling water for an additional 1.5 h on the next day. The now killed bacteria were washed three times with Dulbecco's PBS (0.15M, pH 7) and concentrated by centrifugation to 0.1 original vol. The resulting product had the appearance of skim milk. It was judged to be sterile by failing to cause turbidity in BHI broth tubes after prolonged incubation at 38^{o} C and its failure to produce growth on BGA plates. Storage was in 0.3% formalized PBS at 4^{o} C.

H antigen was prepared from the same BGA plate colonies used for the O antigen. A 250 mL BHI broth bottle was grown out at 38^{o} C until its turbidity reached MacFarland scale No. 3 (approximately 9 x 10^{8} cells per mL). 250 mL of PBS containing 0.6% formaldehyde was added and the mixture was allowed to stand at room temperature for 5 days. Sterility tests were performed as for the O antigen.

Salmonella O and H Antibody Determination

The O type antibody was determined by microtiter agglutination. Bile or plasma was serially diluted in PBS containing 0.3% formalin by transferring it through 96 well U-bottomed plastic plates (Greiner Bio-one). The first well contained 100 uL of the sample diluted to 1:20. Transfers were made through well 11 that contained a 1:20,480 dilution of the original specimen. 10 uL of O antigen was added to all wells including number 12 serving as antigen control. The plates were shaken mechanically and incubated overnight at room temperature. Settling of the antigen produced a typical "blanket" agglutination pattern in

strongly positive wells. Titer was determined as the last well showing clear evidence of "blankets" while the plate was resting horizontally. A second titer was determined by elevating the plates to 45 degrees. After 1 to 2 min antigen in negative wells slid completely to the bottom edge while in positive wells it remained in place or showed clear evidence of delayed settling.

H type antibody was determined in tubes containing 250 µL bile or plasma diluted with PBS containing 0.3 % formalin to 1:10 and 1:50. An equal part of H antigen preparation was added and the tubes were incubated at 38^{o} C for a minimum of 2.5 h. Each was examined for the presence of a "floc" using mirrored fluorescent light. Flocs were scored from 0 to 4+ and the tubes were reincubated at 4^{o} C for an additional 18 h and rescored.

Anti-Gal Antibody Determination

Anti-Gal antibody was determined by the agglutination of rabbit erythrocytes because these cells express high levels of the α-Gal epitope (Cotter et al., 2005). In order to prevent direct lysis of the erythrocytes by bile, the rabbit cells were first stabilized by exposure to glutaraldehyde (Barrett, 1985). Cells were packed by gentile centrifugation and washed three times with PBS. Five mL packed RBC were mixed with 100 mL PBS containing 0.25 % glutaraldehyde. The mixture was placed on a laboratory rotator for 5 min after which the cells were removed from the glutaraldehyde by gentle centrifugation (2,000 RPM, 5 min). Residual glutaraldehyde was removed by repeated PBS washes. The cells were stable as judged by the absence of haemoglobin loss during several weeks of storage at 4^{o}C.

Bile was added to microtiter plates so that the first well contained a 1:20 dilution. Doubling dilutions were made in PBS by transferring 25 uL through well 11 leaving well 12 as an antigen control. Stabilized rabbit cells (10 uL) were added to each well and the plates were shaken mechanically for several seconds followed by incubation overnight at room temperature.

Statistics

Microtiter and tube test data were analysed using the GLM procedure of SPSS version 11.5 for windows (SPSS Inc.).

5.3 RESULTS

Agglutination of flagella (H) antigen by pooled plasma is given in Table 5.1. The absence of flocs on day 0 indicates that H-specific agglutinins were not present in any of the experimental groups prior to SE exposure. This status changed by day 9 because 4+ flocs were detected in the plasma of both R- and R+ hens challenged with live *Salmonella* (S+). The levels of H agglutinins waned in both hen types through day 29 as indicated by the decline in floc scores. This appeared to occur at a slower rate in the R- type.

Heat killed antigen challenge resulted in transient production of weak flagella agglutinins in plasma from the R+ hens but these were not detected in the R- type. Both S- groups were re-challenged with heat killed antigen on day 23 by s.c. injection. This resulted in the production of strongly positive flagella agglutinins in R- hens by day 29 compared to moderate levels in the R+ type (Table 5.1).

TABLE 5.1 Tube test results for "Widal" H-type agglutination (floc score) in plasma pools obtained from hens differing in metabolic efficiency (R) and SE exposure (S)[1]

	Sample day				
Group[2]	0	9	16	23	29
R-S-	- / -	- / -	- / -	- / -[3]	4+/4+
R-S+	- / -	4+/4+	3+/4+	3+/3+	2+/3+
R+S-	- / -	± / +	± / +	- / -[3]	2+/3+
R+S+	- / -	4+/4+	2+/2+	+ /3+	+ /2+

[1] Plasma specimens were pooled from approximately 10 hens per treatment. Tubes were diluted to 1:100 in 0.3% formalized PBS. Incubation was for 2.5 hr at 36° C followed by 18 h at 4°C. The numerator is the 2.5 h score, the denominator is the 18 h score. Score: - = no floc, +/- = weak positive, to 4+ = large, cloud like floc

[2] R - = feed efficient hens, R+ = non-efficient hens; S-, = hens exposed to heat killed SE, S+ = hens exposed to live SE

[3] S - were re-challenged with heat-killed antigen on day 23

Plasma somatic (O) agglutinin development is shown in Table 5.2. As was the case with flagella agglutinins, each treatment group was negative on day 0 but by day 9 positive results were detected. Agglutinin levels were highest in the live challenge groups (S+) but O

agglutinins were also found in heat-killed challenge (S-) groups, although at lower levels. Rechallenge on day 23 resulted in higher somatic titers in R+ hens.

TABLE 5.2 O-type microtiter agglutination in plasma pools obtained from hens differing in metabolic efficiency (R) and SE exposure (S)[1]

	Sample day				
Group[2]	0	9	16	23[3]	29
R-S-	0/0	0/10[4]	0/10	0/0	20/20
R-S+	0/0	80/160	80/80	40/40	40/40
R+S-	0/0	20/40	20/40	0/20	80/60[5]
R+S+	0/0	80/160[6]	80/80	40/40	40/40

[1] Microtiter plates were read after 5 h (numerator) and 18 h (denominator) incubation at 20°C. Plasma specimens were pooled from approximately 10 hens per treatment. Well 1 was diluted to 1:20 followed by doubling dilutions in 0.3% formalized PBS.

[2] R - = feed-efficient hens, R + = non-efficient hens; S - = hens exposed to heat-killed SE, S+ = hens exposed to live SE.

[3] R-S- and R+S- hens were rechallenged with heat-killed antigen by s.c. injection on day 23.

[4] Well 1 became +/- at 18 h and was assigned a titer of 1:10.

[5] Agglutination in well 3 (1:80) was intermediate at 18 h.

[6] antigen aggregates were present.

The results of bile tests are presented in Tables 5.3 and 5.4. Widal test scores for (H) antibody depend on the production of distinct “floc” type agglutination. The antibody combines with flagella protruding from the surface of the bacterial cell. The resulting agglutination is loose, cloud like, and remains partially suspended. The results indicate that SE challenged hens produced significantly more floc-type agglutinins than non-challenged hens (Table 5.3). At the 1:10 dilution there was a greater difference in challenged vs. non-challenged R- hens compared with R+ hens but this was not significant ($P \leq 0.09$).

TABLE 5.3 Widal tube test results (mean values) for H agglutinins in individual bile specimens obtained from hens differing in metabolic efficiency (R) and SE exposure (S)[1].

		Dilution	
Group	n	1:10	1:50
R-S-	13	1.54	0.15
R-S+	14	3.14	0.11
R+S-	16	2.63	0.09
R+S+	15	3.07	0.20
Pooled SEM		0.18	0.04
ANOVA 1:10		P values	
R		0.1	
S		0.004	
R x S		0.1	

[1] Bile was diluted to 1:10 and 1:50 dilutions using 0.3% formalized PBS. Results are expressed as mean floc scores based on a scale from 0 to 4.

[2] H agglutinins were measured in bile from efficient (R -) and non-efficient (R+) birds either exposed to SE (S+) or non-exposed (S-).

[3] n = number of bile specimens tested.

Somatic (O) agglutination data are given in Table 5.4. This type of agglutination depends on the antibody's ability to bind with structures located on the cell wall surface. In some cases the agglutination results in the end-to-end alignment of the cells as was observed by microscopic study (data not shown). Palisades were also observed and it is likely that these further strengthened the overall agglutination. Part of the palisade process may involve reactions with non O non H antigens remaining after heat or formalin treatments. The agglutinated cells settle on the bottom of the wells forming "blankets" most probably caused by reactions predominated by IgM. Weaker agglutination reactions are detected by elevation of the microtiter plates to 45 degrees. Wells showing weak agglutination appear negative at first when the plates are lying flat. After elevation weakly agglutinated antigen remains in place or exhibits a conspicuous delay in settling. Non-agglutinated antigen begins to slide to the bottom edge of the well almost immediately. Weak agglutinins are most probably predominated by IgG.

R- hens produced higher O titers when exposed to salmonella compared to R+ hens (2.3 vs. 1.9, P = 0.06, Table 5.4). Efficiency types did not differ in the absence of exposure, nor did they differ in the production of weak agglutinins.

TABLE 5.4 Microtiter means for "blanket" type somatic (O) agglutinins in bile obtained from hens differing in metabolic efficiency (R) and SE exposure (S).

Group[1]	n[2]	Microtiter mean
R-S-	13	1.3[3]
R-S+	14	2.3
R+S-	16	1.8
R+S+	15	1.9
Pooled SEM		0.126
ANOVA		P values
R		0.9
S		0.04
R x S		0.06

[1] Efficient (R -) or non-efficient hens (R +) exposed to SE (S+) or non-exposed (S-).

[2] n = number of specimens tested.

[3] Bile was serially diluted in PBS containing 0.3% formalin, well 1 was diluted to 1:20 followed by doubling dilutions.

Rabbit agglutinin ("anti-Gal") data presented in Table 5.5 shows that bile contained this antibody in high titer. Efficiency types did not differ in anti-Gal levels, but titers were significantly higher in groups exposed to SE ($P \leq 0.02$).

TABLE 5.5 Mean rabbit cell (anti-Gal) agglutinin microtiter results in bile samples obtained from hens differing in metabolic efficiency (R) and SE exposure (S).

Group[1]	n[2]	Microtiter mean
R-S-	13	9.2[3]
R-S+	14	9.8
R+S-	16	8.9
R+S+	14	10.2
Pooled SEM		0.197
ANOVA		P values
R		0.9
S		0.02
R x S		0.31

[1] Efficient (R -) or non-efficient hens (R +) exposed to SE (S+) or non-exposed (S-)

[2] n = number of bile specimens tested

[3] Well 1contained bile samples diluted to 1:20 followed by doubling dilutions in PBS. Rabbit cells were glutaraldehyde stabilized

5.4 DISCUSSION

The present experiments were designed in part to determine if phenotypic selection for metabolic efficiency results in hens having a compromised immune status. It has been assumed that genetic selection for improved production efficiency may have negative consequences with respect to animal health issues (Dunnington, 1990) and long term selection for growth was negatively correlated with the immune response to SRBC antigen (Dunnington and Siegel, 1996). Genetic selection for improved broiler performance resulted in a decrease in the adaptive arm of the immune response but an increase in the cell-mediated and inflammatory responses (Cheema et al., 2003). Our experiments were not a direct test of the consequences of genetic selection because efficiency status was determined on phenotype alone. Our observations may appear to contrast with those reporting a negative relation between fast growing meat strains and immunity. However the hens of the present study were commercial layers approaching maturity when challenged with SE. Some were already at the point of lay by 20 weeks when the experiment was terminated. Presumably any possible negative relation between efficiency status and immunity existing during the rapid growth phase was minimized.

However the present observations may bear on such issues indirectly because the questions addressed are related. *Salmonella* O and H antibody production represent tests of specific immunity while "anti-Gal" antibody represents innate immunity and both were measured in hens already separated phenotypically by metabolic criteria.

Animal resources are limited; energy devoted to one physiologic process is thought not be available for another (Beilharz et al., 1993) so some compensation may be required especially in environments that are less than optimal. If metabolic efficiency is attained at the expense of immunity high residual feed intake hens (R+) might be better equipped to deal with infectious challenges. This would be because they may possess reserves not found in R- types. Metabolic efficiency might have a hidden cost. If selection for production traits followed a similar principle genetic gains in performance might also be accompanied by weakened immunity. As a consequence such animals might become more dependent on antibiotics or vaccines to maintain a healthy status.

Alternatively, some components of immunity might have such a high priority that metabolic efficiency cannot be achieved at their expense; at least this seems to be the case for O and H antibodies, as measured by the Widal method, and also anti-Gal. The nutrient demand for maintaining the immune system, during an infectious challenge, is very small

relative to the demands of growth or egg production. It is likely that the acute phase response is a more significant consumer of nutrients than the immune system itself (Klasing, 1998). Presumably live oral challenge with SE caused some type of inflammatory response but perhaps it was mild.

The transient development of H agglutinins in the plasma of R+ hens challenged with heat killed antigen (Table 5.1) might be interpreted as illustrating inefficiency. This would be true in either of two instances. In typhoid disease H agglutinins are thought to develop later during the course of infection than do O type agglutinins (Pai et al., 2003) and flagella are a component of the virulence factors of *S.* Enteritidis in chicks (Parker and Guard-Petter, 2001). A challenge by a heat killed antigen will not result in disease, thus there is no infection. If R+ hens devote resources to the synthesis of unnecessary (H) antibody while R- hens do not, it could be argued that this represents inefficiency. Live challenge, on the other hand, resulted in higher production of bile H antibody in R- hens compared with their non-challenged counterparts (Table 5.3). Since control of SE disease at the mucosal surface depends on secretory IgA this would appear to represent efficiency.

O type agglutinins of typhoid develop early and are mostly IgM (Pai et al., 2003). Based on the "blanket" form of the plasma agglutination products (Table 5.2) this was probably true with SE as well. IgM could not have been an important component of bile agglutinins because it was detected in only 2 of 59 hens (Cotter and Van Eerden, 2006). More likely IgA provided the bulk of the O agglutinins as this isotype was found in all bile samples as was IgG (data not shown). R- hens produced higher levels of "blanket" type agglutinins, presumably IgA, than R+ (Table 5.4). R+ hens on the other hand produced more "weak" agglutinins; presumably IgG. If it is argued that IgA is the more important isotype in defence of mucosal tissue then it follows that the response of R- hens is more efficient.

It is believed that anti-Gal antibodies arise as a result of a stimulus provided by gut microbes including enterobacteria (Weiner, 1951). Perhaps *Salmonella* exposure stimulated the observed rise in anti-Gal found here (Table 5.5). The precise functions of natural antibodies are unknown but they are believed to play a role in preventing the dissemination of potential pathogens (Ochsenbein et al., 1999). Our observations are consistent with this idea and perhaps by temporarily raising anti-Gal levels hens acquire a broader spectrum of protection. This would occur in addition to the heightened protection due to pathogen specific responses.

Few studies on natural anti-Gal of chickens have been reported and we are not aware of any including data on bile. Our results show that this antibody is present in bile and in high

quantity. It is our belief that anti-Gal represents the singular most important antibody type. This opinion is based both on its quantity, its presence in bile as demonstrated here, and its distribution in avian species as reported earlier (Cotter, 1998).

In summary our data suggest that metabolic efficiency in R- hens was attained without any apparent compromise in their specific immune capacity. On the contrary it appears that metabolic efficiency was accompanied by immune efficiency rather than at its expense. Whether the same would occur with genetic selection remains to be determined.

5.5 REFERENCES

Barrett, T. J. 1985. Improvement of the indirect hemagglutination assay for Salmonella typhi antibodies by use of glutaraldehyde-fixed erythrocytes. J. Clin. Microbiol. 22(4): 662-663.

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: Is our understanding of genetics sufficient to explain evolution? J. Anim. Breed. Genet.110:161–170.

Campbell, D. H., J. S. Garvey, N. E. Cremer, D. H. Sussdorf, 1963. Preparation of antigens. Pages 65-95 in Methods in Immunology. W. A. Benjamin, Inc.

Cheema, M. A., M. A. Qureshi, and G. B. Havenstein. 2003.A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1519-1529.

Cotter P. F. 2000**.** Analysis of chicken bile by gel precipitation reactions using a lectin in the place of antibody. Poult. Sci. 79:1276-1281.

Cotter, P. F. 1998. Naturally occurring rabbit erythrocyte agglutinins in fowl sera. Poult. Sci. 77 (Supp. 1) 100 (Abstr.)

Cotter, P. F., J. Ayoub, and H. K. Parmentier. 2005**.** Directional selection for specific sheep cell antibody responses affects natural rabbit agglutinins of chickens. Poult. Sci. 84: 220-225.

Cotter P. F and E. Van Eerden. 2006. Salmonella challenge affects the antibody isotype profile of bile in hens differing in metabolic efficiency. Poult. Sci. 85:861-865.

Dunnington, E. A. 1990. Selection and homeostasis. Pages 5–12in Proceedings of the 4thWorld Congress on Genetics Applied to Livestock Production. Edinburgh, UK.

Dunnington E.A., and P.B. Siegel. 1996. Long-term divergent selection for eight-week body weight in white Plymouth Rock chickens. Poult. Sci. 75:1168-79.

Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77:1119-1125.

Ochsenbein, A. F., T. Fehr, C. Luta, M. Suter, F. Bromacher, H. Hengartner, and R. M. Zinkernagel. 1999. Control of early viral and bacterial distribution and disease by natural antibodies. Science 286:2156-2159.

Pai, A. P, G. V. Koppikar, S. Deshpande. 2003. Role of modified Widal test in the diagnosis of enteric fever**.** J. Assoc. Physicians India. 51:7-8.

Parker, C. T., J. Guard-Petter. 2001. Contribution of flagella and invasion proteins to pathogenesis of *Salmonella enterica* serovar enteritidis in chicks. FEMS Microbiol. Lett. 20: 287-291.

Sheela, R. R., U. Babu, J. Mu, S. Elankumaran, D. A. Bautista, R. B. Raybourne, R. A. Heckert, W. Song, 2003. Immune responses against *Salmonella enterica* serovar enteritidis infection in virally immunosuppressed chickens. Clin. Diagn. Lab. Immunol. 10(4): 670-679.

Van Eerden, E., H. Van Den Brand, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004a. Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. Poult. Sci. 83:1602–1609.

Van Eerden E., H. Van Den Brand, G. De Vries Reilingh, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004b. Residual feed intake and its effect on *Salmonella enteritidis* infection in growing layer hens. Poult. Sci. 83:1904–1910.

Widal, F. 1896. Serodiagnostic de la fièvre typhoide à-propos d'une modification par M. M. C. Nicolle et A. Halipre. Bull. Mem. Soc. Med. Hop. Paris 13:561–566.

Weiner, A. S. 1951. Origin of naturally occurring hemagglutinins and hemolysins. A review. J. Immunol. 66:287-295.

CHAPTER 6

Maintenance resources, dietary efficiency, and their implications for immunocompetence

E. Van Eerden, P. F. Cotter, E. A. M. Graat, H. Van Den Brand,
M. C. M. De Jong, B. Kemp

ABSTRACT

The immune system is part of an animal's maintenance processes. If there are more resources available for maintenance processes, theoretically these processes, including the immune system, could function better. Thus, the amount of maintenance resources may affect responses to an infectious challenge. Differential availability of maintenance resources can be achieved by differences in high or low residual feed intake (RFI). RFI is also a measure for dietary efficiency: non-efficient animals have a high RFI, and they have a higher availability of maintenance resources than efficient animals with a low RFI. We investigated how differences in dietary efficiency, i.e. differential availability of maintenance resources, affected humoral and cellular immune responses and bacterial shedding dynamics after experimental *Salmonella* Enteritidis challenge in chickens (*Gallus gallus domesticus*). Our data showed that the immune status of non-efficient animals was higher in unchallenged conditions, but immune responses after *Salmonella* challenge were lower than in efficient animals. We conclude that the higher level of maintenance resources in non-efficient animals is linked to a more active immune system in unchallenged conditions, but this strategy seems to diminish the response after challenge. It remains unclear whether non-efficient animals are unable or do not need to elicit a stronger response.

6.1 INTRODUCTION

In a limiting environment, resources are expected to be allocated to various fitness traits in such a way that a fitness optimum is reached (Beilharz et al., 1993). When priority for one trait increases, relatively more resources are reallocated to this trait at the expense of resources to another trait. This reallocation results in a negative correlation between these traits, and becomes apparent as a trade-off. Usually, trade-offs occur between maintenance and production processes, as illustrated by trade-offs between the immune system and brood care (in blue tits; Råberg et al., 2000), thermoregulation and growth (in domestic chickens; Hangalapura et al., 2003), or immunocompentence and growth (in magpies; Soler et al., 2003). With a constant amount of resources, differential allocation between two traits, say 1 and 2, causes differential availability of resources to these traits: more resources to trait 1 results in fewer resources to trait 2, and vice versa.

There is an alternative theory that explains why positive correlations rather than negative correlations (i.e. trade-offs) are found sometimes. This theory was first described by Van Noordwijk and De Jong (1986) and rests on the concept of resource acquisition in addition to resource allocation. A positive correlation between traits may arise when there is relatively high variation in resource acquisition and low variation in resource allocation between animals. In this situation, the amount of acquired resources is not equal for each individual, because some individuals have the ability to acquire relatively more resources; they are thus able to allocate more resources to every trait. Here, it is differential acquisition of resources, of which equal proportions are allocated to traits 1 and 2, which causes differential availability of resources to these traits: more acquired resources results in more resources to both traits 1 and 2, whereas fewer acquired resources results in fewer resources to both traits 1 and 2.

In this paper we hypothesize that there is yet another situation, in which differential acquisition and differential allocation of resources occur simultaneously. If total amount of resources allocated to trait 1 remain constant, more acquired resources results in more resources allocated to trait 2, whereas fewer acquired resources results in fewer resources allocated to trait 2. In this situation, differential acquisition causes differential availability of resources only to trait 2.

Differential resource acquisition is seen experimentally as high and low residual feed intake (RFI). RFI is defined as the difference between observed and expected feed intake based on maintenance (metabolic body weight) and production processes (e.g. growth or egg

production) (Bordas and Merat, 1974; Luiting and Urff, 1991). Animals that eat less than expected have a low residual feed intake (R-), whereas animals that eat more than expected have a high residual feed intake (R+). Thus, RFI is also a measure for dietary efficiency: R- animals are considered efficient and R+ animals are considered non-efficient. Studies on energy partitioning have shown that R+ animals were characterized by a higher feed intake, higher heat production, higher energy expenditure for maintenance processes (Bordas and Minvielle, 1999; Gabarrou et al., 1998; Luiting et al., 1991), and higher heart and liver weights than R- animals (Van Eerden et al., 2004a). For some reason, R+ animals acquire more resources than R- animals in the same environment, and the additional resources are mainly spent on maintenance processes.

It may be hypothesized that the immune system, as part of maintenance processes, in R+ animals may benefit from the fact that there are more resources available for maintenance processes. Dietary efficiency, as in R- animals, may thus come at the cost of a weakened immune system. Therefore, we carried out an experiment to investigate whether selection for dietary efficiency by means of RFI, i.e. selection for differential acquisition of maintenance resources, affects humoral and cellular immune responses to a challenge with an infectious agent, *Salmonella* Enteritidis.

6.2 MATERIALS AND METHODS

Animals and housing

This experiment was carried out in 8 trials with domestic chickens (*Gallus gallus domesticus*) as a model, because this animal species is well-known for research on RFI and can be kept relatively easy in sufficiently large numbers to perform a high-low phenotypic selection experiment. In each trial, 176 female Lohmann Brown chickens were individually housed and fed ad libitum from 4 until 14 weeks of age. Body weight and feed intake were recorded once a week. Residual feed intake was calculated per animal using the procedure as described in Van Eerden et al. (2004b). From this group, 8 most efficient (R-) and 8 least efficient (R+) birds were selected at 15 weeks of age, and they were housed individually during 5 weeks in 2 identical, open circuit, climate respiration chambers (Verstegen et al., 1987) that were used as isolation units. Every chamber contained either 8 R- or 8 R+ birds. Temperature was maintained at 21°C and relative humidity at 60% in both chambers. Lighting scheme was 9 hours light: 15 hours dark during the whole experiment.

Treatment

The first week in the chambers was an adaptation week. At 16 weeks of age (day 0), the birds were challenged by oral inoculation of 10^8 colony forming units (CFU) of *Salmonella* Enteritidis directly into the crop; this treatment was designated S+ and was applied in 4 trials. The other 4 trials served as controls and were designated S-. The 8 trials were sequential in time, and were alternately S+ or S-. The experiment consisted of 4 treatment combinations, arranged as a 2 × 2 factorial design with inoculation, S+ and S-, and RFI, R+ and R-, as factors.

A blood sample (1 ml) of each bird was taken from the wing vein on day 0 before inoculation and on day 7 and 21 after inoculation. Cells from fresh blood samples were analysed in a Lymphocyte Stimulation Test (LST). Plasma was removed and stored at –20°C until further processing. Each trial ended on day 28 after inoculation. The birds were killed through i.v. administration of Euthasan® (Anisane, Pet Health Products, Raamsdonksveer, The Netherlands).

Salmonella* Enteritidis*, somatic (O) and flagella (H) antigen preparation

A nalidixic acid resistant strain of *Salmonella* Enteritidis was grown overnight at 37°C on a Brilliant Green Agar (BGA) plate containing 0.01% nalidixic acid (all culture media used in this experiment: Oxoid Ltd, Basingstoke, Hampshire, England). For preparation of the inoculation dose a few colonies were transferred into 0.5 L of buffered peptone water and grown overnight at 37°C. The bacterial suspension was centrifuged at 3,000 × g for 15 min, and washed twice in sterile PBS. The final concentration was 2 × 10^8 CFU/mL. The inoculation dose was 0.5 mL per animal.

The procedure for preparation of somatic O and flagella H antigens is described in detail in Cotter and Van Eerden (2006). In short, somatic O antigen was prepared by immersing *Salmonella* bacteria in a boiling water bath for several hours. The killed bacteria were washed three times with PBS and concentrated to 0.1 of the original volume. Storage was in PBS with 0.3% formaldehyde at 4°C.

Flagella H antigen was prepared by adding 250 mL of PBS containing 0.6% formaldehyde to 250 mL of a *Salmonella* culture that was grown to 9.5 × 10^8 CFU/mL in Brain Heart Infusion (BHI) broth. The mixture was allowed to stand at room temperature for 5 days. Both O and H antigens were judged sterile, because culture of the end products in BHI broth was negative.

Faeces samples and bacteriological examination

In S+ trials, a clean plate was put underneath the cages every morning. This procedure allowed us to take 1 g of fresh faeces from each animal for examination of shedding. Individual faeces samples were homogenized in 9 mL of sterile PBS and incubated for 1.5 h at 37°C (starting dilution). Samples were 1:10 serial diluted in sterile PBS, and a 100 µL drop of each dilution was plated onto BGA plates containing 0.01% nalidixic acid. All BGA plates and the remainder of the starting dilution were incubated at 37°C. Colonies, typical of *Salmonella*, were counted after 24 h. When a sample in the lowest dilution was negative on BGA, a 100 µL drop of the starting dilution was put on a Modified Semisolid Rappaport Vassiliadis (MSRV) plate, incubated for another 24 h at 42°C, and evaluated for growth. The number of bacteria on BGA plates was expressed as the ^{10}log of the counted colonies in the highest dilution on the BGA plate multiplied by the dilution factor. When a sample was positive on MSRV, it was assumed to have 10 CFU/g; i.e. a ^{10}log value of 1. Bacterial counts per animal per day were plotted in a graph. It resulted in a shedding curve for each animal that was used to calculate the area under the curve (AUC).

O and H agglutination procedure

All plasma samples were tested for somatic agglutinins using the O antigen. Agglutination of antibodies with O antigen was determined in all plasma samples, using 96-well U-bottomed plastic plates. Plasma was 1:20 diluted in PBS with 0.3% formaldehyde in the 1st well, and then 1:1 serial diluted in PBS with 0.3% formaldehyde, through well 11. A volume of 10 µL of O antigen was added to all wells including well 12 that served as antigen control. The plates were shaken mechanically and incubated overnight at room temperature. The antigen-antibody complex, designated as strong agglutination, was detected as "blanket-type" agglutination in positive wells. The titre was determined as the last well showing agglutination while the plate was resting horizontally. A second titre, designed to detect weak agglutination, was determined by elevating the plate at 45°. This method allowed discrimination between "dot-type" positives from true negatives. The former remain aggregated in place, while the latter form streaks by cascading to the bottom edge of the well within 2 minutes.

Flagella (H) specific antibody in day 7 plasma samples was determined by scoring the degree of flocculation in tubes containing H antigen. A volume of 250 µL of H antigen was added to glass tubes containing 250 µL plasma diluted in PBS with 0.3% formaldehyde (final dilutions 1:50 and 1:100). The tubes were first incubated at 37°C for 4 h, and then incubated

overnight at 4°C. Tubes were visually scored by 1 observer who was uninformed about the experimental status of the tubes. Scores were 0 for negative agglutination, or 1, 2, 3 for - increasing - positive agglutination to 4 for complete flocculation.

***ELISA procedur*e**

Specific antibody titres to *Salmonella* Enteritidis lipopolysaccharide (LPS), O antigen, and H antigen, and natural antibodies to Keyhole Limpet Hemocyanin (KLH) were determined as total immunoglobulin (Ig), i.e. IgG plus IgM, by indirect ELISA in plasma of all birds. IgM to O and H antigen was determined in day 7 samples. In short, 96-well plates were coated with 1 µg of LPS/mL, 10^6 cfu of O antigen/mL, 6×10^7 CFU of H antigen/mL, or 1 µg of KLH/mL, respectively; all were diluted in carbonate buffer. Plates were incubated overnight at 4°C. Two-step serial dilutions of plasma in PBS, 0.05% Tween and 0.5% neonatal calf serum were added, starting at 1:40 for KLH and 1:80 for the *Salmonella* antigens. Plates were incubated for 1 h at room temperature. Between all incubation steps, the plates were washed with water and 0.05% Tween. Binding of antibodies was detected using rabbit-anti-chicken for total Ig and goat-anti-chicken for IgM, coupled to peroxidase (RACh/IgG_{H+L}/PO: Nordic, Tilburg, The Netherlands; GACh IgM: Bethyl Laboratories, Inc., Montgomery, TX), both diluted 1:20,000. Plates were incubated for 1 h at room temperature; tetramethylbenzidine and 0.05% H_2O_2 were then added. The reaction was stopped after 10 min with 1.25 M H_2SO_4. Extinctions were measured at a wavelength of 450 nm using a Multiskan® reader (Labsystems, Helsinki, Finland). Titres were expressed as the ^{2}log values of the highest dilution giving a positive reaction, derived from a serial diluted standard positive plasma sample that was present in duplicate on each plate.

Lymphocyte Stimulation Test (LST)

T-cell activity in fresh blood samples was measured as the ratio between stimulated and non-stimulated lymphocytes. Concanavalin A (ConA; Sigma Chemical Co., St. Louis, MO) was used for non-specific stimulation. After dilution to 1:60 with RPMI, supplemented with penicillin and streptomycin (Sigma Chemical Co., St. Louis, MO), every sample was tested twice in triplicate in 96-well flat bottom plates; once non-stimulated (only RPMI) and once stimulated (RPMI with an additional 4 µg ConA per well). The plates were incubated for 48 h at 41°C and 5% CO_2. Then 20 µl of tritium (0.5 µCi methyl-^{3}H-thymidine, ICN Biomedicals, Inc., Aurora, OH) was added per well, and the plates were incubated for another 24 h at 41°C and 5% CO_2. The cells were harvested, and the amount of incorporated tritium was counted

on a β-scintillation counter. The stimulation index per sample was calculated as mean counts in stimulated ConA triplicates divided by the mean counts in non-stimulated triplicates.

Statistical Analysis

All analyses were carried out with the SAS package (SAS Institute, 2004). For the inoculated animals the amount of bacteria shed at peak and day of peak shedding were tested using linear regression (PROC GLM) with RFI and trial as fixed effects (interactions between RFI and trial were not significant):

$$Y = \mu + R + trial + e.$$

Y = amount of bacteria shed at peak, or day of peak shedding; μ = overall mean; R = efficiency type (R- or R+); trial = S+ trials (2, 3, 5, or 7); e = error.

The areas under the shedding curves (AUC) were calculated per animal per week, using SlideWrite® Plus V6. Data of shedding per day and AUC were not normally distributed, and were therefore analysed with the non-parametric Wilcoxon Rank Sum test (PROC NPAR1WAY).

Antibody titres and stimulation indexes were tested for all animals using linear regression (PROC GLM) per sampling day.

$$Y = \mu + S + trial\ (S) + R + R \times S + e.$$

Y = antibody titre, or stimulation index; μ = overall mean; S = *Salmonella* treatment (S- or S+); trial(S) = trial nested within *Salmonella* treatment; R = efficiency type (R- or R+); R×S = interaction between efficiency type and treatment; and e = residual error. Effects of *Salmonella* treatment was tested with trial(S) as error term. Effects of efficiency type and its interaction were tested against the residual error.

Multivariable logistic regression (PROC LOGISTIC) was used for O and H agglutination to test differences between efficiency type and *Salmonella* treatment. Due to the relatively low presence of scores equal to or larger than 1, all scores were transformed to 1 for positive and 0 for negative agglutination. Interactions between dietary efficiency and *Salmonella* treatment could not be tested, due to sparseness or absence of positive agglutination scores in the S- treatment.

6.3 RESULTS

Salmonella shedding

Five birds (4 R- and 1 R+) were removed from the experiment after the adaptation week before *Salmonella* treatment was started, because they had an extremely low feed intake. In total 123 birds remained, of which 61 S+ birds for analysis of shedding data. Over the course of the experiment, *Salmonella* was detected in 60/61 (98%) of the birds. *Salmonella* was undetected in only one bird, which was R+. Shedding dynamics of the two kinds of birds appeared to differ (Figure 6.1). *Salmonella* was detected more frequent and in higher number in faeces of efficient birds compared with non efficient birds during the first 7 to 10 days. Shedding declined during the course of the experiment in both efficiency types. After reaching their peak the rate of decline appeared steeper in efficient birds, both with respect to frequency of positive birds and in the absolute numbers of shed bacteria. However, this was not confirmed at a $P < 0.05$ level in statistical analyses. Expressed as ^{10}log CFU values, peak shedding was 4.49 for R- and 3.79 for R+ birds (SEM = 0.34; P = 0.16). Day of peak shedding was 5.97 for R- and 7.53 for R+ birds (SEM = 0.90; P = 0.23). Areas under the shedding curves (AUC) were calculated per week: Wilcoxon scores for week 1 were 20.9 vs. 27.3 (P = 0.11) for R+ and R-, respectively, for week 2: 22.9 vs. 25.1 (P = 0.59), for week 3: 32.0 vs. 29.9 (P = 0.65), and for week 4: 37.1 vs. 24.3 (P = 0.004).

O and H agglutination

All day 0 samples had the score of 0, except for 1 sample (R-S+) with the score of 1; therefore, day 0 was not further analysed. Interactions between R and S in day 7 and 21 samples could not be tested, moreover, they were most likely not present, because estimates for R were not affected when S was included in the model. For strong agglutination in day 7 samples (Table 6.1), R+ animals had an odds ratio smaller than 1, meaning that strong O agglutination occurred less in R+ animals compared to R- animals (4.8% vs. 16.7%; P = 0.04). Strong O agglutination in S+ animals occurred more often than in S- animals (16.4% vs. 4.8%; P = 0.04). Strong O agglutination on day 21 was absent in the S- group (0% vs. 63.3% for S+; $P < 0.001$). In the S+ situation, agglutination did not differ between R+ and R- (64.5% vs. 62.1%; P = 0.79).

Weak O agglutination at day 7 (Table 6.2) occurred less in R+ than in R- animals (7.9% vs. 18.3%; P = 0.07). Efficiency types did not differ for weak O agglutination at day 21

(Table 6.3). The risk on weak O agglutination was 5.7 and 55.9 times increased in S+ animals compared to S- animals on day 7 and day 21, respectively.

For H agglutination, all S- animals had negative scores, regardless of dilution. In the S+ situation, more R- animals (19/29; = 66%) than R+ animals (11/32; = 34%) showed H agglutination in 1:50 diluted samples after 24 hours of incubation (with R+ as reference: odds ratio = 3.63; 95% confidence interval = [1.26 – 10.44]; P = 0.02). H agglutination for the 1:100 dilutions occurred in 59% of R- and in 34% of R+ animals (with R+ as reference: odds ratio = 2.70; 95% confidence interval = [0.96 – 7.64]; P = 0.06).

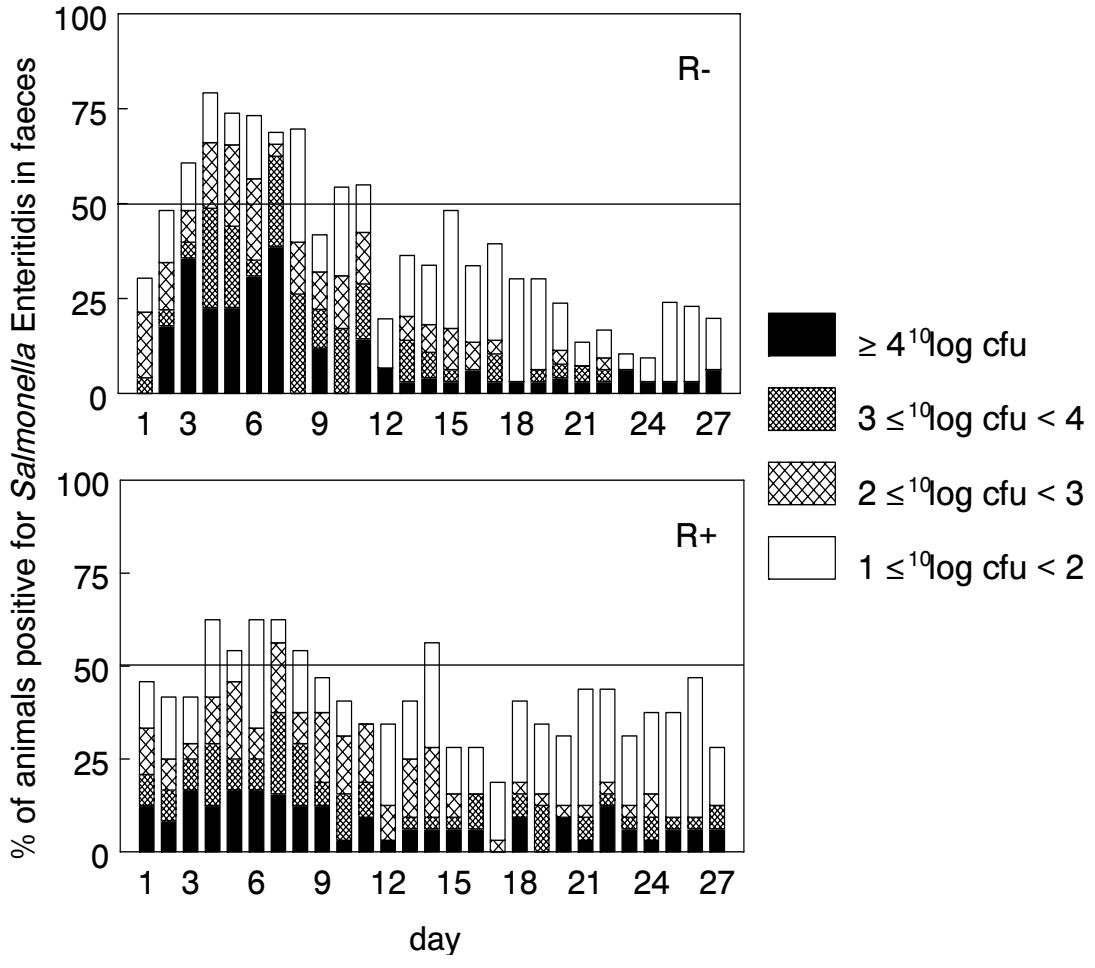


FIGURE 6.1 *Salmonella* Enteritidis shedding in dietary efficient R- and non-efficient R+ chickens from day 1 until 27 after inoculation. Numbers of bacteria are expressed as ^{10}log values of the number of colony forming units (CFU). The 50% line is added for reference.

ELISA

For total Ig against KLH and *Salmonella* O and H antigens, there were no significant interactions between efficiency type and *Salmonella* treatment, and there were no efficiency type effects at any day (P > 0.05). At day 7, S+ birds had more antibodies against *Salmonella* O and H antigen than S- birds (3.45 vs. 2.98; P = 0.03 for O antigen; 4.81 vs. 4.00; P = 0.04 for H antigen), but S+ birds had less natural antibodies to KLH than S- birds (1.55 vs. 1.72; P = 0.03). At day 21 these results were even more pronounced in the *Salmonella* antigens: S+ birds had more antibodies against *Salmonella* O and H antigen than S- birds (5.65 vs. 3.39; P = 0.0002 for O antigen; 7.33 vs. 4.44; P = 0.003 for H antigen), but S+ birds had less natural antibodies to KLH than S- birds (1.73 vs. 2.17; P = 0.11).

At day 7 there were interactions between efficiency type and treatment for total Ig against *Salmonella* LPS (P = 0.14) and for IgM against *Salmonella* O and H antigen (P = 0.07 and 0.15, respectively). Although not significant at a P < 0.05 level, interactions were all in the same direction: R+ chickens had more total Ig to LPS and more IgM to *Salmonella* O and H antigen than R- chickens in control situations (2.07 vs. 1.97 for LPS; 5.23 vs. 5.07 for O antigen; 4.28 vs. 4.03 for H antigen). However, R- chickens had more total Ig to LPS and more IgM to O and H antigen than R+ chickens when exposed to *Salmonella* (3.45 vs. 2.91 for LPS; 5.89 vs. 5.31 for O antigen; 4.66 vs. 4.47 for H antigen).

Lymphocyte Stimulation Test

Stimulation indexes of trial 3 differed significantly from the other trials, as concluded from Scheffe's multiple-comparison procedure. Therefore, trial 3 was omitted from further analysis. At day 0, R+ birds tended to have higher indexes than R- birds (R+: 24.46 vs. R-: 17.12; SEM 3.09; P = 0.10). There was a slight interaction between efficiency type and *Salmonella* treatment at day 7 (P = 0.10; pooled SEM 5.78): R+ chickens had higher indexes than R- when unexposed to *Salmonella* (R+: 30.44 vs. R-: 24.72), whereas R- had higher indexes than R+ when exposed to *Salmonella* (R-: 41.62 vs. R+: 27.93). At day 21, efficiency type effects, *Salmonella* treatment effects, and their interactions were not significant (P > 0.05).

TABLE 6.1 Strong O agglutination in plasma samples taken at day 7 from non-efficient (R+) and efficient (R-) chickens, inoculated with *Salmonella* Enteritidis (S+) or in a control situation (S-).

treatment	number	% positive agglutination	odds ratio	95% confidence interval	p-value
R+	63	4.8	0.23	0.06 – 0.90	0.035
R-	60	16.7	reference	-	-
S+	61	16.4	4.20	1.07 – 16.52	0.040
S-	62	4.8	reference	-	-

TABLE 6.2 Weak O agglutination in plasma samples taken at day 7 from non-efficient (R+) and efficient (R-) chickens, inoculated with *Salmonella* Enteritidis (S+) or in a control situation (S-).

treatment	number	% positive agglutination	odds ratio	95% confidence interval	p-value
R+	63	7.9	0.35	0.11 – 1.11	0.074
R-	60	18.3	reference	-	-
S+	61	21.3	5.70	1.51 – 21.54	0.010
S-	62	4.8	reference	-	-

TABLE 6.3 Weak O agglutination in plasma samples taken at day 21 from non-efficient (R+) and efficient (R-) chickens, inoculated with *Salmonella* Enteritidis (S+) or in a control situation (S-).

treatment	number	% positive agglutination	odds ratio	95% confidence interval	p-value
R+	63	35.5	1.24	0.46 – 3.36	0.67
R-	60	31.7	reference	-	-
S+	61	65.0	55.92	12.39 – 252.3	<0.0001
S-	62	3.2	reference	-	-

6.4 DISCUSSION

Non-efficient animals, defined as animals with high residual feed intake, have more resources available for maintenance processes. It implies, from a resource allocation perspective, that these animals can put more resources into, e.g., the immune system, which

should allow them to cope better with an immune stressor, compared to animals with low residual feed intake. This could have been reflected in higher agglutination titres, higher stimulation indexes, higher ELISA titres, fewer animals that were shedding or shedding lower numbers of bacteria, or a combination of these responses. Indeed, we found an efficiency type effect in day 0 plasma samples tested in the LST, with R+ having higher indexes than R-, and it also appeared that less R+ chickens were shedding *Salmonella* in the first week after inoculation, compared to R- chickens. However, we found (tendencies towards) interactions between dietary efficiency and *Salmonella* treatment in day 7 plasma samples that put things in a different perspective. Anti-LPS titres from the ELISA and stimulation indexes from the LST showed that non-efficient animals have a more active immune status than efficient animals in an unchallenged situation, but efficient animals respond stronger than non-efficient animals to a *Salmonella* challenge. These responses were not always significant at a $P < 0.05$ level, but they were always in the same direction. A previous study on O and H type agglutinins in bile from the same animals also showed interactions between *Salmonella* treatment and dietary efficiency (Cotter and Van Eerden, 2006), with differences pointing in the same direction, i.e. R- animals responding stronger than R+ animals after *Salmonella* infection. As the bile samples could be taken only after termination of each trial, it is noteworthy that the interaction between dietary efficiency and *Salmonella* treatment was still present locally, 28 days after infection, whereas this interaction was no longer present systemically in this experiment, 21 days after infection.

Dietary efficiency and immune responsiveness thus appear to be linked. Metabolism of R+ animals acts on a higher level than in R- animals, as indicated by the higher feed intake, the higher maintenance needs at the same production level, the higher heat production, and the heavier hearts and livers (Van Eerden et al. 2004a). The immune status seems to differ likewise between R+ and R- animals in an unchallenged state, as the immune system in R+ animals appears to be more active. One may argue that R+ animals readily invest more resources in an immune system that is always active, but this strategy may come at the cost of not being able to a strong immune response in the first days after an infection. R- animals seem to rely on an immune system that is less active in a non-challenged situation, but which responds strongly when challenged.

The fact that R+ animals keep immune activity high in an unchallenged state can be considered as a sign of inefficiency. It would seem to represent an “unnecessary” allocation of resources to the immune system, especially when the lower immune responsiveness after challenge in R+ relative to R- animals is considered as a form of exhaustion. The fact that R+

animals were prolonged *Salmonella*-shedders may support this idea. However, judging by the results in plasma samples taken at day 21 after infection, exhaustion did not appear to be the case, because at that time immune responses between R+ and R- animals did not differ.

It must be noted that the model we used, a *Salmonella* Enteritidis challenge, generally does not lead to mortality in 16-week-old chickens. Therefore, the situation may be different if an animal comes across a more pathogenic antigen with a high risk of mortality; in that case, immediate action of the immune system is needed in order to survive. Here, R+ animals may benefit from an immune system that is already more active, whereas R- animals may suffer from an immune system that needs time to get activated.

Collectively, the results from this experiment suggest that the immune system acts differently in animals that differ in dietary efficiency, measured by residual feed intake. An active immune system in an unchallenged state, as in R+, may represent a strategy analogous to the function of the innate part of the immune system. The strong response in R- animals after challenge could represent a strategy analogous to the adaptive part of the immune system. Future experiments should address this issue more specifically. Furthermore, it remains to be established whether the lower immune responsiveness after *Salmonella* challenge in R+ relative to R- animals is a matter of not being able to respond strongly, or a matter of not requiring a strong response. Considering the fact that differences in immune responsiveness between efficiency types had disappeared systemically by day 21 after infection, future experiments should focus on the first 7 days after an immune challenge, which seems to be a critical period to detect differences in immune capacity between R+ and R- animals.

We gratefully acknowledge Ger De Vries Reilingh for technical assistance during the experiments. The Institutional Animal Care and Use Committee of Wageningen University approved all experimental protocols.

6.5 REFERENCES

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? Journal of Animal Breeding and Genetics, 110:161-170.

Bordas, A., and P. Merat. 1974. Genetic variation in laying hens and phenotypic correlations of feed consumption corrected for body weight and egg production. Annales de Genetique et de Selection Animale, 6:369-379.

Bordas, A., and F. Minvielle. 1999. Patterns of growth and feed intake in divergent lines of laying domestic fowl selected for residual feed consumption. Poult. Sci. 78:317-323.

Cotter, P. F., and E. Van Eerden. 2006. Natural anti-Gal and Salmonella-specific antibodies in bile and plasma of hens differing in diet efficiency. Poult Sci, 85:435-440.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. British Poultry Science, 39:79-89.

Hangalapura, B. N., M. G. Nieuwland, G. de Vries Reilingh, M. J. Heetkamp, H. van den Brand, B. Kemp, et al. 2003. Effects of cold stress on immune responses and body weight of chicken lines divergently selected for antibody responses to sheep red blood cells. Poult Sci, 82:1692-1700.

Luiting, P., J. W. Schrama, W. v. d. Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. British Poultry Science, 32:763-782.

Luiting, P., and E. M. Urff. 1991. Optimization of a model to estimate residual feed consumption in the laying hen. Livestock Production Science, 27:321-338.

Råberg, L., A. Nilsson Jan, P. Ilmonen, M. Stjernman, and D. Hasselquist. 2000. The cost of an immune response: Vaccination reduces parental effort. Ecology Letters. 2000;, 3:382-386.

SAS Institute. 2004. SAS/STAT® 9.1 *User's Guide* SAS Institute Inc., Cary, NC.

Soler, J. J., L. De Neve, T. Perez Contreras, M. Soler, and G. Sorci. 2003. Trade-off between immunocompetence and growth in magpies: An experimental study. Proceedings of the Royal Society Biological Sciences Series B. [print] 7 February 2003 2003;, 270:241-248.

Van Eerden, E., H. Van den Brand, G. De Vries Reilingh, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004a. Residual feed intake and its effect on Salmonella enteritidis infection in growing layer hens. Poult Sci, 83:1904-1910.

Van Eerden, E., H. Van Den Brand, H. K. Parmentier, M. C. M. De Jong, and B. Kemp. 2004b. Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. Poult Sci, 83:1602-1609.

Van Noordwijk, A. J., and G. De Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist, 128:137-142.

Verstegen, M. W. A., W. Van Der Hel, H. A. Brandsma, A. M. Henken, and A. M. Bransen. 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. Pages pp 21-48 in Energy metabolism in farm animals: Effects of housing, stress and disease. M. W. A. Verstegen and A. M. Henken eds. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

CHAPTER 7

General Discussion

7.1 INTRODUCTION

Residual feed intake (RFI) was first recognized as a trait of interest for animal breeders, because it allowed them to use sources of variation in feed efficiency not accounted for in traditionally used selection programs. RFI is defined as the difference between observed feed intake and expected feed intake. Expected feed intake is based on metabolic body weight and production, such as body weight gain and egg production.

In this thesis, residual feed intake was used as a model to discriminate pullets on feed efficiency. Efficiency was defined as the amount of feed needed to reach a certain growth level at a given metabolic body weight. Non-efficient animals with a high residual feed intake (R+) mainly differ from efficient R- animals in the amount of energy spent on maintenance processes. It was hypothesized that R+ animals would, thus, have more resources available that could be used to cope with increased demands on maintenance processes, such as the immune system. Inoculation with *Salmonella* Enteritidis was used as a challenge with an infectious agent to put pressure on the immune system. It was hypothesized that an infection would force animals to direct resources towards the immune system, in which case R+ animals may be better off than R- animals.

7.2 RFI AS A PHENOTYPIC TRAIT IN PULLETS

Almost all available literature on RFI in chickens describes effects of genotypic selection on RFI for adult hens. It was shown that R+ and R- chickens that were divergently selected for several generations differed in a number of characteristics (feed intake, heat production, lipid metabolism, carcass fatness, body weight, the endocrine system, and reproduction parameters; for an overview see Table 1.2 in Chapter 1); part of the differences may be a result of accidental correlated responses. It is conceivable that the immune capacity also could have been changed as a correlated response to divergent RFI selection. In view of the aim to investigate the relationship between resource availability for maintenance processes and immune capacity, it was not desirable to use genetically RFI selected chickens. Therefore, it was necessary to use phenotypic selection instead of genotypic selection.

When RFI selection is used for breeding purposes in layers, it is essential to include egg mass as a production trait in the selection procedure. Therefore, genetic selection for RFI in layers takes place at an adult age, more specifically, around peak egg production (See Table

1.1 and references therein in Chapter 1). RFI selection in cocks includes only metabolic body weight and body weight changes, but even then the selection moment is at an adult age: in French selection lines RFI was calculated with data obtained between 33 and 37 weeks of age for both sexes (Bordas et al., 1992). The experiments described in this thesis did not aim specifically for egg mass production, but they rather used RFI as a tool to discriminate dietary efficient and non-efficient animals. For that reason it was assumed that growth, i.e. body weight gain, as a production trait in pullets could be suitable. Furthermore, growth was thought to have a high priority in young animals. It was, thus, hypothesized that growth was not likely to be traded off against other processes, unless they also have high priority. The immune system was considered as one of those processes that also have high priority for an animal, especially when an animal has to respond to a challenge with an infectious agent.

As there were no data available that described phenotypic selection for RFI in pullets, the contrast in RFI between phenotypically selected R- and R+ pullets needed to be established first. This contrast in RFI depended on the fraction of animals that were selected from the population and the variation in RFI. The experiments described in Chapter 2 used 50 pullets per group (high or low RFI) out of 350 pullets (= 14.3%). Mean RFI values after the 10-week selection period were -158.8 g for the selected R- pullets and +159.6 g for the selected R+ pullets, resulting in an average difference in RFI of 4.5 g/d for the whole 10-week selection period. Expressed as a percentage of the population mean total feed intake, the difference was 7%. This difference is much lower than in the results of Luiting et al. (1991). They performed 2 trials, and selected per line 6 out of 145 (= 4.1%) and 5 out of 92 (= 5.4%) hens, and the differences in RFI were 15.1 g/d and 18.2 g/d, respectively; expressed as percentage of the population mean total feed intake, differences were 15.0% and 19.4%, respectively. If we had selected a smaller proportion of animals per group, say 5%, then the difference in RFI would have been 6.0 g/d, which is 9.3% of the population mean total feed intake. Therefore, in the next experiments a smaller proportion of pullets per group were used. For the energy balance trials, described in Chapter 4, we selected 8 pullets per group out of 176 pullets (= 4.6%), except in trial 1. The established values for RFI, measured over the 10-week period, are shown in Table 7.1.

TABLE 7.1 Mean RFI values for selected R- and R+ pullets after a 10-week selection period

trial	R- (g/d)	R+ (g/d)	difference (g/d)	% of feed intake
1	-2.64	2.59	5.23	7.8
2	-3.11	3.66	6.77	9.9
3	-3.25	3.41	6.66	10.0
4	-3.31	3.38	6.69	10.2
5	-2.97	2.93	5.90	9.4
6	-4.29	4.21	8.50	12.9
7	-3.66	3.59	7.25	10.8
8	-4.24	4.72	8.96	13.5

It appears from Table 7.1 that the established differences were indeed in the expected range, except for trial 1. In trial 1 there were 39 pullets selected per line out of 352 pullets (=11.1%), because part of those animals were allocated to another experiment. As a result the difference in RFI was smaller in trial 1 compared to the other trials. Nevertheless, the differences in RFI in the other trials remained smaller than in the experiments of Luiting et al. (1991). It can be concluded that selection at an adult age (about 50 weeks of age in Luiting et al., 1991), thus including egg mass production as a trait in the selection procedure, probably leads to more variation in RFI.

7.3 RFI AND ENERGY PARTITIONING

7.3.1 Introduction

Energy partitioning is visualized in Figure 7.1. Metabolizable energy for maintenance is 100% converted to heat. The arrows that point to net energy (NE) for production processes (protein [P] and fat [F] deposition in egg and body weight gain [bwg]) are accompanied by numbers. These numbers represent partial efficiencies through which deposition into net energy is achieved. The arrows that point to heat production are the resulting inefficiencies of that particular production process.

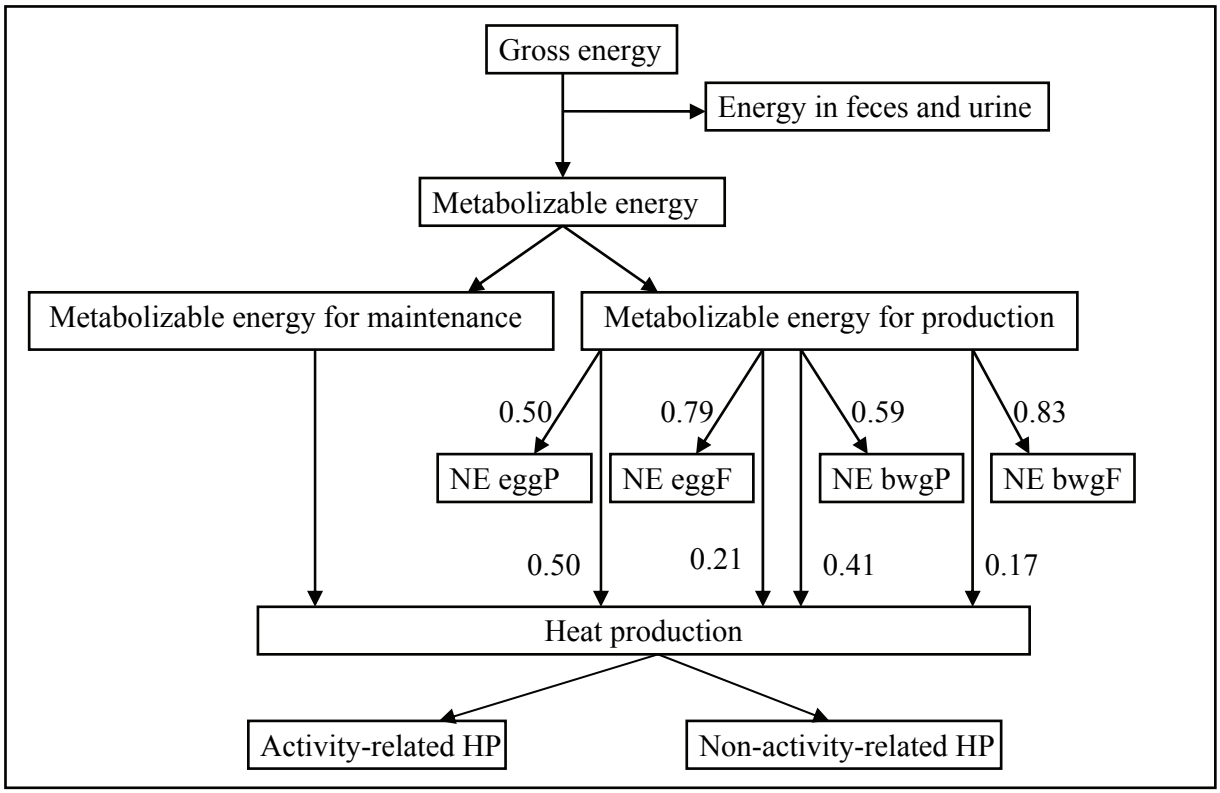


FIGURE 7.1 Schematic representation of energy partitioning. NE eggP = net energy for egg production as protein; NE eggF = net energy for egg production as fat; NE bwgP = net energy for body weight gain as protein; NE bwgF = net energy for body weight gain as fat; HP = heat production. Partial efficiencies for NE for egg production are derived from Chwalibog (1985); partial efficiencies for NE for body weight gain are derived from Chwalibog and Thorbek (1980).

7.3.2 Activity-related heat production

From an energy partitioning point of view, there were not many differences between the genotypically and phenotypically selected RFI lines. Results in our phenotypically selected pullets (see also Chapter 4) agreed for a number of characteristics with results in chickens from genetically selected RFI lines: R+ chickens have higher gross energy intake, higher ME intake, higher ME for maintenance, and they produce more heat. Figures 7.2 and 7.3 represent energy partitioning by week for R+ and R- pullets, respectively.

Although activity-related heat production was not significantly different in their R+ and R- chickens, Luiting et al. (1991) concluded that differences in heat production could be attributed for a large part to differences in activity-related heat production, which agreed with behavioral observations of Braastad and Katle (1989) that R+ hens were more active. Luiting et al. (1991) found that activity-related heat production in 2 replicates was 54% and 29% of total heat production, whereas non-activity-related heat production was 46% and 71% of total heat production. Results from the experiments described in this thesis show that activity-

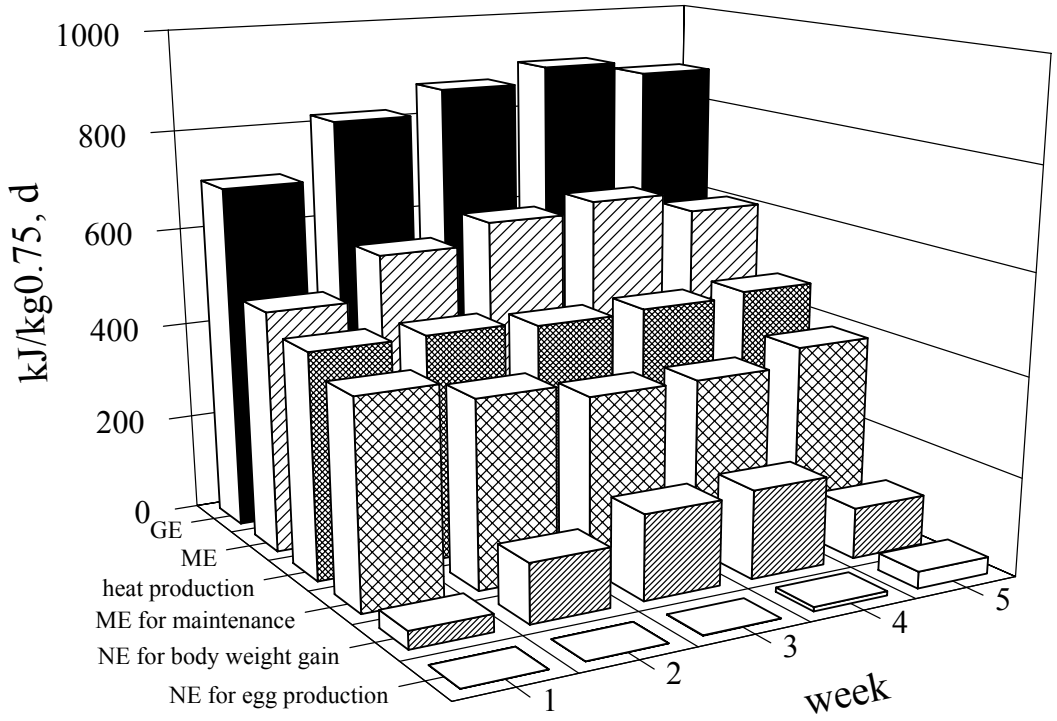


FIGURE 7.2. Energy partitioning of R+ pullets during a 5-week trial. GE = gross energy; ME = metabolizable energy; NE = net energy

related heat production made up only 18% and non-activity-related heat production 82% of total heat production. It is concluded that the difference in activity-related heat production as established in Chapter 4 do not appear to explain much of the difference in total heat production, compared to the results from Luiting et al. (1991). However, it must be noted that the general activity level in our pullets was lower than in the hens from Luiting et al. (1991): activity-related HP was 54.4 kJ/kg$^{0.75}$ per day in R- pullets and 60.1 kJ/kg$^{0.75}$ per day in R+ pullets (Chapter 4), whereas activity-related HP in 2 replicates was 83.9 and 85.3 kJ/kg$^{0.75}$ per day in R- hens, and 103.4 and 163.1 kJ/kg$^{0.75}$ per day in R+ hens (Luiting et al., 1991). Although the hens and pullets in both studies were kept individually, our pullets had less freedom of movement, because Luiting et al. had fewer chickens in the climate respiration chambers (5 or 6 hens of about 1.65 kg each, as opposed to 8 pullets of about 1.74 kg each in our experiments, on 0.8 m^2 total floor space). Also important with respect to activity in poultry is the light period. Luiting et al. (1991) used 16 hours of light per day in their

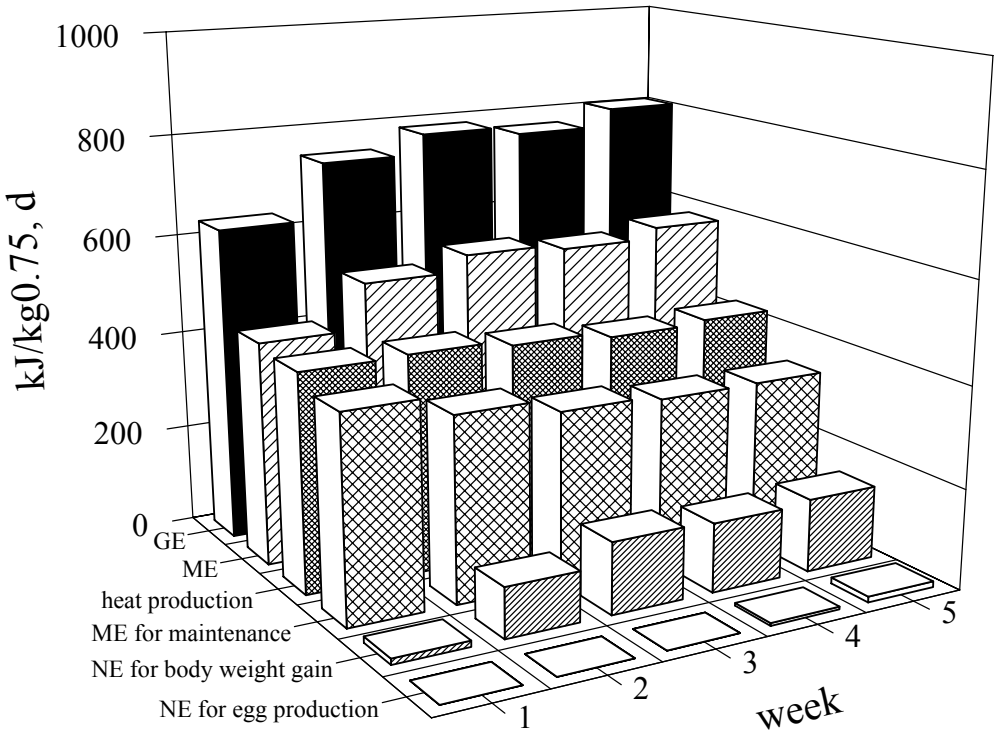


FIGURE 7.3. Energy partitioning of R- pullets during a 5-week trial. GE = gross energy; ME = metabolizable energy; NE = net energy

experiments, whereas our pullets had only 9 hours of light. Probably the most important explanation is the fact that pre-laying agitation in hens in the experiments from Luiting et al. (1991) has accounted for a considerable part to differences in activity-related heat production between R+ and R- hens. It is exactly for this type of behavior that Braastad and Katle (1989) find the most typical differences between R+ and R- hens.

7.3.3 Diet-induced thermogenesis

It was proposed that differences in heat production between R+ and R- animals from genetically selected RFI lines were due to differences in diet-induced thermogenesis, in particular in the regulatory part (Gabarrou et al., 1997). Diet-induced thermogenesis (DIT) is heat that is produced as a result of digestion and absorption of nutrients. DIT can be calculated as the difference between heat production in a fed state and heat production in a fasted state. DIT could not be established in the experiments described in this thesis, because the pullets had ad libitum access to feed at all times. However, DIT could be estimated from

non-activity-related heat production in the dark period (representing a short-term fasted state) and in the light period (representing a fed state) during 4 days in the second week of the balance trials. Estimated DIT was 119.8 kJ/kg$^{0.75}$ per day for R+ pullets and 113.0 kJ/kg$^{0.75}$ per day for R- pullets (P = 0.75). Expressed as fraction of ME intake, estimations of DIT were 20.7 % for R+ pullets and 21.1% for R- pullets (P = 0.29). This result is of the same magnitude as in R- chickens from the 17th generation in the study from Gabarrou (1997) (20.9% in R- cockerels and 27.5% in R+ cockerels). Although DIT was not properly measured in long-term fasted and fed pullets, it is concluded from our data that phenotypically selected R+ and R- pullets do not differ significantly with respect to DIT.

7.3.4 Organs and body composition

There are remarkably few reports on organ weights in chickens selected for RFI. Tixier et al. (1988) measured liver weights in young male chickens from the 10th generation of RFI selection, and they found that R+ cockerels had higher proportional liver weights than R- cockerels. Results from the experiments described in this thesis show that heart, liver, and spleen weights at the age of 15 weeks did not differ significantly between phenotypically selected R+ and R- pullets. At 20 weeks of age, R+ animals had heavier hearts and livers, heavier ovaries and stroma, and more large yellow follicles (> 10 mm) on the ovary. At 29 weeks of age, heart weights in R+ chickens were still slightly higher, although not significantly (P = 0.17). Body weight, liver, and spleen weights did not differ significantly between R+ and R- chickens at that age. The fact that we found significant differences in heart and liver weights at 20 weeks of age, therefore, seems to point at a developmental difference that is expressed just before the onset of lay. Further evidence is found in the difference in reproductive development: R+ pullets are ahead of R- pullets, as shown by heavier ovaries and stromas and more large yellow follicles, resulting in an earlier onset of lay in R+ pullets. However, the age at first egg was not significantly different between R- and R+ pullets (see also Table 2.1 in Chapter 2 and Table 3.2 in Chapter 3).

It has been reported that genetic selection for RFI was accompanied by a change in relative fatness and the amount of adipose tissue. R+ animals were leaner and had less abdominal fat than R- animals (El Kazzi et al., 1995; Tixier et al., 1988; Zein-el-Dein et al., 1985). The method used in these references is excision of the abdominal fat pads around proventriculus, gizzard, abdominal wall and cloaca. In the phenotypically selected pullets that were used in the experiments described in Chapter 4, the amount of fat was calculated and expressed as the amount of net energy partitioned as fat for body weight gain. The results

show that there were no differences between R+ and R- in the amount of fat partitioned to body weight gain. However, a comparison of results from an excision method and calculation through energy partitioning trials should be made reservedly; as can be deducted from Figure 7.1, calculation of net energy for body weight gain as fat depends heavily on the partial efficiency used. More importantly, the same partial efficiency is used for both R- and R+ pullets, but it is not clear whether partial efficiencies differ between both lines. It is likely that the pullets at the age of 20 weeks indeed did not differ in the amount of abdominal fat, because net energy for body weight gain at this age is most likely to be spent on accretion of protein. Even if there were a latent presence of differences in fat partitioning, then the chances of finding this difference were likely to be small, because our pullets can be considered the first generation of selection. Differences in adiposity were established only after several generations of selection. Hens in the first generation of RFI selection did not differ in the amount of abdominal fat (Merat et al., 1980).

We found that livers from R+ pullets were heavier than from R- pullets and that there were no differences in the amount of energy partitioned to body weight gain or eggs as fat. It was suggested that a difference in liver fat content could have masked a difference in carcass fatness. Therefore, livers that were collected after the energy balance trials were investigated by hydrodensitometry as an approximation of their fat content. Hydrodensitometry is a method that is used to estimate body fat percentage. By measuring body weight and underwater body weight, and by using densities for fat mass and fat free mass, the fat percentage can be calculated. It must be noted that this method is not developed for establishing fat percentage of organs; in that case, a chemical fat extraction would be the most appropriate method. However, our interest was not the actual fat percentage, but the relative difference in liver fat content between R+ and R- pullets, and, possibly, effects of Salmonella treatment. Therefore, hydrodensitometry was considered a satisfying and faster alternative than a fat extraction. There were considerable individual differences in appearance (yellowish to brown-red) that could be related to a difference in fat percentage, but there were no significant differences in fat percentage between R+ and R- pullets (15.4% and 14.7%, respectively; $P = 0.48$) and no differences between infected and control animals (14.6% and 15.5%, respectively; $P = 0.63$). We conclude that the difference in liver weight between R+ and R- pullets and the lack of differences in net energy partitioning to body weight gain as fat is not attributable to a difference in liver fat content.

7.3.5 Conclusion

As a conclusion we state that the general principles that are established for genetically selected chickens also hold for the phenotypically selected pullets, such as differences in gross energy intake and metabolizable energy intake. Considering the fact that growth is equal for R+ and R- pullets, the differences in energy intake must be attributable to differences in maintenance expenditures, resulting in differences in total heat production; these conclusions indeed confirm the results that were already found for adult chickens (Gabarrou et al., 1998; Luiting et al., 1991). Theoretically, differences in maintenance expenditures can be related to differences in activity-related heat production, but Luiting et al. (1991) or our results (Chapter 4) could not confirm that this is the case. Another probability for explaining differences in maintenance expenditures could be differences in diet-induced thermogenesis, but our results could not confirm this either.

The fact that some differences were smaller or not found in the pullets compared to adult chickens, such as differences in fatness and DIT, can be subscribed to the fact that the phenotypically selected pullets have to be considered a "parent" generation in which contrasts between pullets with high and low RFI were not that large. Furthermore, the physiological state of the pullets differed between moment of selection and moment of the balance trials; selection took place when the pullets were still in the growth phase, whereas during the balance trials the pullets reached puberty and became reproductive.

7.4 RFI AND THE IMMUNE SYSTEM

R+ pullets spend more resources on maintenance processes. It can, thus, be hypothesized that maintenance processes in R+ pullets will function better. This hypothesis was tested by challenging the immune system in R+ and R- pullets through immunizations with non-replicating antigens such as KLH, *Mycobacterium* and heat-inactivated *Salmonella*, and through inoculation with live, replicating *Salmonella* bacteria. The difficulty in interpreting the results from these challenge experiments, however, is to assess the true meaning with respect to functionality and efficiency of the immune system. In other words: does a high antibody level or a high stimulation index represent a "better" immune system? Therefore, from a practical point of view we considered the animal's capacity to respond to a challenge, and we assumed that a high response is associated with a better capacity of the immune system.

The experiments described in this thesis show that effects of RFI differences on immune responses are not dramatic. Effects on specific humoral immune responses are particularly unaffected, as shown by the non-significant differences in antibody responses to KLH, *Mycobacterium butyricum* and *Salmonella* Enteritidis LPS (Table 7.2). There are significant effects on cellular responses and on agglutination; however, both are in opposite directions with respect to RFI: R+ pullets have higher cellular responses than R- pullets, but R- pullets show more agglutination than R+ pullets. There are also no indications that R+ and R- pullets differ in their innate immune responses. The natural anti-KLH response in one experiment was higher in R- pullets, but this result was not confirmed in another experiment or in the anti-α-Gal response. As a conclusion we state that the immune responses of R+ and R- pullets are not consistently different. Instead of considering R- chickens to be suffering from fewer resources for the immune system, perhaps we should change our reasoning: having a low RFI is not attained at the expense of the immune system.

TABLE 7.2 Summarized effects of differences in residual feed intake on humoral and cellular immune responses and on agglutination reactions

Responses	Chapter	
Specific anti-KLH antibody response	2	NS[1]
Natural anti-KLH antibody response	3; 6	R- > R+; NS
Specific anti-*Mycobacterium* antibody response	2	NS
Specific anti-*S.* Enteritidis LPS antibody response	2; 6	NS
Specific anti-*S.* Enteritidis antibody response	3	R+ > R-
S. Enteritidis O agglutination	6	R- > R+
S. Enteritidis H agglutination	6	R- > R+
Anti-α-Gal response (in bile)	5	NS
Stimulation index (LST) before infection	6	R+ > R-

[1] NS = not significant

A finding that deserves more attention than merely the height of the response, and which is probably more determinative for the capacity and functionality of the immune system, is the speed with which a response is raised. Considering this, it appears from our results that R+ pullets do not have a better functioning of the immune system, per se; perhaps it is even the other way around. This suggestion is inspired by the fact that for several immune responses there were interactions between RFI and challenge with *Salmonella*. All these interactions were in the same direction, and they were found in different materials (plasma and bile). They

were manifested as R- pullets having lower responses than R+ pullets in non-infected situations and R- pullets having higher responses than R+ pullets after challenge with *Salmonella* (see Figures 7.4, 7.5, 7.6, and 7.7). It suggests that selection for RFI has a modulating effect on the immune system, resulting in R+ and R- pullets having different strategies in coping with immune challenges. It must be noted that interactions in the same direction were already found by Katle et al. (1988).

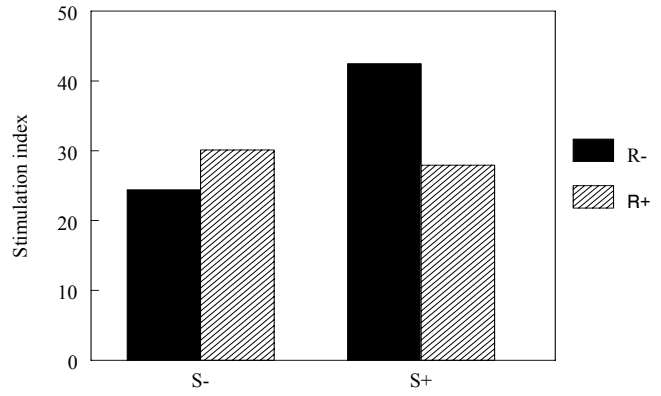
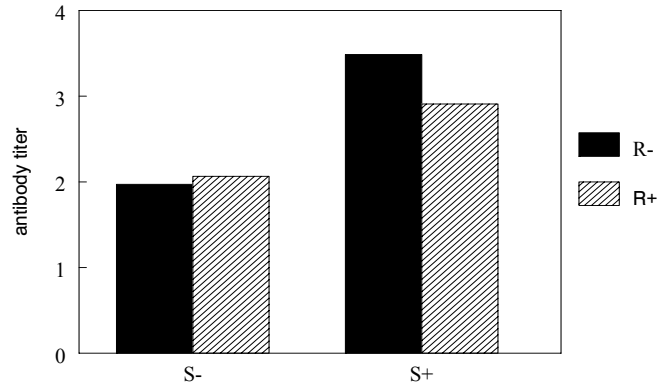


FIGURE 7.4 Stimulation indexes of R- and R+ pullets in a control situation (S-) or after *Salmonella* challenge (S+).



FIGRUE 7.5 Antibody responses in plasma to *Salmonella* Enteritidis LPS of R- and R+ pullets in a control situation (S-) or after *Salmonella* challenge.

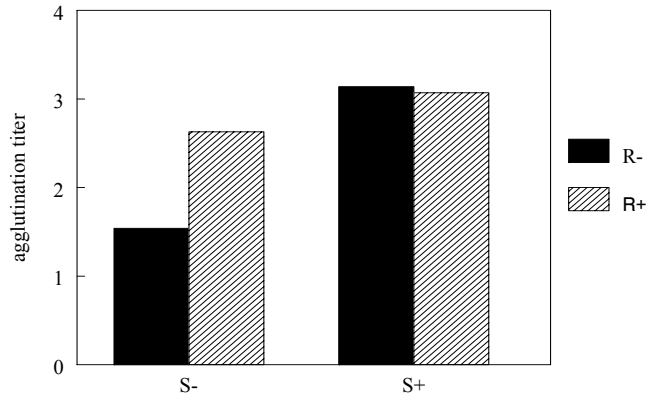


FIGURE 7.6 Flagella agglutination in bile for R- and R+ pullets in a control situation (S-) or after *Salmonella* challenge (S+).

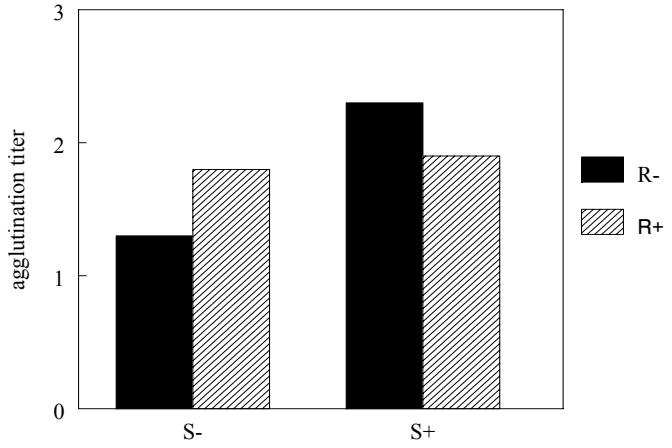


FIGURE 7.7 Somatic agglutination in bile for R- and R+ pullets in a control situation (S-) or after *Salmonella* challenge (S+).

7.5 *SALMONELLA* INOCULATION AND ENERGY PARTITIONING

Salmonella Enteritidis was used as a challenge with an infectious agent, in order to force the animals to divert energy to maintenance processes. Inoculation with a bacterium like *Salmonella* was thought to be energetically costly, because it causes an infection and will, thus, trigger the immune system. The energy partitioning experiments described in Chapter 4, however, showed that there were no effects of *Salmonella* infection at all: not a single energy partitioning parameter was affected by the *Salmonella* challenge. There may be several explanations for this somewhat surprising result. Two of them are already described in Chapter 4: it may have been caused by the fact that we used chickens that were relatively old,

or that the time span between two balance periods may have been too long to show short-term acute effects. There are, however, other possibilities to explain why the inoculation did not lead to changes in energy partitioning.

Firstly, one may argue that the infection was not successful, and did not lead to a sufficiently triggering of the immune system. This argument appears not to be true. As was shown in Chapter 6, the inoculation actually did lead to colonization, because nearly all inoculated animals excreted *Salmonella* in their feces at least once; *Salmonella* remained undetected in only one animal. Moreover, the results in Chapter 6 show that the inoculated animals had significantly higher anti-*Salmonella* specific antibodies.

Secondly, one may argue that the acute phase response, which is considered to be the energy-demanding part of an immune response, was too short-lived to induce significant effects with respect to energy partitioning. This argument also appears not to be true. Plasma samples from both infected and control animals that had been taken 7 days after infection, were tested for the presence of alpha1-acid glycoprotein (α1-AGP). This acute phase protein was shown to be elevated in chickens that were infected with *Salmonella* Enteritidis (Holt and Gast, 2002). Results from our tests showed that α1-AGP levels were significantly higher in infected chickens (602 μg/ml in infected chickens and 352 μg/ml in control chickens; $P < 0.0001$), but there were no RFI effects. It is, therefore, concluded that, although *Salmonella* Enteritidis infection in 16-wk-old chickens was quite mild as an immune stressor, it definitely caused an acute phase response, which had not disappeared 7 days after infection, and which was even sustained until 21 days after infection (467 vs. 332 μg/ml; $P < 0.0001$).

Although α1-AGP is only one of many acute phase proteins (such as ovalbumin, serum amyloid A, haptoglobin, transferrin), we must consider a third option: possibly, an acute phase response is just not energetically costly. This is a challenging hypothesis, because there are many references that state that immune responses are energetically costly (Ardia et al., 2003; Deerenberg et al., 1997; Lochmiller and Deerenberg, 2000; Raberg et al., 2000; Sheldon and Verhulst, 1996), although some references make a distinction between responses to relatively benign, non-replicating antigens and antigens that induce an acute-phase response and fever (Demas, 2004; Klasing, 1998). The latter state that acute-phase responses and fever are the energy-consuming part of an immune response. However, it was already stated by Demas (2004) that there are hardly any data on true energetic costs of immunity. Researchers mainly refer to indirect costs, such as adverse effects on certain reproductive traits when the immune system is triggered (sometimes with non-relevant antigens such as sheep red blood cells), but not to energetic costs in terms of kilojoules.

7.6 CONCLUDING REMARKS

The project described in this thesis started from the general idea that efficient animals "sacrifice" themselves, by reducing maintenance costs, and still be able to sustain a given production level. From this logic, non-efficient animals are considered prodigally, especially with regard to maintenance processes. However, these squandered resources were also regarded as reserves that could be mobilized in bad times. Non-efficient animals, thus, were thought to have an advantage over efficient animals. Residual feed intake was an attractive model to discriminate efficient and non-efficient animals, because differences in RFI mainly rely on differences in maintenance expenditure.

The results in this thesis show that R+ pullets are not prodigally just like that: they put more resources in organ development, in a faster reproductive development, and in a slightly higher mean egg weight. Moreover, the "extra" resources are not to be considered as reserves that can be mobilized to additionally supply maintenance processes. Therefore, the immune system in R+ pullets in general does not benefit and is not better off than the immune system in R- pullets. The question remains why there was not a trade-off between growth and the immune system, and why the only indication for a trade-off was the fact that ovary development was affected by the *Salmonella* infection.

An important thing that is lacking in the ideas of Beilharz et al. (1993) regarding resource allocation is the aspect of prioritizing. It is conceivable that certain processes are so crucial for survival that they will be provided with resources at all times. Maintenance processes in general, and the immune system in particular, are likely candidates to have high priority, because they are directly related to homeostasis and survival. However, growth was also thought to have a high priority in young animals, and was, thus, assessed as a potential trade-off with the immune system (Muller et al., 2005). Without doubt, growth is an important process in young animals, but it is not vital, because growth retardation can be compensated relatively easily. In contrast, a process that is not compensated easily, is, for example, taking care of offspring. Taking care of offspring has a high priority, because a reduction of energy spent on offspring ultimately leads to a reduced fitness, due to a reduced contribution of this genotype to the next generation. Apparently, (production) processes that are not immediately necessary for survival of the individual or the species are relatively easy traded-off in favor of the immune system. Future experiments should take this prioritizing into consideration.

7.7 REFERENCES

Ardia, D. R., K. A. Schat, and D. W. Winkler. 2003. Reproductive effort reduces long-term immune function in breeding tree swallows (Tachycineta bicolor). Proc Biol Sci, 270:1679-1683.

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? Journal of Animal Breeding and Genetics, 110:161-170.

Bordas, A., M. Tixier Boichard, and P. Merat. 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode Island red laying hens. British poultry science, 33:741-754.

Braastad, B. O., and J. Katle. 1989. Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. British Poultry Science, 30:533-544.

Chwalibog, A. 1985. Studies on energy metabolism in laying hens Statens Husdyrbrugsforsøg, Copenhagen, Denmark.

Chwalibog, A., and G. Thorbek. 1980. Nitrogen retention and energy cost of protein retention in chickens kept at different temperatures. Pages 318-328 in Protein metabolism and nutrition. H. J. Oslage and K. Rohr eds. European Association of Animal Production, Braunschweig.

Deerenberg, C., V. Arpanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proceedings of the Royal Society of London B, 264:1021-1029.

Demas, G. E. 2004. The energetics of immunity: a neuroendocrine link between energy balance and immune function. Horm Behav, 45:173-180.

El Kazzi, M., A. Bordas, G. Gandemer, and F. Minvielle. 1995. Divergent selection for residual food intake in Rhode Island red egg-laying lines: Gross carcase composition, carcase adiposity and lipid contents of tissues. British Poultry Science, 36:719-728.

Gabarrou, J. F., P. A. Geraert, N. Francois, S. Guillaumin, M. Picard, and A. Bordas. 1998. Energy balance of laying hens selected on residual food consumption. British Poultry Science, 39:79-89.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. Journal of Nutrition, 127:2371-2376.

Holt, P. S., and R. K. Gast. 2002. Comparison of the effects of infection with Salmonella enteritidis, in combination with an induced molt, on serum levels of the acute phase protein, alpha1 acid glycoprotein, in hens. Poult Sci, 81:1295-1300.

Katle, J., N. Hamet, L. Durand, P. Rombauts, and P. Merat. 1988. Lignées divergentes pour la consommation alimentaire "résiduelle" des pondeuses: réponse des poussins à une inoculation par *Eimeria acervulina* et comparaison de paramètres biologiques Génét. Sél. Evol., 20:387-396.

Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. Poultry Science, 77:1119-1125.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos . Jan., 2000;, 88:87-98.

Luiting, P., J. W. Schrama, W. v. d. Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. British Poultry Science, 32:763-782.

Merat, P., A. Bordas, and F. H. Ricard. 1980. Composition anatomique, production d'oeufs et effficacité alimentaire de poules pondeuses. Corrélations phénotypiques. Ann. Génét. Sél. anim., 12:191-200.

Muller, W., T. G. Groothuis, A. Kasprzik, C. Dijkstra, R. V. Alatalo, and H. Siitari. 2005. Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks. Proc Biol Sci, 272:1971-1977.

Raberg, L., A. Nilsson Jan, P. Ilmonen, M. Stjernman, and D. Hasselquist. 2000. The cost of an immune response: Vaccination reduces parental effort. Ecology Letters, 3:382-386.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution, 11:317-321.

Tixier, M., A. Bordas, and P. Merat. 1988. Divergent selectionfor residual feed intake in laying hens: effects on growth and fatness. Pages 129-132 in Leanness in domestic birds: Genetic, metabolic and hormonal aspects. B. Leclercq and C. C. Whitehead eds. INRA and Buttersworth, London, U.K.

Zein-el-Dein, A., A. Bordas, and P. Merat. 1985. Selection divergente pour la composante "residuelle" de la consommation alimentaire des poules pondeuses: effets sur la composition corporelle. Archiv für Geflügelkunde, 49:158-160.

SUMMARY

Fitness is the relative contribution of a genotype to the next generation, and is expressed as the ability to survive and produce offspring that is fit enough to survive and reproduce. Assuming that fitness consists of fitness components that all need resources, then, in a limiting environment, resources have to be allocated in such a way that optimal fitness is obtained. However, modern production animals are selected for decades to reach high production levels and to be very efficient in reaching these levels. It is thought that this selection has led to animals that are "programmed" to put a lot of resources in production processes, at the expense of resources for maintenance processes. Maintenance processes are physiological processes to maintain homeostasis, such as thermoregulation or the immune system. In other words: efficient animals spend fewer resources on maintenance processes.

Although production level and efficiency in production animals has increased over generations, it is still possible to distinguish efficient and non-efficient animals within populations. Therefore, the leading hypothesis in this thesis is that non-efficiently producing animals within a given population have more resources for maintenance processes than efficiently producing animals or non-efficiently producing animals may be better able to divert resources from production processes to maintenance processes than efficiently producing animals. In this thesis, we investigated whether non-efficient animals are better able to cope with an immune stressor than efficient animals.

We used residual feed intake as a model to phenotypically select efficient and non-efficient animals within a population. Residual feed intake (RFI) is defined as the difference between observed feed intake and expected feed intake. Expected feed intake is based on metabolic body weight and body weight gain. Metabolic body weight accounts for maintenance processes and body weight gain accounts for production processes. Animals that eat less than expected have a low (negative) RFI, and are considered efficient (R-), whereas animals that eat more than expected have a high (positive) RFI and are considered non-efficient (R+).

Our study objects were pullets. Pullets are young, growing female chickens that do not produce eggs yet. Young animals have a priority for growth and we tested whether growth was traded-off against immune responses. Our hypothesis was that efficient pullets were more prone to this trade-off than non-efficient pullets. As immune stressors we used different types of antigens. Some were relatively harmless (Keyhole Limpet Hemocyanin, *Mycobacterium butyricum*, and heat-killed *Salmonella* Enteritidis), because they were non-replicating and did not cause inflammatory reactions. Others were less harmless, because they were live, replicating bacteria (*Salmonella* Enteritidis) that cause an infection.

Our hypothesis was tested in a number of experiments. The first aim was to investigate the possibility of using residual feed intake as a trait in pullets, because until then residual feed intake was only used as a trait in adult chickens. Therefore, an experiment was set up, in which a population of 352 4-week-old pullets were individually housed and fed. They were weighed weekly during 10 weeks and individual feed records were kept (Chapter 2). The data were used in a nonlinear regression of metabolic body weight and body weight gain on feed intake to calculate the error term, which represents residual feed intake. Two groups of 50 R+ and 50 R- pullets were selected. Body weight and body weight gain was equal for both groups, but feed intake and RFI were significantly higher in R+ than in R- pullets. It was concluded that non-efficient pullets need more feed than efficient pullets to reach the same body weight.

Both groups of 50 pullets were rearranged in 2 groups of 30 pullets each and 2 groups of 20 pullets each for further experiments. The 2 groups of 30 pullets each were used to investigate humoral immune responses to 3 different types of antigens (Chapter 2). The antigens used were KLH, *Mycobacterium butyricum*, and heat-killed *Salmonella* Enteritidis. KLH is a T-helper-2 antigen, associated with humoral immune responses; *M. butyricum* is a T-helper-1 antigen, associated with cellular immune responses. Heat-killed *S.* Enteritidis was used to investigate its immunogenic properties without arousing an inflammatory response. The results showed that the pullets mounted strong antibody responses to the antigen they were immunized with. However, there were no differences between R+ and R- pullets in antibody responses to KLH, *M. butyricum*, and *S.* Enteritidis lipopolysaccharide, but antibody responses to whole cell *Salmonella* antigens were significantly higher in R+ pullets than in R- pullets.

The 2 groups of 20 pullets each were used in a combined immunization/infection experiment (Chapter 3). Ten R- and 10 R+ pullets were immunized with heat-killed *S.* Enteritidis, and the other 10 R- and 10 R+ pullets were inoculated with live *S.* Enteritidis bacteria. Blood samples from individual pullets were collected and analyzed for specific and natural antibody responses. After termination of the experiment, heart, liver, spleen, gizzard, bursa, ovary, oviduct, and stroma weights were recorded for each animal. Intestinal length was also measured and the number of large and small yellow follicles on the ovary was counted. All pullets mounted strong antibody responses to *S.* Enteritidis lipopolysaccharide and whole cell *Salmonella* antigens, but there were no significant differences between R+ and R- pullets. All pullets were also checked for natural antibodies to KLH and it was shown that R- pullets had significantly higher natural antibody titers than R+ pullets. Furthermore, it was

shown that R+ animals had higher heart, liver, oviduct, ovary, and stroma weights. *Salmonella*-infected pullets had significantly lower heart, liver, gizzard, oviduct, ovary, and stroma weights than immunized pullets. It was concluded that R+ pullets put more energy in organ and reproductive development than R- pullets. *Salmonella* infection appeared to have a delaying effect on organ and reproductive development in both groups of pullets.

The results from Chapter 2 and 3 indicated that R+ and R- pullets seemed to differ in energy expenditure, although specific antibody responses did not appear to differ between both groups. These results were the basis of a large energy partitioning experiment (Chapter 4). We hypothesized that a *Salmonella* infection is energy-demanding and would cause, thus, a shift in energy from production to maintenance processes. Therefore, we performed an experiment consisting of 8 energy balance trials of 5 weeks each. In 4 trials, all pullets were infected at 16 weeks of age with *Salmonella* Enteritidis and in the other 4 trials all pullets remained uninfected and served as controls. The results showed that R+ pullets have a significantly higher energy intake and a higher total and non-activity-related heat production than R- pullets. Metabolizable energy for maintenance was also significantly higher in R+ pullets than in R- pullets. No differences between R+ and R- pullets were found with respect to net energy for production processes. Energy partitioning did not differ significantly for *Salmonella*-infected or control pullets, although the infected pullets had a short-term higher non-activity-related heat production (between 21 and 27 hours after inoculation) than control pullets. R+ pullets had heavier hearts, livers, ovaries, and more large yellow follicles than R- pullets; however, a delaying effect of *Salmonella* infection on organ development - other than ovary development- as in Chapter 3, was not confirmed. It was concluded that *Salmonella* infection did not result in an increase in energy for maintenance processes.

Bile was collected to see whether R+ and R- pullets differed in anti-alpha-Gal antibody responses (Chapter 5), indicative for the level of natural antibodies. Results showed that this was not the case. It is concluded that efficiency in terms of RFI was not attained at the expense of natural antibodies. Further analyses on the bile samples showed that there were (tendencies towards) interactions between RFI and *Salmonella* infection: in control situations R+ pullets had more agglutinins to *Salmonella* somatic and flagella antigens than R- pullets, whereas in *Salmonella*-infected conditions, R- pullets had more anti-*Salmonella* somatic and flagella agglutinins than R+ pullets. It was concluded that R+ and R- pullets may differ in the way they cope with an immune stressor.

During the energy partitioning trials, blood samples from all pullets were taken and investigated for humoral and cellular immune responses (Chapter 6). Furthermore, individual

feces samples from each *Salmonella*-infected pullet were taken daily during the entire trial to determine *Salmonella* shedding characteristics. The results showed that there were (tendencies towards) interactions between RFI and *Salmonella* infection in several immune parameters, and in the same directions as previously noted for the bile: in control situations R+ pullets had a more active immune status than R- pullets, whereas in *Salmonella*-infected conditions R- pullets had higher immune responses than R+ pullets. Fecal shedding dynamics also appeared to differ between R+ and R- animals, although not significantly. R- pullets showed a higher peak, but also a stronger decline than R+ pullets, both with respect to the frequency of *Salmonella*-shedding pullets and in the numbers of shed bacteria. It is suggested that the immune system acts differently in R- and R+ pullets. R+ and R- pullets may, thus, have different "immune coping styles".

In conclusion, the difference between R+ and R- pullets relies on a difference in maintenance resources. R+ pullets have more maintenance resources than R- pullets, but R+ pullets do not by definition cope better with an immune challenge, just because of that. The immune stressor used, a *Salmonella* Enteritidis infection, did not cause a reallocation of resources from production to maintenance processes. Hence, there were no trade-offs. This may be due to the fact that *S*. Enteritidis is a challenge that 16-week-old pullets can easily deal with, or it may point to the high priority for the immune system.

SAMENVATTING

"Fitness" is de relatieve bijdrage van een genotype aan de volgende generatie, en wordt uitgedrukt als het in staat zijn om te overleven en nakomelingen te produceren die "fit" genoeg zijn om te overleven en zich voort te planten. Aangenomen dat "fitness" bestaat uit fitnesscomponenten die allen "resources" (energiebronnen) nodig hebben, dan moeten resources, in een omgeving die beperkend is, zodanig verdeeld worden dat een optimale fitness kan worden bereikt. Moderne productiedieren worden echter al sinds generaties geselecteerd om hoge productieniveaus te bereiken en om dat zo efficiënt mogelijk te doen. Men zou kunnen aannemen dat deze selectie heeft geleid tot dieren die als het ware "geprogrammeerd" zijn om veel resources in productieprocessen te steken, ten koste van resources voor onderhoudsprocessen. Onderhoudsprocessen zijn fysiologische processen om de homeostase in stand te houden, zoals thermoregulatie en het immuunsysteem. Met andere woorden: efficiënte dieren besteden minder resources aan onderhoudsprocessen.

Hoewel het productieniveau en de efficiëntie van productie is toegenomen over de generaties, is het mogelijk om efficiënte en inefficiënte dieren te onderscheiden binnen populaties. Daarom is de belangrijkste leidraad in dit proefschrift de hypothese dat inefficiënt producerende dieren binnen een populatie meer resources hebben voor onderhoudsprocessen dan efficiënt producerende dieren, of dat inefficiënt producerende dieren beter in staat zijn om resources te herverdelen van productieprocessen naar onderhoudsprocessen dan efficiënt producerende dieren. In dit proefschrift hebben we onderzocht of inefficiënte dieren beter kunnen omgaan met een immuunstressor dan efficiënte dieren.

We hebben residuele voeropname gebruikt als model om efficiënte en inefficiënte dieren binnen een populatie fenotypisch te selecteren. Residuele voeropname (RVO) is gedefinieerd als het verschil tussen de werkelijke voeropname en de verwachte voeropname. De verwachte voeropname is gebaseerd op het metabole lichaamsgewicht en groei. Het metabole lichaamsgewicht representeert onderhoudsprocessen en groei representeert productieprocessen. Dieren die minder eten dan verwacht hebben een lage (negatieve) RVO en worden beschouwd als efficiënte dieren (R-), terwijl dieren die meer eten dan verwacht een hoge (positieve) RVO hebben en beschouwd worden als inefficiënt (R+).

In ons onderzoek gebruikten we opfokhennen. Opfokhennen zijn jonge, groeiende vrouwelijke kippen die nog geen eieren produceren. Jonge dieren hebben prioriteit voor groei en we hebben onderzocht of groei onderhevig is aan trade-offs met immuunresponsen. Onze hypothese was dat efficiënte opfokhennen gevoeliger zijn voor een dergelijke trade-off dan inefficiënte opfokhennen. We gebruikten verschillende typen antigenen. Een aantal daarvan waren relatief onschuldig (Keyhole Limpet Hemocyanin (KLH), *Mycobacterium butyricum*,

en hitte-geïnactiveerde *Salmonella* Enteritidis), omdat ze zich niet vermenigvuldigen en geen ontstekingsreacties veroorzaken. Andere antigenen waren minder onschuldig, omdat het levende, zich vermenigvuldigende bacteriën waren (*Salmonella* Enteritidis) die een infectie veroorzaken.

Onze hypothese werd getest in een aantal experimenten. Het eerste doel was te onderzoeken of residuele voeropname geschikt was als selectiekenmerk in opfokhennen, omdat tot dan toe residuele voeropname alleen gebruikt was als kenmerk in volwassen kippen. Daartoe werd een experiment opgezet, waarin een groep van 352 kuikens van 4 weken oud individueel werd gehuisvest en gevoerd. Lichaamsgewicht en individuele voeropname werden wekelijks gedurende 10 weken geregistreerd (Hoofdstuk 2). De data werden gebruikt in een nonlineaire regressie van metabool gewicht en groei op voeropname om de errorterm te berekenen, die RVO representeert. Twee groepen van 50 R+ en 50 R- opfokhennen werden geselecteerd. Het lichaamsgewicht en de groei waren gelijk voor beide groepen, maar de voeropname en de RVO waren significant hoger in R+ dan in R- hennen. We concludeerden dat R+ hennen meer voer nodig hebben dan R- hennen om hetzelfde lichaamsgewicht te bereiken.

Beide groepen van 50 hennen werden gehergroepeerd in 2 groepen van elk 30 hennen en 2 groepen van 20 dieren voor vervolgexperimenten. De 2 groepen van 30 dieren werden gebruikt om humorale immuunresponsen tegen 3 verschillende antigenen te onderzoeken (Hoofdstuk 2). De gebruikte antigenen waren KLH, *Mycobacterium butyricum*, en hitte-geïnactiveerde *Salmonella* Enteritidis. KLH is een T-helper-2 antigeen dat geassocieerd is met humorale immuunresponsen; *M. butyricum* is een T-helper-1 antigeen dat geassocieerd is met cellulaire immuunresponsen. Hitte-geïnactiveerde *Salmonella* Enteritidis werd gebruikt om diens immunogene eigenschappen te onderzoeken, zonder dat er een ontstekingsreactie werd opgeroepen. De resultaten lieten zien dat de hennen sterke antilichaamresponsen opbouwden tegen het antigeen waarmee ze geïmmuniseerd waren. Er waren echter geen verschillen tussen R+ en R- hennen met betrekking tot antilichaamresponsen tegen KLH, *M. butyricum* en *Salmonella* Enteritidis lipopolysaccharide (LPS), maar antilichaamresponsen tegen hele-cel *Salmonella* antigenen waren significant hoger in R+ dieren dan in R- dieren.

De 2 groepen van 20 dieren werden gebruikt in een gecombineerd immunisatie/infectie experiment (Hoofdstuk 3). Tien R- en 10 R+ dieren werden geïmmuniseerd met hitte-geïnactiveerde *S.* Enteritidis en de overige 10 R- en 10 R+ dieren werden geïnoculeerd met levende *S.* Enteritidis bacteriën. Er werden bloedmonsters van elk dier genomen en geanalyseerd voor specifieke en natuurlijke antilichaamresponsen. Na beëindiging van het

experiment werden hart-, lever-, milt-, maag-, bursa-, ovarium-, oviduct- en stromagewichten bepaald voor elk dier. Darmlengte en het aantal grote en kleine follikels op het ovarium werden ook bepaald. Alle opfokhennen hadden sterke antilichaamresponsen tegen *S.* Enteritidis LPS en hele-cel *Salmonella* antigenen opgebouwd, maar er waren geen significante verschillen tussen R+ en R- opfokhennen. Alle hennen werden onderzocht op de aanwezigheid van natuurlijke antilichamen tegen KLH en het bleek dat R- hennen significant meer natuurlijke antilichamen hadden dan R+ hennen. Verder werd aangetoond dat R+ hennen een zwaarder hart, lever, oviduct, ovarium en stroma hadden. *Salmonella*-geïnfecteerde hennen hadden een significant lichter hart, lever, maag, oviduct en stroma dan geïmmuniseerde hennen. Hieruit werd geconcludeerd dat R+ hennen meer energie steken in orgaan- en reproductieontwikkeling dan R- hennen. *Salmonella* infectie leek een vertragend effect op de reproductieontwikkeling te hebben in beide groepen dieren.

De resultaten van Hoofdstuk 2 en 3 laten zien dat R+ en R- hennen lijken te verschillen in de manier waarop ze met hun energie omgaan, hoewel specifieke antilichaamresponsen niet lijken te verschillen tussen beide groepen. Deze resultaten vormden de basis voor een groot energiebalans experiment (Hoofdstuk 4). De hypothese was dat een *Salmonella* infectie energie vraagt en zo een verschuiving veroorzaakt in energie van productie- naar onderhoudsprocessen. Daarom werd een experiment uitgevoerd, bestaand uit 8 energiebalansproeven van elk 5 weken. In 4 proeven werden de hennen op 16 weken leeftijd geïnfecteerd met *Salmonella* Enteritidis en in de overige 4 proeven werden de dieren niet geïnfecteerd en dienden zo als controle. De resultaten lieten zien dat R+ hennen een significant hogere energie opname hadden en een hogere totale en niet-activiteit gerelateerde warmteproductie hadden dan R- hennen. De metaboliseerbare energie voor onderhoudsprocessen was ook significant hoger in R+ hennen dan in R- hennen. Er waren geen significante verschillen tussen R+ en R- hennen met betrekking tot energie voor productieprocessen. De energiebalans was niet significant verschillend tussen *Salmonella*-geïnfecteerde en controle hennen, hoewel de geïnfecteerde hennen kortdurend een hogere niet-activiteit gerelateerde warmteproductie hadden (tussen 21 en 27 uur na inoculatie) dan controle hennen. R+ hennen hadden een zwaarder hart, lever, ovarium en meer grote gele follikels op het ovarium dan R- hennen; een vertragend effect van *Salmonella* infectie op orgaanontwikkeling – anders dan ovariumontwikkeling- zoals in Hoofdstuk 3, werd echter niet gevonden. Hieruit werd geconcludeerd dat een *Salmonella* infectie niet resulteert in een toename van energie voor onderhoudsprocessen.

Er werd ook gal verzameld, om te onderzoeken of anti-α-Gal antilichaamresponsen (Hoofdstuk 5), die een maat zijn voor het niveau van natuurlijke antilichamen, verschillend waren voor R+ en R- hennen. De resultaten lieten zien dat dit niet het geval was. Hieruit werd geconcludeerd dat efficiëntie in termen van RVO niet bereikt werd ten koste van natuurlijke antilichaamproductie. Aanvullende analyses op de galmonsters lieten zien dat er (tendensen voor) interacties tussen RVO en *Salmonella* infectie waren: in controle situaties vertoonden R+ hennen meer agglutinatie tegen *Salmonella* somatisch en flagellair antigeen dan R- hennen, terwijl in *Salmonella*-geïnfecteerde toestand R- hennen meer anti-*Salmonella* somatische en flagellaire agglutinatie vertoonden dan R+ hennen. Hieruit werd geconcludeerd dat R+ en R- hennen mogelijk verschillen in de manier waarop ze omgaan met een immuunstressor.

Tijdens de energiebalansproeven werden ook bloedmonsters van alle hennen genomen en onderzocht op humorale en cellulaire immuunresponsen (Hoofdstuk 6). Tevens werden individuele mestmonsters genomen van elke *Salmonella*-geïnfecteerde hen gedurende de hele proef om karakteristieken van *Salmonella* uitscheiding te bepalen. De resultaten lieten zien dat er (tendensen voor) interacties tussen RVO en *Salmonella* infectie waren voor verschillende immuunparameters, en alle in dezelfde richting als al gevonden was voor gal: in controle situaties was het immuunsysteem actiever in R+ dan in R- hennen, terwijl in *Salmonella*-geïnfecteerde toestand de immuunresponsen hoger waren in R- dan in R+ hennen. De *Salmonella* uitscheiding leek ook te verschillen tussen R+ en R- hennen, hoewel die verschillen niet significant waren. R- hennen hadden een hogere piek maar ook een sterkere daling dan R+ hennen, zowel voor het aantal dieren dat *Salmonella* uitscheidde als voor het aantal uitgescheiden bacteriën. Dit veronderstelde dat het immuunsysteem verschillend werkt in R+ en R- hennen. R+ en R- hennen kunnen op die manier een verschillende “coping style” van het immuunsysteem hebben.

Concluderend: het verschil tussen R+ en R- hennen berust op een verschil in de hoeveelheid resources die aan onderhoudsprocessen worden besteed. R+ hennen hebben meer resources voor onderhoud, maar ze gaan daarom niet per definitie beter om met een immuun challenge. De gebruikte immuunstressor, een *Salmonella* Enteritidis infectie, veroorzaakte geen herverdeling van resources van productie- naar onderhoudsprocessen. Er waren dus geen trade-offs. Dit kan veroorzaakt worden door het feit dat 16-weken-oude kippen gemakkelijk kunnen omgaan met een *Salmonella* Enteritidis infectie, of het kan duiden op de hoge prioriteit die het immuunsysteem geniet.

DANKWOORD

Na een dynamische vierjarige periode is het goed om op deze plek even de tijd te nemen, stil te staan en terug te blikken. Terug te blikken op een project dat op mijn lijf geschreven leek en waar ik veel plezier aan heb beleefd, en op fijne collega's die ik veel dank ben verschuldigd.

Allereerst het driemanschap dat verantwoordelijk was voor de begeleiding. Bas, het vertrouwen dat je in me had en de vrijheid die je me gaf bij de uitvoering van het project, hebben heel veel voor me betekend. Henry, je enthousiasme en betrokkenheid waren super. Ik vond het bijzonder prettig om met je samen te werken. Mart, je kritische en scherpzinnige inbreng, vooral in het laatste jaar, heb ik erg gewaardeerd. Heren, hartelijk dank voor jullie inspanningen en geduld!

Lisette, Henk Parmentier en Aart, jullie waren geen officiële begeleiders, maar ik wil jullie toch graag als zodanig beschouwen, omdat jullie een grote bijdrage hebben geleverd. Lisette, je hulp en uitleg bij de statistiek waren onontbeerlijk. Henk en Aart, jullie waren erg belangrijk als discussiepartner en jullie hebben me veel geleerd over immunologie en onderwijs. Mijn dank aan jullie drieën is groot.

Dear Paul Cotter, you came to Wageningen with the intention to work on something completely different than on *Salmonella*. After you had learned –accidently- about my project, you had a simple request: please collect some bile from your chickens? It was the beginning of a very fruitful cooperation, and I am honoured to have had a personal coach like you, who has spent so many hours of discussing science, literature, results and hypotheses with me. Many, many thanks for your support!

Ger, jij neemt een heel speciale plek in. Wat had ik zonder jou gemoeten? Je hulpvaardigheid, je creatieve oplossingen, je innovatieve ideeën (microtiterkweek!) en je gezelligheid maakten je een fantastische Naaste-collega! Je blijft mijn steun en toeverlaat tot en met de promotie; bedankt dat je mijn paranimf wilt zijn.

Niet te vergeten zijn daar natuurlijk ook nog de Tuinkabouters: Emmy, Bjorge, Frits, Mike, Koos, Ilona, Rudie, Marcel en Henk Brandsma, het was plezierig om bij jullie in de kantoortuin te mogen huizen. Voor jullie, en ook voor Nicoline, Wouter, Klaas, Pieter, Joanne, Nanette, Lora, Liesbeth, Jorine, Johanna en collega-AIO's Basav, Ariëtte, Francisca, Judith, Laura, Rozemarijn, Marieke, Inge, Roos en Adriana geldt: bedankt voor de vele gezellige en collegiale momenten.

De klimaatrespiratiecellen maken een belangrijke relatie zichtbaar tussen de leerstoelgroep Adaptatiefysiologie en de vakgroep Veevoeding. Daarom een speciaal woord

van dank aan de “respirators” van Veevoeding, Walter, Tamme, Sven en Joost, voor jullie theoretische input en praktische hulp bij mijn proeven.

Tja, mijn proeven, daar moesten heel wat kippen en voer voor gewogen worden. Het begrip “zakkenvullers” heeft voor mij nu toch wel een heel speciale betekenis. Dank je wel, Ries, Marleen, André, Peter, Ben, Roel en Willem, voor de verzorging van de dieren en voor jullie hulp bij al het wegen. Ook wil ik graag de dierverzorgers van de Pluimvee- en Klimaataccommodatie in Lelystad bedanken voor hun hulp bij de proef die daar is uitgevoerd. Studenten en student-assistenten hebben zich ook niet onbetuigd gelaten: Ria, Suintha, Inge, Ann, Margot en Annelies, hartelijk dank voor jullie bijdragen in de stal en op het lab.

Ook een welgemeend dankjewel aan Saskia van Laar, die verantwoordelijk was voor de gang van zaken op het lab van Veevoeding, en aan Paul Koene en Hans Swarts, wier hulp onmisbaar was bij de uitvoering van, respectievelijk, tonic immobility testen en radio-immuno assays.

Vrienden, familie en nieuwe collega’s van het IRAS, bedankt voor jullie interesse; Ekelijn, moge onze samenwerking tot nog meer mooie dingen leiden, en Annemarie, de muzikale ontspanning was meer dan eens heel erg welkom, dank je wel. Yvette, bedankt voor je jarenlange vriendschap; ik ben heel blij dat je mijn paranimf wilt zijn.

Mijn laatste dankwoorden gaan uit naar 5 belangrijke personen in mijn leven. Pap en mam, ik kan het jullie nu zwart op wit geven: ik heb het geweldig met jullie als ouders getroffen. Heel erg bedankt voor jullie niet aflatende steun en vertrouwen.

Har, Ties en Maud, de overgang was voor jullie groot toen ik aan mijn AIO baan begon, en ik realiseer me dat er veel is gevraagd van jullie adaptatievermogen. Dit proefschrift is mede mogelijk gemaakt door jullie. Bedankt voor alles!

ABOUT THE AUTHOR

Ellen van Eerden werd op 15 augustus 1970 geboren in Winterswijk. Ze behaalde haar VWO diploma aan de Rijksscholengemeenschap Hamaland in Winterswijk. In september 1989 begon ze aan de studie Zoötechniek aan de Landbouwuniversiteit Wageningen. Ze deed onderzoek naar de parasieten *Cooperia* en *Ostertagia* in kalveren, naar glucose-intolerantie in hoogdrachtige zeugen, en naar de parasiet *Neospora* in schapen. Ze studeerde af in 1996 in de richting Gezondheidsleer en Reproductie. In september 2002 begon ze als Assistent in Opleiding bij de leerstoelgroep Adaptatiefysiologie van Wageningen Universiteit. Het daar uitgevoerde onderzoek staat beschreven in dit proefschrift. Sinds oktober 2006 werkt ze als postdoc onderzoeker bij het Institute for Risk Assessment Sciences, divisie Veterinary Public Health, van de Universiteit Utrecht.

LIST OF PUBLICATIONS

PAPERS IN PEER-REVIEWED JOURNALS

E. Van Eerden, H. Van Den Brand, H. K. Parmentier, M .C. M. De Jong, B. Kemp. 2004. Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. Poultry Science 83:1602-1609

E. Van Eerden, H. Van Den Brand, G. De Vries Reilingh, H. K. Parmentier, M .C. M. De Jong, B. Kemp. 2004. Residual feed intake and its effect on *Salmonella enteritidis* infection in growing layer hens. Poultry Science 83:1904-1910

P. F. Cotter and E. Van Eerden. 2006. Natural anti-Gal and *Salmonella*-specific antibodies in bile and plasma of hens differing in diet efficiency. Poultry Science 85:435-440

P. F. Cotter and E. Van Eerden. 2006. *Salmonella* challenge affects the antibody isotype profile of bile in hens differing in metabolic efficiency. Poultry Science 85:861-865

E. Van Eerden, H. Van Den Brand, M. J. W. Heetkamp, E. Decuypere, B. Kemp. 2006. Energy partitioning and thyroid hormone levels during *Salmonella enteritidis* infection in pullets with high or low residual feed intake. Poultry Science 85:1775-1783

E. Thomas, A. Bouma, E. Van Eerden, W. J. M. Landman, F. Van Knapen, A. Stegeman, A. A. Bergwerff. 2006. Detection of egg yolk antibodies reflecting *Salmonella enteritidis* infections using a surface plasmon resonance biosensor. Journal of Immunological Methods 315:68-74

CONFERENCE PROCEEDINGS AND ABSTRACTS

E. Van Eerden, H. Van Den Brand, H. K. Parmentier, M. De Jong, B. Kemp. 2004. Effect of phenotypic selection for residual feed intake on antibody responses in growing layer hens. Proceedings of the XXII World's Poultry Congress, 8-13 June 2004, Istanbul, Turkey. WPSA, 3 pp. (CD-ROM).

E. Van Eerden. 2005. Effect of metabolic efficiency on kinetic differences in *Salmonella* Enteritidis shedding rates. Page 30 in Proceedings of the 28th Poultry Science Symposium, 15-17 September 2005, Bristol, United Kingdom (abstract). WPSA UK Branch.

E. Van Eerden, H. Van Den Brand, B. Kemp. 2005. Dietary efficiency: waste not, want not? Page 28-29 in Proceedings of the 31st Studiedag Nederlandstalige Voedingsonderzoekers (abstract).

E. Van Eerden, H. Van Den Brand, B. Kemp. 2006. Profits and losses of dietary efficiency with respect to immune reactivity. Page 494-495 in Proceedings of the XII European Poultry Conference, 10-14 September, Verona, Italy (abstract). World's Poultry Science Journal vol. 62 (supplement); Romboli, Flock, Franchini (editors).

Training and Supervision Plan	
Name PhD student	Ellen van Eerden
Project title	Colonization, stress, and transmission: Unravelling the interrelationships in an animal model
Group	Adaptation Physiology
Daily supervisor(s)	H. van den Brand
Supervisor(s)	B. Kemp
Project term	01-09-2002 until 30-09-06
Submitted	24-08-2006; first plan / midterm/**certificate**

EDUCATION AND TRAINING (minimum 30, maximum 60 credits)		
The Basic Package (minimum 3 credits)	year	credits*
WIAS Introduction Course (mandatory)	2003	
Course on philosophy of science and/or ethics (mandatory)	2003	
Subtotal Basic Package		**3**
Scientific Exposure (conferences, seminars and presentations, minimum 8 credits)	year	
International conferences *(minimum 3 credits)*		
22th World's Poultry Congress, June 8-13, 2004, Istanbul	2004	
28th Poultry Science Symposium, WPSA UK Branch; Avian Gut Function, Health and Disease; September 15-17, 2005 Bristol	2005	
12th Benelux Congress of Zoology, October, 26-28, 2005, Wageningen	2005	
12th European Poultry Congress, September 10-13, 2006, Verona	2006	
Seminars and workshops		
WIAS Science Day, March 27, 2003; February 17, 2005; March 9, 2006	03/'05-'06	
Meeting Poultry Coordination Centre, Lelystad, October 31	2002	
PhD retreat, Nunspeet, December 12-13, 2002; Nijmegen, May 13-14, 2004	02/'04	
Meeting Poultry Coordination Centre, Lelystad, August 28	2003	
PhD Day Poultry Coordination Centre, Wageningen, December 16	2004	
WIAS minisymposium, Selection of chickens; an approach to unravel specific and innate immune competence, Wageningen, March 19	2005	
WIAS minisymposium, Hamburger Disease, the ins and outs of E.coli O157, Wageningen, October 14	2005	
Symposium of Dutch speaking nutrition researchers, NVO, Rotterdam, April 7	2006	
Current themes in ecology: influenza ecology and pandemics, Wageningen, April 19	2006	
Presentations *(minimum 4 original presentations of which at least 1 oral, 1 credit each)*		
Effect of phenotypic selection for residual feed intake on antibody responses in growing layer hens, Istanbul, June 11, poster	2004	
Effect of phenotypic selection for residual feed intake on antibody responses and body characteristics after experimental Salmonella enteritidis infection in growing layer hens, Wageningen, February 17, oral	2005	
Effect of metabolic efficiency on kinetic differences in Salmonella enteritidis shedding rates, Bristol, September 15, poster and oral	2005	
Dietary efficiency: waste not, want not, Rotterdam, April 7, oral	2006	
Profits and losses of dietary efficiency with respect to immune reactivity, Verona, September 11, poster	2006	
Subtotal International Exposure		**14**

In-Depth Studies (minimum 6 credits, of which minimum 4 at PhD level)	year	
Disciplinary and interdisciplinary courses		
WIAS/VLAG course Ecophysiology of the gastrointestinal tract	2005	
VLAG course Management of Microbial Hazards in Foods	2005	
Dutch Association for Immunology (NVvI) course Self-Nonself Recognition	2005	
WIAS course Biological basis for improved management and selection tools	2005	
Advanced statistics courses *(optional)*		
WIAS course Advanced statistics course Experimental Design	2005	
MSc level courses *(only in case of deficiencies)*		
MSc course Microbial Physiology	2005	
Subtotal In-Depth Studies		**9**
Statutory Courses	year	
Use of Laboratory Animals (mandatory when working with animals)	2002	
Laboratory Use of Isotopes (mandatory when working with radio isotopes)	2003	
Subtotal Statutory Courses		**6**
Professional Skills Support Courses (minimum 3 credits)	year	
Course Techniques for Scientific Writing (advised)	2003	
Course Supervising MSc thesis work (advised when supervising MSc students)	2003	
Time planning and Project Management	2004	
Course Didactic Skills	2005	
Course Communication Skills	2005	
Course Lecturing skills	2005	
Subtotal Professional Skills Support Courses		**8**
Didactic Skills Training (optional)	year	
Lecturing *(real time including preparation)*		
lecture ADP-1, 2004, 2005, 2006	04-'06	
Supervising practicals and excursions *(real time)*		
supervising practical Immunology, 2005, 2006	05-'06	
supervising practical Thermoregulation	2006	
Supervising theses *(max 2 credits per MSc major, 1.5 c MSc minor, 1 c BSc thesis)*		
Supervising three MSc major students	03-'04	
Subtotal Didactic Skills Training		**9**
Management Skills Training (optional)	year	
Organisation of seminars and courses		
Organisation PhD retreat 2004, Nijmegen	2004	
Subtotal Management Skills Training		**2**
Education and Training Total (minimum 30, maximum 60 credits)		**51**

* one ECTS credit equals a study load of approximately 28 hours

The research described in this thesis was part of the Adaptation and Resistance research programme.

Printed by: Ponsen & Looijen BV, Wageningen

Cover design: Ger de Vries Reilingh