The factors affecting the dispersion of gastro-intestinal parasites in birds, specifically the nematode *Heterakis gallinarum* in the ring-necked pheasant *Phasianus colchicus.*

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Abstract

This thesis aimed to identify and parameterise the factors affecting host susceptibility to parasitism.

The model system chosen was the caecal nematode *Heterakis gallinarum*, infecting the ring-necked pheasant *Phasianus colchicus*. It has become increasingly important to examine the characteristics within hosts that affect susceptibility to parasitism as current control methods are becoming more restricted. Non-invasive parasite control procedures for game birds could solve many of the problems associated with large-scale anthelmintic usage and resultant resistance.

The research was undertaken using pheasants naive to parasite infection that were orally challenged with *H. gallinarum* eggs, or using individuals previously exposed to parasitism that were again naturally exposed.

The results showed that host susceptibility to parasitism was affected by variation in the T-cell mediated immune response of the pheasant host, possible acquired immune resistance of the pheasant to *H. gallinarum* parasitism, nutritional stress interacting with body condition, and possible trade-offs between condition, spleenic response, secondary sexual ornaments and *H. gallinarum* parasitism. *H. gallinarum* did not seem to affect pheasant morbidity subsequent to parasite challenge.

Statement of Originality

The work described in this thesis is original research carried out by myself and has not been submitted for consideration previously for a higher degree at this or any other university. Any references henceforth used have been appropriately acknowledged.

K. Marie McIntyre

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Contents

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The factors affecting the dispersion of gastro-intestinal parasites in birds, specifically the nematode *Heterakis gallinarum* in the ring-necked pheasant *Phasianus colchicus.*

Chapter 1 General introduction and methodology

General introduction

Parasites are typically aggregated within host populations (Anderson, 1974; Anderson and May, 1978; May, 1977; Nilssen et al., 1998; Pacala and Dobson, 1988; Pennycuick, 1971; Shaw and Dobson, 1995; Shaw et al., 1998; Wilson, 1983). Simply described, this means that within a population there are a few heavily parasitised host individuals in which the main proportion of parasite reproduction occurs. This reproduction allows further transmission of the parasite and plays an important role in the persistence of parasitism (Anderson and May, 1985a; Woolhouse et al., 1997). Therefore, it is important to identify both the causes of variation in parasite aggregation and the effects of these causes upon parasite dispersion. Host community structure and dynamics can be mediated by parasites through apparent competition (Holt and Lawton, 1994; Hudson and Greenman, 1998). The potential for apparent competition can occur when two host species in an ecosystem that do not compete for resources are colonized by the same parasite species. Parasite density increases because one of the hosts is highly susceptible to the parasite, which means parasite reproduction can occur within this host, which consequently increases the density within the other host. If the second host species experiences greater morbidity as a result of the parasite then apparent competition can occur (Tompkins and Wilson,

1998). This type of competition has the potential to reduce the population growth rate and create morbidity for both species (Tompkins et al., 2002a). Parasite persistence in the second species can be discouraged if the amplification of the parasite population can be reduced in the often more common, less affected host, as has been suggested by recent work (Holt and Lawton, 1994; Tompkins et al., 2002c, 2003).

Variation in the exposure of the host to parasitism, and in host susceptibility to parasites are suggested to be the main factors affecting parasite aggregation.

Differential exposure of the host to the parasite can be influenced by heterogeneous host behaviour caused by host sex (Bundy, 1988; Tinsley, 1989), host density (Hart, 1994, 1997; Jaenike and Anderson, 1992; Mooring and Hart, 1992; Rubenstein and Hohmann, 1989), host migration (Altizer et al., 2000; Folstad et al., 1991; Pfennig and Tinsley, 2000) or parasitism itself (Florez-Duquet et al., 1998; Gilbert, 1997; Holmes and Zohar, 1990; Hutchings et al., 2002a, 1998, 1999, 2002b; Karban, 1998; Monagas and Gatten, 1983; Moore and Gotelli, 1990, 1996; Poulin, 1994a, 1994b; Rubenstein and Hohmann, 1989; Thompson, 1990; Thompson and Kavaliers, 1994). Exposure can also be influenced by stochastic environmental and topographic differences such as seasonality (temperature and precipitation) (Boxshall, 1974; Gibbs, 1986; Jaenike, 1994; Michael, 1974; Moss et al., 1993; Rubenstein and Hohmann, 1989; Shaw and Dobson, 1995; Shaw et al., 1998; Shaw and Moss, 1989b) and habitat type and size (Gibbs, 1986; Hutchings et al., 1998, 1999; Jaenike, 1994; Jaenike and Anderson, 1992; Keymer and Anderson, 1979; Michael, 1974) creating heterogeneity in the distribution of the infective stages of the parasite (Harvey et al., 1999).

Host susceptibility is affected by not just innate phenotypic and genetic variation (Boulinier et al., 1997; Burdon, 1991; Henter and Via, 1995; Jaenike, 1993; Jarosz and Burdon, 1990; Kolmer, 1996; May and Anderson, 1990; Smith et al., 1999; Stear and Wakelin, 1998; Thompson and Burdon, 1992; Webster and Woolhouse, 1999; Yan and Norman, 1995; Yu et al., 1998), but also by variation in host body condition (Brinkhof et al., 1999; Cook, 1991; Holmstad and Skorping, 1998; Stear et al., 1998; Wilson, 1994), host immune functioning, such as the ability to generate an acquired immune reaction (Anderson and May, 1985a, 1985b, 1991; Baron and Weintraub, 1987; Crombie and Anderson, 1985; Dobson et al., 1990; May and Anderson, 1983; Roitt et al., 1998; Wakelin and Apanius, 1997), host sex (Alexander and Stimson, 1988; Bundy, 1988; Zuk, 1990) and host age (Dobson et al., 1990; Gregory et al., 1992; Hudson, 1992; Hudson and Dobson, 1995) (affecting relative host size; Quinnell, 1992). Stresses caused by social interactions (although this is difficult to differentiate from increased hormone levels; Barnard et al., 1993, 1994) or environmental stress as a result of the weather (Nelson and Demas, 1996) or changes in habitat (in the form of foraging quality and availability; Gulland, 1992; de Lope, 1993) and host density creating crowding stress (Barnard et al., 1994; Cote and Poulin, 1995; Loye and Carroll, 1995; Poiani, 1992; Poulin, 1991; Shields and Crook, 1987) may also affect host fitness and immune functioning, consequently influencing susceptibility to parasitism.

Susceptibility has been shown to be important in influencing parasite virulence (Bull, 1994; May and Anderson, 1983, 1990), but its importance may differ between high (co-evolved) and low (generalist parasite) specificity parasite/host systems (Gandon et

al., 1996). Within a system in which the parasite has relatively high host specificity, as suggested for *Heterakis gallinarum* parasitism in the pheasant, there must be variation in the susceptibility of the host (dependent upon parasite prevalence and virulence) for resistance to the parasite species to develop (Minchella, 1985). Without such variation, the host and parasite species would allocate increasing amounts of the resources obtained from food, into resisting parasitism or more virulently parasitising the host (Wakelin and Apanius, 1997). This would reduce the resources available for other life history traits such as reproduction (Gustafsson et al., 1994; Lochmiller and Deerenberg, 2000; Nordling et al., 1998; Oppliger et al., 1996), development (Lochmiller and Deerenberg, 2000), competitive ability or long-term survival. Variation in the level of immunity may therefore be dictated by differences in the natural susceptibility of individuals in combination with resource allocation (Ferdig et al., 1993; Gustafsson et al., 1994; Lochmiller and Deerenberg, 2000; Norris and Evans, 2000; Schmid-Hempel, 2003; Sheldon and Verhulst, 1996; Svensson and Merila, 1996; Wakelin et al., 2002) to optimize resource trade-offs for the most favorable life-history strategy for the host (Anderson and May, 1982; Owens and Wilson, 1999).

Thesis aims

Host susceptibility and resistance are often assumed to be related to, and to affect parasite numbers. Documented examples of this are lacking because insufficient information on host parasitism history inhibits straightforward examination. The goal of this thesis was to identify and parameterise the determinants of host susceptibility to parasitism, in a non-domesticated bird/helminth system. Although different factors of susceptibility have been investigated in wild birds, no single system study has yet

successfully established the most formative influences upon susceptibility and equated the magnitude of those influences to parasite aggregation, as recommended by Grenfell and Dobson (1995).

Model system

The model system chosen was the caecal nematode *Heterakis gallinarum*, infecting the ring-necked pheasant *Phasianus colchicus*. This is justified from conservation and agricultural perspectives because endoparasitic (usually gastro-intestinal) species of parasites have been shown to negatively affect life history in domesticated or semi-domesticated avian hosts (Tompkins and Begon, 1999).

Currently, much of the knowledge on the immunology of wild bird species stems from analogy with domesticated animals, but domesticated species are not hampered by the same environmental stresses, such as those imposed by low nutrition (Grenfell and Dobson, 1995). Studies on domesticated species also do not untangle within-host immunodynamics, and their results are often subject to disparities from wild species because of artificial selection (affecting vertical transmission of parasites through additive genetic resistance to infection) and the effects of husbandry (creating 'neonatal tolerance' to infection; Grenfell and Michael, 1992; Lloyd, 1995).

Control of *H. gallinarum* parasitism in pheasants is economically important because ground heavily contaminated with the parasite often also harbours the concurrent flagellate protozoan, *Histomonas meleagridis*. *H. gallinarum* is the secondary host of *H. meleagridis* and together they cause the 'blackhead' infection. This infection impacts upon the pheasant by thickening the caecal mucosa, causing it to bleed and

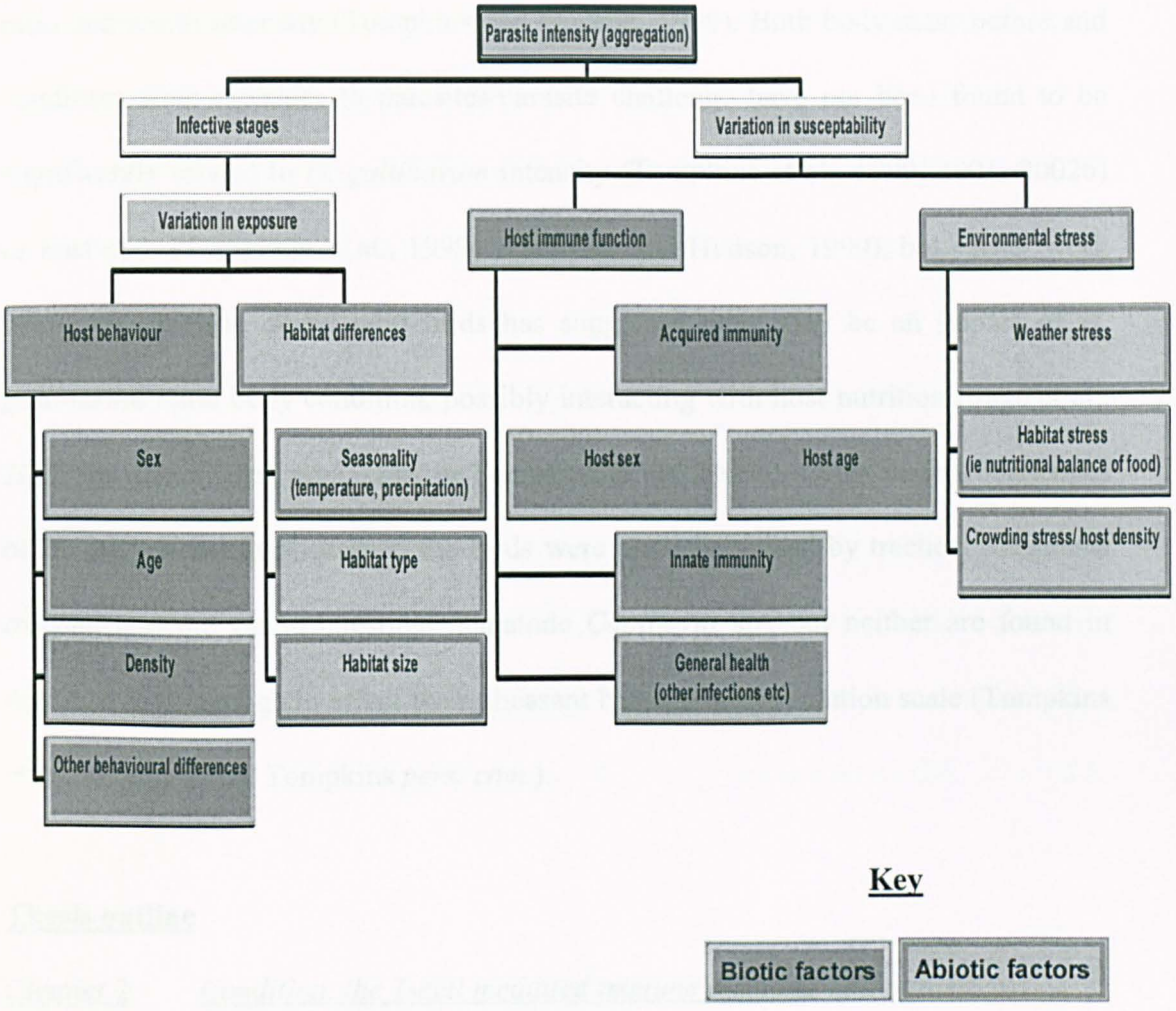
inhibit food digestion, with a consequent loss of host body condition. *H. gallinarum* has also been implicated in causing grey partridge (*Perdix perdix*) declines in the UK, via parasite-mediated competition with pheasants. Variation in the susceptibility of the two hosts to the parasite may have resulted in partridge exclusion from certain areas, due to the dissemination of infective eggs from only a few, heavily parasitised pheasants (Tompkins et al., 1999, 2000a, 2000b).

It has become increasingly important to examine the characteristics within hosts that affect susceptibility to parasitism as current control methods are becoming more restricted (for example the recent ban of sale on Emtril, used to control Blackhead in the UK). Non-invasive parasite control procedures for game birds could solve many of the problems associated with large-scale anthelmintic usage and resultant resistance (Wakelin et al., 2002).

Content of chapters

Within this study I investigated the factors affecting host susceptibility to parasitism (Figure 1), examining aspects of host body condition and susceptibility (Chapter 2), acquired immunity (Chapter 3), the effects of nutritional stress upon parasitism (Chapter 4) and the relationships between condition, parasitism and secondary sexual display characteristics (Chapter 5). The consequences for the host of parasite-mediated morbidity were also examined (Chapter 6) and general conclusions drawn (Chapter 7).

Figure 1 Factors affecting parasite dispersion within host populations.



Past research

Much work on *H. gallinarum* parasitism in pheasants has already been undertaken by D. M. Tompkins and M. Woodburn. Such work has established the occurrence of *H. gallinarum* mediated morbidity in the pheasant, with birds experiencing a slight reduction in caecal activity (Tompkins et al., 2001) and in fecundity (M. Woodburn, *pers. com.*). A significant difference was found between the host sexes and *H. gallinarum* intensity (Tompkins et al., 1999; Tompkins and Hudson, 1999) but not female worm length (indicative of fecundity) (Tompkins et al., 1999; Tompkins and Hudson, 1999), which may indicate that intensity differences are due to variation in male versus female behaviour and are not linked to immunity. No correlation has been

found between either the proportion of parasite adults versus larvae or parasite sex ratio and worm intensity (Tompkins and Hudson, 1999). Both body mass before and condition after exposure to parasites/parasite challenge have not been found to be significantly related to *H. gallinarum* intensity (Tompkins et al., 1999, 2001, 2002b) or host sex (Tompkins et al., 1999; Tompkins and Hudson, 1999), but earlier work using anthelmintic dosed wild birds has suggested there may be an impact of *H. gallinarum* upon body condition, possibly interacting with host nutrition (Sage et al., 2002; M. Woodburn, *pers. com.* in Tompkins et al., 2000b). Often during challenges of *H. gallinarum* in pheasants, the birds were also parasitised by tracheal *Syngamus trachea* and the gastro-intestinal nematode *Capillaria* sp., but neither are found in densities high enough to affect their pheasant hosts at the population scale (Tompkins et al., 2002b; D. M. Tompkins *pers. com.*).

Thesis outline

Chapter 2 *Condition, the T-cell mediated immune response and parasitism*

Variations in susceptibility are likely to be affected by trade-offs between immune defence, condition and life history traits (Ferdig et al., 1993; Gustafsson et al., 1994; Hamilton and Zuk, 1982; Lochmiller and Deerenberg, 2000; Nordling et al., 1998; Norris and Evans, 2000; Oppliger et al., 1996; Schmid-Hempel, 2003; Sheldon and Verhulst, 1996; Svensson and Merila, 1996; Wakelin et al., 2002). Within this chapter I investigated the relationships between condition, the T-cell mediated immune response in naive individuals and the intensity of parasitism. Within the investigation, individuals naive to infection were used as their level of immunity would not be influenced by an acquired immune response.

The predictions tested were that:

- variation in the T-cell mediated immune response before parasite challenge would be predicted by variation in the condition of individual hosts, and
- variation in the T-cell mediated response prior to challenge would predict variation in the number and size of parasites in the host after exposure.

Within the pheasant system, immunity may be likely to help regulate resistance and consequent susceptibility to parasitism as mating systems are polygamous and populations are highly fragmented (Giesel et al., 1997; Scribner et al., 1989); the effects of heritable resistance could therefore be comparatively small. This is because greater genetic mixing and diversity within harems due to male-male competition might lead to lower rates of selection for resistance, compared to that of a more monogamous species.

Chapter 3 *Acquired immunity and parasitism*

Upon infection, the innate immune response initially defends against disease. In short-lived species, this defence may be the only mechanism of immune response. But this preliminary reaction may not always have a sufficiently detrimental effect upon the pathogen, and could divert host immune defence away from important functional antigens, immunosuppress the later induced response or may even promote immunopathology. As a result, in some longer-lived species a second line of resistance has evolved to moderate or prohibit further parasite subsistence; the antigenic acquired immune response.

An acquired immune response has been suggested to be the most important physiological defence to have evolved against parasites (Roitt et al., 1998) and its expression has been shown to effectively control parasite loads (Baron and Weintraub, 1987; Wakelin and Apanius, 1997). Such a response is characterised by a reduction in parasite intensity or prevalence when a host is re-infected by a parasite species.

An assumption of no acquired immune resistance of the pheasant to *H. gallinarum* parasitism was made by examining earlier work on another game bird – the red grouse (Hudson and Dobson, 1997; Shaw and Moss, 1989a; Wilson, 1983). This was used within the apparent parasite-mediated competition model that suggested *H. gallinarum* as one mechanism for grey partridge decline in the UK. But, because resistance to nematode infection does occur in some hosts (Keymer and Tarlton, 1991; Stear et al., 1999), the work undertaken within Chapter 3 aimed to investigate possible acquired immunity of the pheasant to *H. gallinarum.*

The prediction tested was that parasite intensity and fecundity would differ between experimental treatment groups.

Chapter 4 *Food stress and susceptibility to parasitism*

Over wintering maintenance diets for game birds are based loosely around research undertaken on broiler chickens and other domesticated bird species. They are designed to promote optimum growth and development conditions: criteria suggested to be insufficient even within the broiler industry (Gous, 1998). These diets have not been developed to ensure long-term health (Dietert and Lamont, 1994) or depress disease and parasitism, which are usually treated when necessary with the use of appropriate

drugs. Another method of control could be through amino acid and protein manipulation in feeds.

Hand-reared, released pheasants have lower breeding (Brittas et al., 1992; Hill and Robertson, 1988; Hillgarth, 1991; Leif, 1994), continuing survival (Brittas et al., 1992; Hill and Robertson, 1988; Leif, 1994; Woodburn, 1993) and re-nesting rates (Hoodless et al., 1999) than their wild counterparts. These differences are suggested to be a result of poor nutrition (Hoodless et al., 1999), which may be responsible for reductions in health and body condition (Robertson, 1990; Robertson et al., 1990), and would be exacerbated by resource trade-offs caused by *H. gallinarum* parasitism and perhaps antagonized (but not investigated in this instance) by reduced immune functioning combined with depressed body condition.

In chapter 4, the effects of protein and amino acid deficiencies in the diet of host pheasants, and also the effects of a linseed oil additive were examined in relation to *H. gallinarum* parasitism.

Protein was manipulated because current levels are suggested to be influenced by research from the broiler industry and by customer preference and they may therefore be set too high, consequently promoting obesity and in turn lowering individual immune fitness, thereby allowing parasite intensity to rise and possibly creating a better source of food for parasites.

The amino acids lysine and methionine were selected for manipulation as their supplementation in a reduced protein diet (reduced to 90% of the recommendation)

has been suggested as the best method of improving amino acid balance, and consequently gut health and individual immune defence in poultry (Ferket, 2003).

I tested the effect of a linseed oil additive as a manipulation because a few cases exist of specific dietary additives being used to depress particular parasitic infections such as the use of omega-3 fatty acids from fish oils, to discourage Coccidiosis (IFOMA, 1999).

The predictions tested were that:

- parasite numbers and sizes should differ between hosts maintained on diets that differed in protein content but not amino acid levels,
- body condition should differ between hosts maintained on diets that differed in levels of amino acids,
- variation in host body condition would predict variation in the number and size of parasites within the host, and
- the number and size of parasites within the host should differ between hosts maintained on diets containing differing amounts of linseed oil.

The trial was undertaken during the winter months when the birds were not in breeding status so that protein and amino acid (specifically digestible lysine, methionine and cysteine) levels within a standard maintenance pellet could be manipulated without negatively affecting the health of the birds as a result of their breeding status.

Chapter 5 *Secondary sexual characteristics, condition, immune defence and parasitism*

In chapter 5, the relationships between host immune defence, condition, secondary sexual characteristics and parasite infection were investigated to examine whether the expression of ornaments was related to quality and therefore to see whether they were likely to affect the susceptibility of hosts to *H. gallinarum* through investment in signals.

The predictions were tested that:

- characteristics would to be correlated with each other if they were used in mate sexual selection or male-male intra-sexual interactions,
- immune functioning and condition would affect male showiness, and
- parasitism would also affect male showiness.

Chapter 6 *Effects of* H. gallinarum *parasitism*

Tompkins investigated the effects of the caecal nematode *H. gallinarum*, which is able to parasitise both the grey partridge and the ring-necked pheasant in the UK and is implicated in creating apparent parasite-mediated competition between the species. He concluded that the partridge suffered greater morbidity as a result of *H. gallinarum* than the pheasant, as despite the fact the pheasant had a higher parasite carrying capacity (Draycott *pers. com.*), a higher parasite egg ingestion rate (Tompkins et al., 2000b), higher parasite establishment (Tompkins et al., 2000b) and higher parasite fecundity (Tompkins et al., 2000b), the partridge had greater host mortality (Robertson and Dowell, 1990) and seemed to suffer an increase in mortality as a result of *H. gallinarum* parasitism (Tompkins et al, 1999). The pheasant suffered very little

morbidity as a result of this parasitism, other than a slight reduction in caecal activity (Tompkins et al., 2001) and a reduction in fecundity (M. Woodburn, *pers. com.*). Possible reasons for the greater effects of *H. gallinarum* parasitism upon the morbidity of one host compared to the other are host suitability, which may be affected by host parasite co-evolution (Tompkins *pers. com.*), or differences in host immune defence and parasite virulence.

The aim of this study was therefore to examine the effects of *H. gallinarum* parasitism upon pheasant condition and spleenic response. The results were compared to a previous study on *Trichostrongylus tenuis* parasitism of red grouse (Wilson and Wilson, 1978), to identify evidence of a generalised immune response to endoparasitic infection in avian species.

The predictions were tested that:

- variation in the intensity of *H. gallinarum* parasitism between hosts should predict variation in host body condition, PCV and RBC,
- variation in host liver mass should be predicted by variation in the measures of host body condition, and
- variation in spleen mass would predict variation in the intensity of *H. gallinarum* infection between hosts.

Examining the effects of endoparasitism upon the immunological responses of a pheasant host is important, as responses resulting from parasitism have been suggested to affect host subsequent life history. Ectoparasitic hen flea infestation of great tits during egg laying was shown to induce host responses and increase tolerance

to parasitism, with a consequent reduction in breeding failures, a higher proportion of (earlier) fledglings, nestlings of higher body mass with longer feathers and a greater number of recruits and first year grandchildren (Heeb et al., 1998).

Chapter 7 *Main discussion and conclusions*

Chapter 7 is an overview of the effects of host condition, the T-cell mediated immune response, acquired immunity, food stress and secondary sexual characteristics upon *H. gallinarum* parasitism in the pheasant. Discussion is made of the factors determining host susceptibility to parasitism, how food stress impacts upon parasite aggregation within a host population, and how parasite challenge compared to natural exposure to parasitism affects parasite aggregation.

General methodology

Faecal egg counts

Caecal droppings were collected from the pen, mixed well and half a gram of droppings were suspended in 10 ml saturated salt solution and examined using McMasters chambers under x100 magnification, in 0.1 ml sub-samples to confirm the presence/absence of *H. gallinarum* eggs.

Blood sampling

Birds were blood sampled (<0.5 ml blood) from the brachial vein of the left wing using a 21 gauge, 6.35 mm needle, after first sterilising the skin with surgical spirit. Standard haematological techniques (Howlett, 2000; Wardlaw and Levine, 1983) were undertaken to quantify Red Blood Cell (RBC) counts and Packed Cell Volume (PCV)

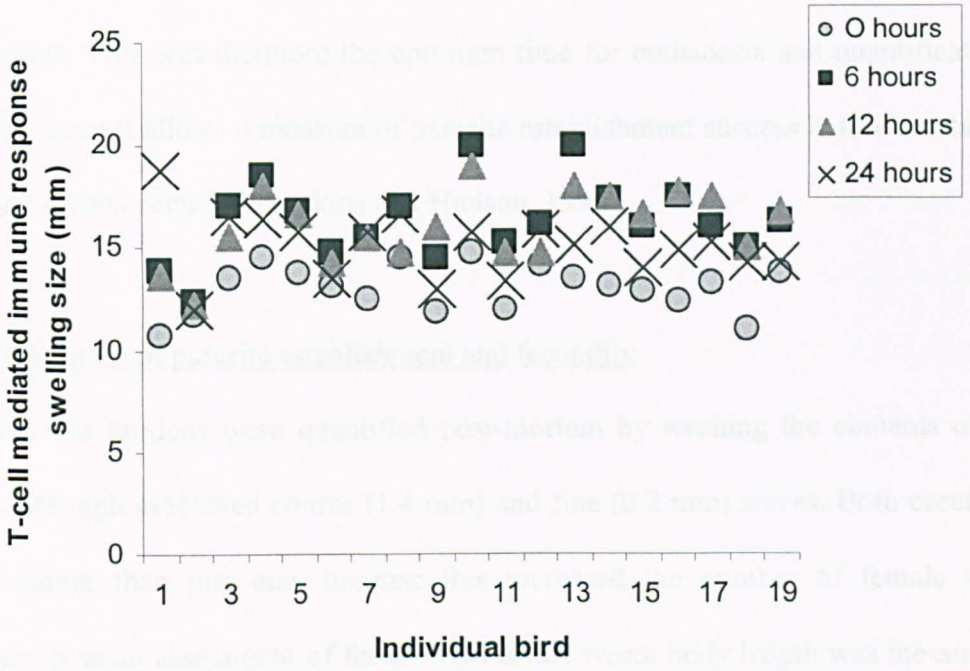
estimates. PCV measures the relative (rather than absolute, as with RBC) amount of red blood cells in blood volume, and reflects oxygen uptake and transfer to tissues.

Testing for T cell mediated immunity

T-cell mediated immunity (CMI) was measured using the phytohaemagglutinin (PHA) skin testing technique (Howlett, 2000; Smits et al., 1999) and was undertaken during the same procedure as the blood sampling, to minimise handling stress to the bird. An injection of PHA stimulates macrophage infiltration as a mitogenic effect upon T-lymphocytes causes their accumulation; the subsequent swelling is indicative of a response (McCorkle et al., 1980). The wing web on the mid-patagium of the right wing was plucked free of feathers and swabbed with alcohol. 6 mg PHA dissolved into 1.2 ml phosphate buffered saline (PBS) was then subcutaneously injected (using a 21 gauge, 6.35 mm needle); this was the dosage calculated for a bird of 600 g body mass, calibrated from preliminary work as the minimal viable for quantification of response. Cutaneous hypersensitivity response was calculated as the difference in wing-web thickness measured with Vernier callipers prior to challenge, and then 6 and 12 hours after. All wing-web thickness measurements were taken twice, and repeatability values were calculated (Lessells and Boag, 1987). Measurements were highly repeatable for both the Chapter 2 and 3 studies (repeatability values of between 0.99 and1.0; P <0.0001). The response 12 hours after injection was subtracted from that at 6 hours, to quantify the CMI response recovery from challenge. Preliminary work on pheasants was undertaken to calculate these time intervals and they were within the period during which the maximal response was observed after PHA challenge (Figure 2). Recent work has shown a control injection of PBS alone,

injected into the opposing wing, can be detrimental to the bird and adds no useful information to the test so this was not undertaken (Smits et al., 1999).

Figure 2 The magnitude of T-cell mediated immune response swelling size subsequent to challenge with PHA.



Parasitic challenge

All challenges to birds were undertaken orally using a tube inserted into the crop. Eggs were obtained from female worms (from pheasants used in earlier work), incubated at 21 °C for 21 days in 0.05 % formalin solution, and then broken down and diluted to the required level in 0.05 % saline solution using a small electric blender and maintained until use at 4 °C. A challenge of 100 *H. gallinarum* eggs is a realistic level of infection as such intensities are commonly found in wild pheasants (Draycott et al., 2000; Tompkins and Hudson, 1999) and previous work has suggested a larger dose may create density-dependent effects (Tompkins and Hudson, 1999). A single

dose rather than continuous challenge was chosen as it has been suggested to be more indicative of a typically highly aggregated natural distribution (Draycott et al., 2000; Tompkins and Hudson, 1999). Furthermore, work on other game bird/nematode systems indicates no difference in the infection resulting from single-dose versus trickle-dose challenges (Shaw and Moss, 1989a). Past research has shown 30 days is sufficient time to allow one generation of *H. gallinarum* larvae to reach maturity in pheasants. This was therefore the optimum time for euthanasia and quantification of burden since it allows a measure of parasite establishment success before mortality of mature worms occurs (Tompkins and Hudson, 1999).

Quantification of parasite establishment and fecundity

Host worm burdens were quantified post-mortem by washing the contents of both caeca through graduated course (1.4 mm) and fine (0.2 mm) sieves. Both caeca were used rather than just one, because this increased the number of female worms measured as an assessment of fecundity. Female worm body length was measured on recovered worms, using an ocular micrometer under x40 magnification to the nearest 0.001 mm. Nematode fecundity has often been found to be related to worm size (Goater, 1992; Michael and Bundy, 1989; Stear et al., 1996) and this method of establishing fecundity has previously been used within this system (Tompkins et al., 1999; Tompkins and Hudson, 1999; Tompkins et al., 2002b). This technique was chosen, as the traditional measurement of *per capita* fecundity using faecal egg production is not representative of *H. gallinarum* burdens in the pheasant and earlier work has determined worm length as an alternative (Anderson and Schad, 1985; Hudson and Dobson, 1997; Keymer and Slater, 1987; Shostak and Scott, 1993).

Worm lengths were averaged per host for analyses, as degrees of freedom were insufficient to include host as a covariate or random factor.

Modelling pectoral muscle mass on live birds and validation of the Bolton technique

A technique for modelling pectoral muscle mass on live birds was used in all experiments reported here (Bolton et al., 1991). This technique estimates keel height from biometrics (step *a*) and measures profile area (step *b*). Within my studies the technique was modified as actual keel heights taken during post-mortem were available and weighed photocopied profiles (on paper standardized for weight) were deemed as good a measurement as profile area. Two profiles from each pheasant were created at each measuring occasion, and their average was obtained. Repeatability analysis was undertaken on the two profiles, which established that the measurement technique was highly repeatable (repeatability values of 0.91, 0.94 and 0.78; P <0.0001 for Chapters 2, 3 and 4, respectively; Lessells and Boag, 1987). A body size score was created using principle component analysis of tarsal, wing (from the 'arm pit' to wing tip), wing chord and head lengths (step *e*). Each was measured twice and repeatability analysis has shown their measurement is highly repeatable (repeatability values of <0.99-1.0>; P <0.0001; Lessells and Boag, 1987).

The pectoral muscle mass is highly correlated with the protein content of game birds, and when body size is controlled for it is a good indicator of body condition (Brittas and Marcstrom, 1982; Tompkins et al., 1999, 2002b). Before euthanasia, this technique was again applied to validate its usefulness using the actual lean wet breast muscle mass.

Liver and spleen masses

The liver and spleen are suggested to play a role in the immunological response of birds, although their specific functions are not altogether understood (John, 1994a, 1994b). The proportion of the spleen used specifically for immune response is much greater than that of the liver (Møller et al., 1998b) and although I investigated both organs, I therefore expected the spleen to show greater affiliations with immune response and parasitism. Liver mass was assumed to be positively associated with condition. Spleen mass/size has been studied to a much greater extent in mammals, with interpretation of its role within disease resistance applied to avian species. Little research has attempted to examine avian spleenic activity and parasitism specifically, but nematode parasitism has been linked to the evolution of relative spleen size (John, 1994a; Morand and Poulin, 2000) and relative spleen size has been linked to the T-cell mediated immune response (due to the conceivable lymphocyte production; John, 1994a). Research has offered differing conclusions as to the relationship between spleen mass/size and the level of parasitism. The presence of ectoparasitic fleas and haematophagous bugs upon host cliff swallows was linked to spleens that were 20% larger than in non-parasitised hosts (Brown and Brown, 2002), a comparative study of birds showed a positive association between larger spleen size and individuals suffering from parasitic infection and signs of disease (Møller et al., 1998a), and a positive relationship was also found between spleen size and parasite-induced host mortality in 21 species of atricial birds (Møller and Erritzoe, 2002). Another study on snow geese found no associations between spleen mass and parasitism (Shutler et al., 1999). Larger spleens have also been linked to colonially living species (which had a consistently larger bursa of Fabricius; Møller and Erritzoe, 1996) probably as they are more likely to encounter parasitism (Brown and Brown, 2002) and would be expected

to invest in immunological defense (Møller, 2001; Møller et al., 1998a), and to species more regularly exposed to parasites (Møller, 1998; Møller and Erritzoe, 1998). Across species of birds, in juveniles there is no sexual dimorphism in spleen mass, but it has been found to be relatively smaller, and more variable, in male than female birds (Møller, 1998). Spleen mass may accordingly be driven by either parasite-induced splenomegaly or as a result of greater immunological investment against parasitism (Brown and Brown, 2002). As the two are difficult to separate, interpretations of mass were only made in comparison with both the level of parasitism and with measures of the immunological response, where heavier spleen mass was indicative of a greater ability to respond immunologically to parasitism (and therefore in a negative relationship with parasitism).

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Chapter 2

Innate condition, the T-cell mediated immune response and their effects upon macroparasite infection: an investigation using the ring-necked pheasant parasitised by the caecal nematode *Heterakis gallinarum.*

ABSTRACT

Much of the present literature on avian immunology explores the physiological trade-offs, driven by limited resources, which affect life-history decisions such as the ability to respond against pathogens/parasites, potential reproduction and the expression of secondary sexual characteristics. This study explored an over-riding concept linking trade-offs with immunocompetence: that ring-necked pheasant *Phasianus colchicus* condition and the immune response are themselves likely to be positively related in naïve individuals, because of resource limitation. The prediction that pectoral muscle mass and red blood cell count as condition indicators would be positively associated, that these would be positively related to the T-cell mediated immune response, and that this would be negatively associated with *Heterakis gallinarum* parasitism, were explored. The condition indicators were correlated, but neither was related to T-cell mediated immune response. Pectoral muscle mass was positively related to parasitism, to which red blood cell count was not associated. T-cell mediated immune response was negatively related to parasite intensity, and positively related to female parasite fecundity. Female parasite fecundity was not related to red blood cell count or pectoral muscle mass. These results are consistent with T-cell mediated immune response influencing parasitism, but not with pheasant condition and immune response being linked in naïve individuals because of resource limitation. T-cell

mediated immunity may however be compromised and limited by life-history characteristics likely to be of a more similar energetic cost, such as reproduction and display of secondary sexual characteristics.

INTRODUCTION

Within a host population, the distribution of parasites is often aggregated with certain hosts carrying a greater proportion of the parasite population and most hosts carrying few or no parasites (for example Anderson and May, 1978; Pacala and Dobson, 1988; Shaw and Dobson, 1995). It is within these heavily parasitised individuals that the main proportion of parasite reproduction occurs. This reproduction allows further transmission of the parasite and plays an important role in the persistence of parasitism (Anderson and May, 1985; Woolhouse et al., 1997).

The causes of parasite aggregation are thought to be both differences in the exposure of the host to the infective stages of the parasite, and in the susceptibility of each host during exposure to the parasite. The susceptibility of the host can be influenced by variations in host innate phenotype, genetic background, body condition, immune functioning, sex, age and stresses caused by social interactions, host density, the environment and habitat (for example Anderson and May, 1991; Barnard et al., 1993, 1994; Brinkhof et al., 1999; Bundy, 1988; Burdon, 1991; Cook, 1991; Cote and Poulin, 1995; Crombie and Anderson, 1985; Gregory et al., 1992; Gulland, 1992; Hudson and Dobson, 1995; Kolmer, 1996; Nelson and Demas, 1996; Poulin, 1991; Roitt et al., 1998; Shields and Crook, 1987; Webster and Woolhouse, 1999; Yan and Norman, 1995; Zuk, 1990).

After the initial infection by a pathogen or parasite, hosts are likely to exhibit variation in their immunological responses and consequent susceptibility to parasitism. This is demonstrated by heterogeneities in parasite prevalence. Although differences may result from the factors mentioned above, they are also likely to be affected by the status of life-history decisions made prior to the initial infection, which affect the relative condition of the individual and its ability to respond immunologically to infection. By this I mean that the limited resources available within one organism drive evolutionary but also mediate physiological trade-offs (Gustafsson et al., 1994), which are optimised for each individual (Norris and Evans, 2000). Previous studies have indicated that these trade-offs involve life-history characteristics such as reproductive potential (Gustafsson et al., 1994; Nordling et al., 1998; Oppliger et al., 1996), the immune response to infection (Folstad and Karter, 1992; Gustafsson et al., 1994; Nordling et al., 1998; Sheldon and Verhulst, 1996) and the expression of secondary sexual characteristics (Johnston and Grafen, 1993; Møller et al., 1996, 2000; Nolan et al., 1998; Saino and Møller, 1996; Saino et al., 1999; Svensson and Merila, 1996; Verhulst et al., 1999; Zahavi, 2003; Zuk, 1996).

This study aimed to examine the hypothesis that the relationships between the condition of the individual, its ability to respond immunologically to infection, and the prevalence and fecundity of parasite infection are in proportion with each other. This is because, as a result of resources being limited, each is likely to impact upon the next. The hypothesis was investigated by quantifying innate condition and immune response, and relating them to the level of macroparasitism after parasite challenge. Such an investigation is important because to my knowledge the current

literature supports little evidence of parasite burdens in 'wild' host species being unambiguously reliant upon host condition or the immune response; the hosts described in previous studies are likely to have been affected by previous life-history events such as reproduction and infection (Brinkhof et al., 1999; Christe et al., 1998, 2000; Nordling et al., 1998; Tella et al., 2000), which would have knock-on effects upon condition and further immune responses, and affect the results. The results of studies which involve measuring life history parameters such as reproductive fecundity, parasite intensity, host condition and immune responses are also likely to be imprecise because these parameters are difficult to untangle from each other, and are therefore difficult to accurately perceive and quantify, especially within a field situation (Norris and Evans, 2000).

The model system chosen for this study was the ring-necked pheasant *Phasianus colchicus*, parasitised by the caecal nematode *Heterakis gallinarum*. More work undertaken within this parasite-host system has implied that pheasants are able to gain an acquired immune resistance to *H. gallinarum*, which in the short-term negatively impacts upon future parasite infection (Chapter 3, this thesis). To ensure the parameters measured within this study were unaffected by and therefore naïve to, previous infection, the study animals were hand-reared in a sterile environment. The chicks were just reaching sexual maturity and their immune responses and condition were also unlikely to have been affected by trade-offs with reproduction or the expression of secondary sexual characteristics. As far as I am aware, this investigation is therefore uniquely placed amongst those exploring the impact of resource limitation upon the immune response and ensuing parasitism in host species.

Within this study, the relationships between the different characteristics of pheasant condition and the T-cell mediated immune response before parasite challenge were first examined. This is important because the immune response involves multiple components within the immune system such as the innate and acquired immune arms. If energetic trade-offs do occur then at least some of these immune response components are likely to be dependent upon, and therefore correlated to, the general condition of an individual. Pectoral muscle mass and red blood cell count were predicted to be positively associated, and positively related to the T-cell mediated immune response. Similar relationships have been found in individuals that have had previous exposure to parasites, such as great tits (Brinkhof et al., 1999), barn swallow nestlings (Saino et al., 1997a), fledgling American kestrels (Tella et al., 2000) and blue tits (Svensson and Merila, 1996).

Because of physiological trade-offs, a negative relationship between the prevalence or intensity of parasitism and individual condition and immune response, has also been postulated (Deerenberg at al., 1997; Saino et al., 1997a, 1997b). This relationship was investigated, with the characteristics of pheasant condition and the T-cell mediated immune response predicted to be negatively related to parasite prevalence and fecundity.

The T-cell mediated response, indicative of the concentration of immunologically mediated T-cells, may be likely to affect caecal nematode parasitism by causing 'spontaneous cure' through worm expulsion during primary infection. Although the process leading to expulsion is not well understood, during *Trichostrongylus columbriformis* parasitic infection of sheep, specialised T-cells attracted basophils

into sites of antigen stimulation and induced amine release. Amine levels in the gut wall and gut lumen have been observed to rise with the onset of worm loss. These rises are coupled with increases in eosinophils and macrophages from bone marrow, which all contribute to the development of a localised inflammatory restriction within the gut. This inflammation can physically, through excess mucus secretion and increased peristalsis, and immunologically, act against parasitism (Wakelin, 1996).

During examination of the T-cell mediated immune response, the recovery from the response as well as the frequently investigated maximal cutaneous hypersensitivity response (Cheng and Lamont, 1998; Christe et al., 2000; Saino et al., 2003), calculated from preliminary investigations, was examined. The recovery from response was interpreted as the response taken at a set time during the decline phase of the cutaneous reaction, subtracted from the maximal response. It may be important because longer maintenance of an inflammatory reaction could indicate a continuing response against parasite infection, or in the antithesis it could indicate that recovery from a response to infection is taking longer, and is therefore of less benefit to a host.

MATERIAL AND METHODS

Experimental design

Fifty ring-necked pheasant chicks were hand-reared from 2-days old in sterile conditions to ensure naivety to infection; half were retained until 12 weeks of age and half to 16 weeks. During this period, all individuals were randomly substituted every two days into one of two identically sized pens (two pens were necessary for animal

husbandry reasons) to ensure that all had been identically treated. Birds were given water and supplied with chick crumbs, they were then retained on standard maintenance pellets *ad-libitum.* At 12 weeks, half were randomly selected and caecal droppings were collected from their pen to verify the absence of *H. gallinarum* prior to challenge. The selected group of birds were then weighed (to the nearest 25 g) and had their tarsus and wing chord lengths measured and pectoral muscle profiles traced, were blood sampled to examine general condition using red blood cell counts, and were challenged with phytohaemagglutinin (PHA) to examine their T-cell mediated immune response.

Immediately after blood sampling and the PHA skin test, all birds were orally challenged using a 2ml single dose suspension of approximately 100 *H. gallinarum* eggs. Birds were randomly split into one of two groups, necessary for animal husbandry reasons; they were then moved into outdoor pens and maintained over a 30-day period. Caecal droppings were collected and examined for eggs to ascertain the success of the challenge.

After this 30-day maintenance period all birds were again weighed and were then euthanased to quantify female *H. gallinarum* length and the intensity of parasite infection. At 16 weeks the remaining poults were treated identically to the first group.

Two groups of birds aged 12 and 16 weeks were used within this study rather than one large group because I was unable to provide accommodation, animal husbandry and process laboratory samples simultaneously for one group.

General methodology

Faecal egg counts

Caecal droppings were collected from the pen, mixed well and half a gram of droppings were suspended in 10 ml saturated salt solution and examined using McMasters chambers under x100 magnification in 0.1 ml sub-samples to confirm the presence/absence of *H. gallinarum* eggs.

Blood sampling

Birds were blood sampled (<0.5 ml blood) from the brachial vein of the left wing using a 21 gauge, 6.35 mm needle after first sterilising the skin with surgical spirit. Standard haematological techniques (Howlett, 2000; Wardlaw and Levine, 1983) were used for red blood cell count, which was used to represent pheasant condition before parasite challenge.

Testing for T-cell mediated immunity

The T-cell mediated immune response measured using the PHA skin testing technique (Howlett, 2000; Smits et al., 1999) was undertaken during the same procedure as the blood sampling to minimise handling stress to the bird. An injection of PHA stimulates macrophage infiltration as a mitogenic effect upon T-lymphocytes causes their accumulation; the subsequent swelling is indicative of a response (McCorkle et al., 1980). The wing web on the mid-patagium of the right wing was plucked free of

feathers and swabbed with alcohol. 6 mg PHA dissolved into 1.2 ml phosphate buffered saline (PBS) was then subcutaneously injected (using a 21 gauge, 6.35 mm needle); this was the dosage calculated for a bird of 600 g body mass, calibrated from preliminary work as the minimal viable for quantification of response. Cutaneous hypersensitivity response was calculated as the difference in wing-web thickness measured with Vernier callipers prior to challenge, and then 6 and 12 hours after. All wing-web thickness measurements were taken twice, and repeatability values were calculated (Lessells and Boag, 1987). Measurements were highly repeatable (repeatability values >0.99; P >0.0001). The response 12 hours after injection was subtracted from that at 6 hours to quantify recovery from the response. Preliminary work on pheasants was undertaken to calculate the time intervals for measurement. Recent work has shown a control injection of PBS alone, injected into the opposing wing, can be detrimental to the bird and adds no useful information to the test so this was not undertaken (Smits et al., 1999).

Parasitic challenge

All challenges to birds were undertaken orally using a tube inserted into the crop. Eggs were obtained from female worms (from pheasants used in earlier work), incubated at 21°C for 21 days in 0.05 % formalin solution, and were then broken down and diluted to the required level in 0.05 % saline solution using a small electric blender and maintained until use at 4°C. A challenge of 100 *H. gallinarum* eggs is a realistic level of infection as such intensities are commonly found in wild pheasants (Draycott et al., 2000; Tompkins and Hudson, 1999) and previous work has suggested a larger dose may create density-dependent effects (Tompkins and Hudson, 1999). A

single dose rather than continuous challenge was chosen as it has been suggested to be more indicative of a typically highly aggregated natural distribution (Draycott et al., 2000; Tompkins and Hudson, 1999). Furthermore, work on other game bird/nematode systems has indicated no difference in the infection resulting from single-dose versus trickle-dose challenges (Shaw and Moss, 1989). Past research has shown 30 days is sufficient time to allow one generation of *H. gallinarum* larvae to reach maturity in pheasants. This was therefore the optimum time for euthanasia and quantification of burden since it allows a measure of parasite establishment success before mortality of mature worms occurs (Tompkins and Hudson, 1999).

Quantification of parasite establishment and fecundity

Host worm burdens were quantified post-mortem by washing the contents of both caeca through graduated coarse (1.4 mm) and fine (0.2 mm) sieves. Both caeca were used rather than just one because this increased the number of female worms measured as an assessment of fecundity. Female worm body length was measured on recovered worms using an ocular micrometer under x40 magnification to the nearest 0.001 mm. Nematode fecundity has often been found to be related to worm size (Goater, 1992; Michael and Bundy, 1989; Stear et al., 1996) and this method of establishing fecundity has previously been used within this system (Tompkins et al., 1999; Tompkins and Hudson, 1999; Tompkins et al., 2002). This technique was chosen as the traditional measurement of *per capita* fecundity using faecal egg production is not representative of *H. gallinarum* burdens in the pheasant, and earlier work has determined worm length as an alternative (Anderson and Schad, 1985; Hudson and Dobson, 1997; Keymer and Slater, 1987; Shostak and Scott, 1993).

Female worm lengths were averaged per host for analyses, as degrees of freedom were insufficient to include host as a covariate or random factor.

Modelling pectoral muscle mass on live birds and validation of the Bolton technique

The pectoral muscle mass is highly correlated with the protein content of game birds, and when body size is controlled for it is a good indicator of body condition (Brittas et al., 1982; Tompkins et al., 1999, 2002). A technique for modelling pectoral muscle mass on live birds was used as an indicator of actual pectoral muscle mass and therefore of body condition (Bolton et al., 1991). This technique estimates keel height from biometrics (step *a*) and measures profile area in a cross-section through the body from an obvious notch on the sternum (step *b*). It was slightly modified as actual keel heights taken during post-mortem were available and weighed photocopied profiles (on paper standardized for weight) were deemed as good a measurement as profile area after preliminary investigation. Two profiles from each pheasant were created and their average was obtained. Repeatability analysis was undertaken on the two profiles, which established that the measurement technique was highly repeatable (repeatability value of 0.91; $P<0.0001$). A body size score was created using principle component analysis of tarsal, wing (from the 'arm pit' to wing tip), wing chord and head lengths (step *e*). Each was measured twice and repeatability analysis has shown that their measurement is highly repeatable (repeatability values >0.99; $P<0.0001$). Before euthanasia, this technique was again applied to validate its usefulness using the actual pectoral muscle mass.

Statistical analyses

All analyses were undertaken using S-PLUS version 6.2.1 for Windows (November 2003)^{TM} Professional Edition Release 2 (S-PLUS : Copyright 1988, 2003 Insightful Corp.) program, unless otherwise stated. This allowed analyses using Gaussian and negative binomial error distributions. Where used, Linear and Generalised Linear models were arrived at using stepwise deletion of the insignificant main effects, excluding those within significant interaction terms. Predicted fits were used to display results controlling for the other terms remaining in the models. The *F* statistics and deviance values presented are from the minimal models for significant terms, or the minimal model with the non-significant term added on to the model for terms dropped from the maximal model. Within Linear and Generalised Linear modelling, pheasant sex and age, pen number and the pheasant body size score created using principal component analysis were included as control factors in analyses. Pen number was included to control for differences in group behaviour arising as a result of competition between the individuals within each pen. Pheasant sex and age and pen number were discarded if non-significant.

The Pearson *r* correlation method was employed to clarify Bolton's modelled muscle mass technique, using the modelled pectoral muscle mass and actual pectoral muscle mass of the birds at the time of euthanasia.

The influence of body condition and T-cell mediated immune response upon parasite intensity was examined using a Generalised Linear model with an explicitly defined negative binomial error distribution (Wilson and Grenfell, 1997; Wilson et al., 1996).

This allowed a valid model to be fitted to the aggregated parasite data (Figure 3), and also allowed for the inclusion of the pheasant body size score, pheasant sex, age, an interaction between them (sex*age), and interactions between red blood cell count*pheasant sex and pectoral muscle mass*pheasant age. A statistical test of negative binomial fit (designed and written by Darren Shaw, University of Edinburgh for the S-PLUS program) was used to examine the error distribution of the worm intensity data.

To investigate associations between the different characteristics of pheasant condition: pectoral muscle mass and the red blood cell count before parasite challenge, a Linear model was used. This allowed for the inclusion of the pheasant body size score, pheasant sex, age, an interaction between them (sex*age) and an interaction of pheasant sex*body size score. Pectoral muscle mass rather than the red blood cell count was used as the response variable because red blood cell count was felt likely to be less variable, operating within greater biological confines than muscle mass, which is recognized as a sound indicator of general condition.

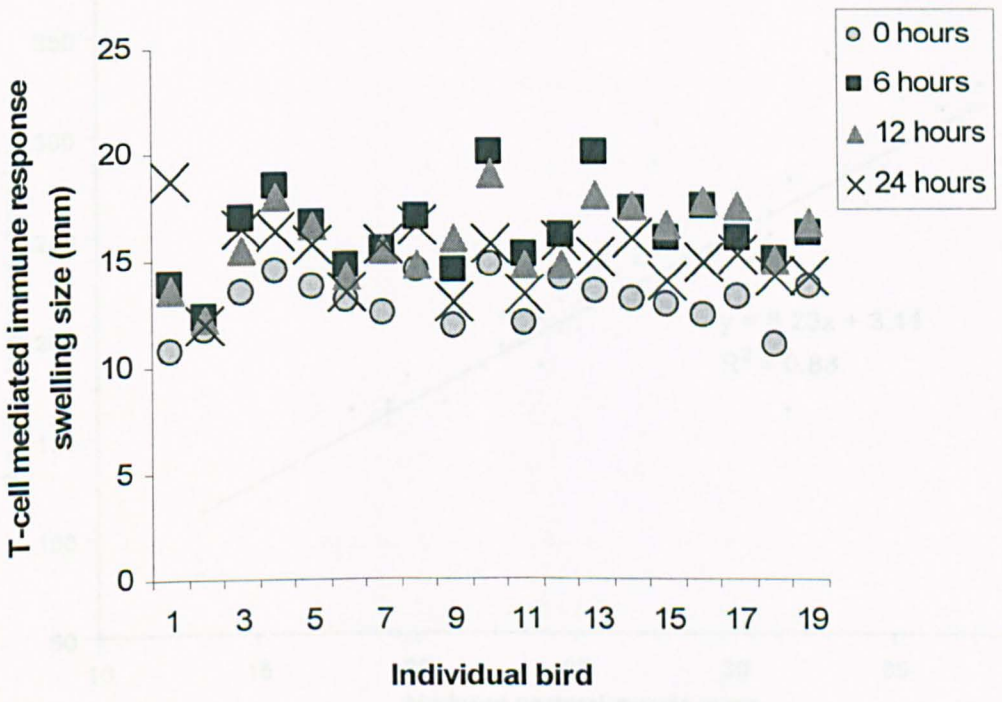
Linear models were used to examine whether variation in T-cell mediated immune response and the recovery from T-cell mediated immune response were influenced by pectoral muscle mass and red blood cell count, and to examine the influence of body condition and T-cell mediated immune response upon female *H. gallinarum* length. This allowed for the inclusion of the pheasant body size score in both analyses and for the inclusion of parasite intensity to control for any density dependent effects in the analysis examining female worm length.

RESULTS

Calculation of the time intervals for measurement of maximal cutaneous hypersensitivity response on the wing web

During preliminary work, the time intervals for measurement of the maximal cutaneous hypersensitivity response, and the recovery from response were calculated. The time interval for the maximal response was within the period during which the maximal reaction was observed, after PHA challenge (Figure 1). The second interval to enable measurement of the recovery from response was taken at a time practical for husbandry reasons: before sundown, in the decline phase of the cutaneous reaction.

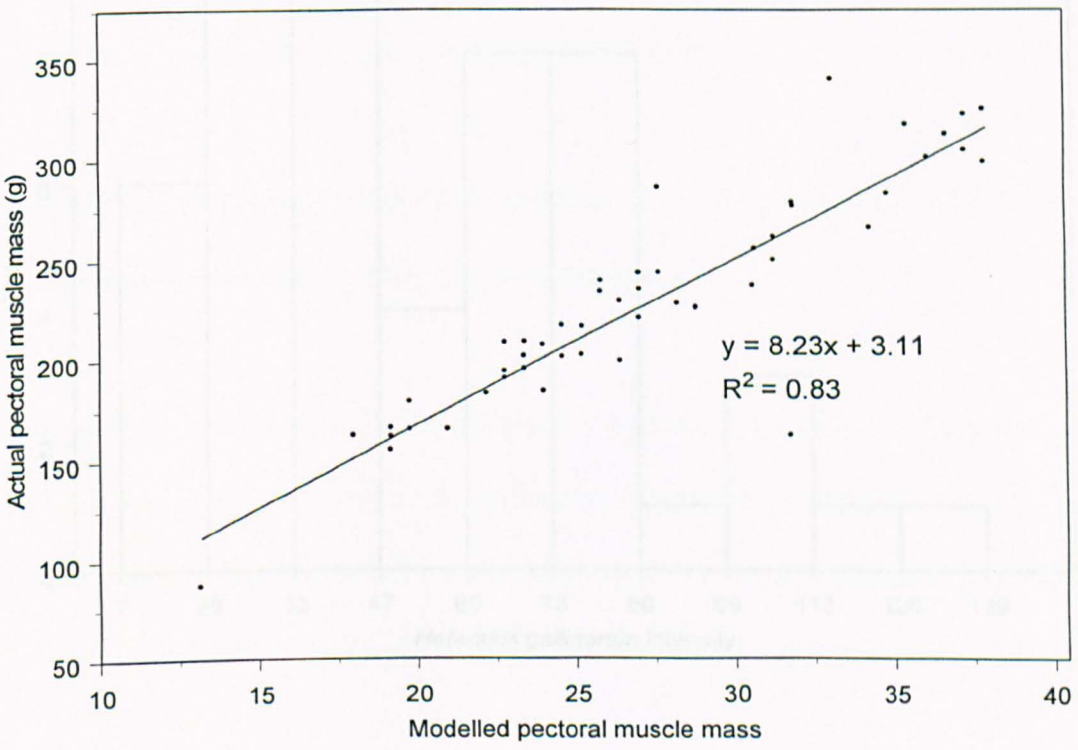
Figure 1. The magnitude of the cutaneous hypersensitivity response on the wing web with time, subsequent to challenge with PHA.



Accuracy of Bolton's modelled pectoral muscle mass technique

The precision of the technique for measuring body condition on live birds was clarified by undertaking a correlation on the modelled pectoral muscle mass and actual pectoral muscle mass of the birds at the time of euthanasia (after the parasite challenge and 30-day maintenance period). The two variables were significantly correlated (Figure 2; Pearson's correlation = 0.91, P <0.001, n = 48), indicating that modelled pectoral muscle mass was a reliable indicator of actual pectoral muscle mass at the time of measurement.

Figure 2. Correlation between the modelled pectoral muscle mass and actual pectoral muscle mass (n = 48).

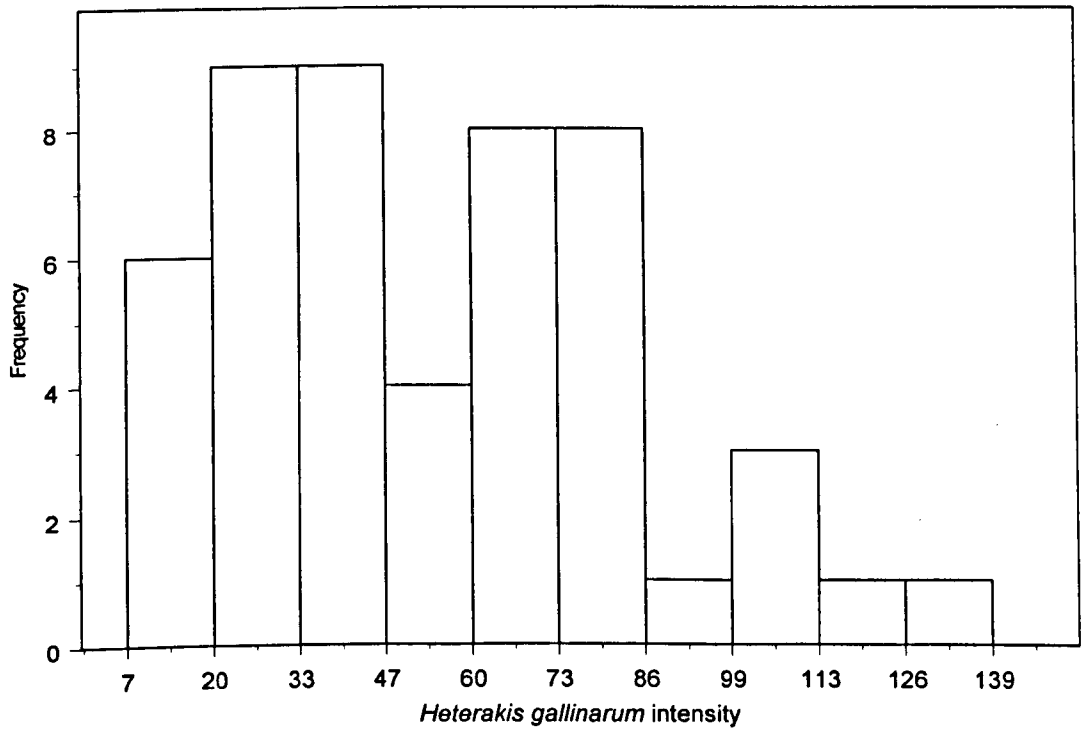


Frequency distribution of *H. gallinarum* intensity

After the 30-day maintenance period 100% of pheasants were infected with *H. gallinarum*, with an overall mean ± SD of 55.84 ± 30.51 worms (Figure 3).

A statistical test of negative binomial fit was used to examine the error distribution of the worm intensity data. It was found that the data did not differ significantly from a negative binomial distribution (P = 0.93), with a best estimate of the aggregation parameter (k) of 3.4 and variance/mean ratio of 16.7.

Figure 3. Frequency distribution of *H. gallinarum* intensity in pheasants (n = 50), 30-days after oral challenge with ~ 100 eggs.



The association between pectoral muscle mass and the red blood cell count before parasite challenge as indicators of pheasant condition

After controlling for pheasant body size score, sex, age and interactions between pheasant sex*age and pheasant sex*body size, variation in pectoral muscle mass was explained by red blood cell count (birds with greater muscle mass had higher red blood cell counts; Appendix 1, Table 1).

The relationship between pheasant condition and the T-cell mediated immune response before parasite challenge

After controlling for pheasant body size score, the variation in T-cell mediated immune response was not explained by either pectoral muscle mass or red blood cell count. It was explained by pheasant body size (birds with greater T-cell mediated immune response were of a smaller body size; Appendix 1, Table 2).

The relationship between pheasant condition and the recovery from T-cell mediated immune response before parasite challenge

After controlling for pheasant body size score, variation in the recovery from T-cell mediated immune response was not explained by either pectoral muscle mass or red blood cell count. It was explained by pheasant body size (birds holding a cutaneous hypersensitivity response for longer were of larger body size; Appendix 1, Table 3).

Effects of T-cell mediated immunity and pheasant condition upon parasite intensity

After controlling for pheasant body size score, sex, age and an interaction between sex*age, *H. gallinarum* intensity was significantly explained by red blood cell count (birds with higher parasite intensities had lower red blood cell counts), pectoral muscle mass (birds with higher muscle mass also had higher parasite intensities), and recovery from T-cell mediated immune response (birds holding a cutaneous T-cell hypersensitivity response for longer had lower parasite intensities) before parasite challenge. Parasite intensity was also significantly explained by interactions between red blood cell count*pheasant sex (with a negative relationship between intensity and red blood cell count in females, and a positive relationship in males; Figure 4) and pectoral muscle mass*pheasant age (with lower parasite intensity and muscle mass in hosts challenged at 12 weeks of age compared with those challenged at 16 weeks of age; Figure 5). Parasite intensity was not significantly explained by T-cell mediated immune response before parasite challenge (Appendix 1, Table 4).

Figure 4. Relationship between *H. gallinarum* intensity and an interaction between red blood cell count*pheasant sex (n = 41).

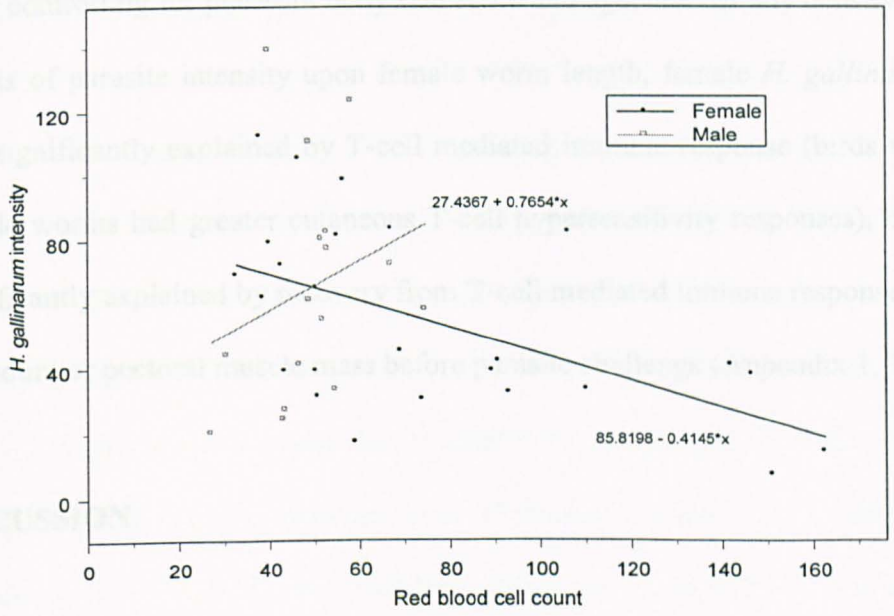
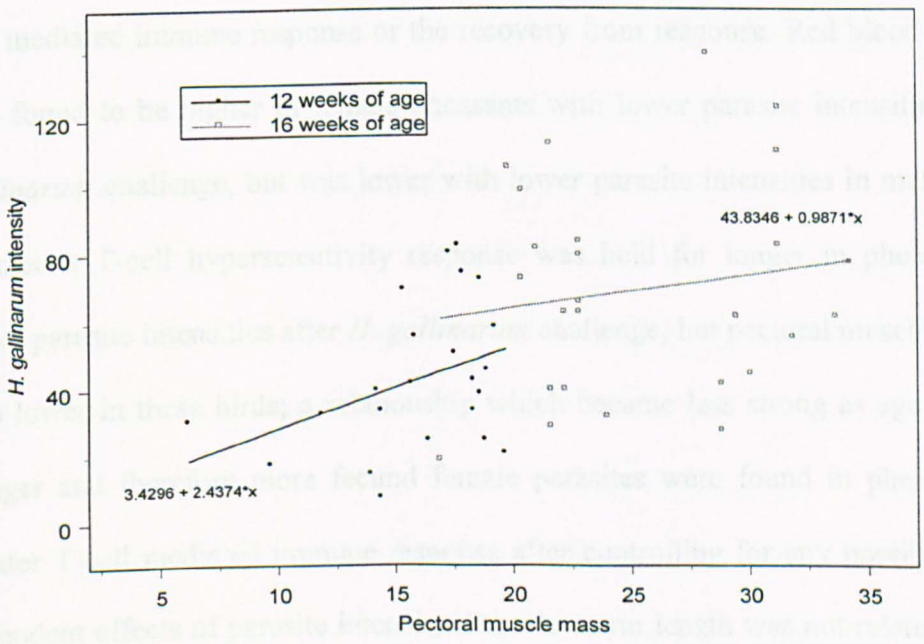


Figure 5. Relationship between *H. gallinarum* intensity and an interaction between pectoral muscle mass*pheasant age (n = 41).



Effects of T-cell mediated immunity and pheasant condition upon female *H. gallinarum* length after challenge with parasite eggs

After controlling for pheasant body size score and age, and for any density-dependent effects of parasite intensity upon female worm length, female *H. gallinarum* length was significantly explained by T-cell mediated immune response (birds with longer female worms had greater cutaneous T-cell hypersensitivity responses), but was not significantly explained by recovery from T-cell mediated immune response, red blood cell count or pectoral muscle mass before parasite challenge (Appendix 1, Table 5).

DISCUSSION

The technique for measuring body condition on live pheasants using pectoral muscle mass was found to be reliable and was therefore included within this study. An association between the different characteristics of pheasant condition; pectoral muscle mass and red blood cell count was established, but neither was related to T-cell mediated immune response or the recovery from response. Red blood cell count was found to be higher in female pheasants with lower parasite intensities after *H. gallinarum* challenge, but was lower with lower parasite intensities in male birds. A cutaneous T-cell hypersensitivity response was held for longer in pheasants with lower parasite intensities after *H. gallinarum* challenge, but pectoral muscle mass was also lower in these birds; a relationship which became less strong as age increased. Longer and therefore more fecund female parasites were found in pheasants with greater T-cell mediated immune response after controlling for any possible density-dependent effects of parasite intensity. Female worm length was not related to T-cell

mediated immune response recovery, red blood cell count or pectoral muscle mass before parasite challenge. Below these findings are discussed in relation to theories about trade-offs between condition, immune response and parasitism, and their impact upon patterns of parasite aggregation.

Within their review paper on 'Ecological immunity', Norris and Evans (2000) suggested that three criteria must be satisfied to demonstrate that a trade-off between life-history decisions and immunocompetence could be of evolutionary significance, because of an impact upon the relationship between hosts and parasites. These were that immunocompetence, the hosts ability to prevent or control infection by pathogens/parasites, must compete with life-history decisions for access to limiting resources; if investment in a particular life-history component is increased then immunocompetence must be reduced; and when it is reduced a reduction in fitness must also occur. In this study, the hypothesis that the relationships between the condition of an individual, their ability to respond immunologically to infection, and the prevalence and fecundity of parasite infection are in proportion to each other, was investigated in order to find evidence of an over-riding concept linking trade-offs with immunocompetence: condition and the immune response must themselves, at the most basic level, be linked because of resource limitations.

Relationships between the characteristics of pheasant condition and T-cell mediated immune response

Before an equilibrium of investment between life-history decisions and immunocompetence can be reached, it would seem reasonable that another, a balance

in the characteristics of general condition, must already have been mediated by physiological trade-offs within an individual. In field studies, indicators of general condition such as pectoral muscle mass, body weight, haematocrit/packed cell volume and red blood cell count are often quantified. Correlations between these variables are rarely reported however, because their significance is generally examined in relation to the immune response or parasitism. In a study of pied flycatchers, haematocrit/packed cell volume and the thickness of breast muscles were positively related in fledgling birds, but haematocrit was not linked to body mass in adults, possibly as a result of trade-offs with chick rearing (Potti et al., 1999). The first aim of this study was therefore to examine the pectoral muscle mass and red blood cell count of pheasants for correlation. Accordingly, we found a positive association, which is less ambiguous than the results of wild studies because the pheasants were naïve to previous reproduction or infection. Neither pectoral muscle mass nor red blood cell count were related to T-cell mediated immune response or the recovery from response though. This differed from the results of wild studies in which T-cell mediated immune response has been positively linked to body mass in fledgling American kestrels (Tella et al., 2000) and nestling great tits (Brinkhof et al., 1999), and to body condition in nestling house martins (Christe et al., 1998). The lack of correlation demonstrated within this study could be of evolutionary significance due to an impact upon other life-history components. For instance, secondary sexual characteristics have often been linked with condition, and the effect of non-honest expression of characteristics would therefore be the selection of inferior genes leading to increased rates of morbidity and mortality, a reduction in future reproductive potential and possibly mutation in future offspring. This result should be treated with caution, however, as a relationship, although insignificant, did exist between T-cell mediated

immune response and pectoral muscle mass. Furthermore, Norris and Evans (2000) suggest that assessments of immunity should be made using techniques to measure more than one response (T-cell mediated, innate and humoral immune response). Due to logistical constraints, unfortunately only the PHA skin testing technique could be employed in this study.

Pheasant condition and T-cell mediated immune response and their effects upon parasitism

Negative associations have often been identified between condition and/or the immune response and parasitism in field studies where the birds have already been exposed to parasites. For example, the concentration of antibodies against Newcastle disease virus and the intensity of *Haemoproteus* infection were negatively related in an experimental manipulation using female collared flycatchers (Nordling et al., 1998). Pryethrin treatment against the haematophagous parasitic house martin bug *Oeciacus hirundinis* also significantly increased the T-cell mediated immune response of the house martin (Christe et al., 2000). This study aimed to examine this relationship by measuring T-cell mediated response before parasite exposure, rather than after. This allowed a judgment of the immune response to be made before it was affected by T-cell proliferation resulting from exposure to parasitism, which could disproportionately impact upon ensuing parasitism. The measured response should therefore have been more representative of the physiological status of a pheasant, than in a previously exposed bird. The results showed that a cutaneous T-cell hypersensitivity response was held for longer in pheasants with lower parasite intensities after *H. gallinarum* challenge, suggesting that they had a better ability to

counter and control parasitism because the longer maintenance of an inflammatory reaction indicated a continuing response. T-cell mediated immune response itself did not however explain variation in parasite intensity.

As variation in pectoral muscle mass did not directly explain variation in T-cell mediated immune response within this study, its relationship with parasite intensity need not be interpreted as confounding the significance of the T-cell mediated immune response/parasite relationship. However, the longer maintenance of a T-cell mediated inflammatory reaction can therefore only be interpreted as indicating a better continuing response against parasitism, rather than being representative of a trade-off between condition and immune response, impacting upon parasitism.

Longer and therefore more fecund female parasites were found in pheasants with greater T-cell mediated immune response, after control for the possible effects of density-dependence caused by parasite intensity. This may be an artefact of the relatively low parasite burdens used within the study, creating favourable conditions for the maturation of female parasites, or may suggest an insignificant density-dependent impact, as parasite intensity was negatively related to T-cell mediated immune response recovery (although female worm length was not directly related to T-cell mediated immune response recovery). Female worm length was not related to red blood cell count or pectoral muscle mass before parasite challenge suggesting it is not directly influenced by the immune response.

The impact of the results upon patterns of parasite aggregation

A lack of correlation between condition indicators and the immune response would impact upon patterns of parasite aggregation by causing, most probably, a reduction in the factors influencing host susceptibility to parasitism (such as body condition, immune functioning and the effects of host age and sex and stresses caused by social interactions). With this reduction, a further reduction in the susceptibility of the individual to parasitism would occur, and the parasites would become more homogenously spread within the host population.

Resource limitation in innate body condition and the immune response, and their effects upon macroparasite infection

The correlation between pectoral muscle mass and red blood cell count identified within this study followed the hypothesis that their maintenance is accountable and may be limited by resources, and suggests that further trade-offs with life-history characteristics could occur. The lack of association between pectoral muscle mass and T-cell mediated immune response suggests that perhaps this immune response is not linked with these particular indicators of condition, except that there did seem to be a relationship which was complicated by the effects of pheasant body size. The implication of resource limitation is that T-cell mediated immunity must be limited more directly, and compromised by, other life-history characteristics. This is implied by the negative relationship between immunity and parasitism. The difference in the energetic costs likely to be used for reproduction, to respond immunologically to parasites and for the display of secondary sexual characteristics compared to the

maintenance of general condition, may perhaps explain why more basic underlying condition constraints could not be identified within this study.

Future work

Future work should aim to further tease apart the complexity of resource relationships within the host individual. This could best be achieved over a longer time span, for instance, by examining the effects of manipulated reproduction, quantified immune responses to infection and secondary sexual characteristics and relating them to general condition before a parasite challenge or reproduction occurred. A better understanding of how relationships are woven could then be gained, by understanding how each parameter is related to, and impacts upon, the next.

Appendix 1

Table 1.

Results of Linear Model testing for an association between pectoral muscle mass and red blood count before parasite challenge. The model included pheasant body size score, sex and age, and interactions between pheasant sex*age and pheasant sex*body size.

	Response~Pectoral muscle mass	d.f., residuals	Value	s.e.	*F* statistic	*P (F)*
Minimal model	Red blood cell count	1,33	0.012	0.012	13.695	<0.001
	Pheasant sex	1,33	-3.034	1.711	8.902	0.005
	Pheasant age	1,33	0.796	1.621	66.936	<0.001
	Pheasant body size	1,33	-4.288	0.731	16.926	<0.001
	Pheasant sex x age	1,33	9.984	1.931	26.726	<0.001
	Pheasant sex x body size	1,33	3.124	1.005	9.672	<0.004
	Terms dropped excluding interactions					
	Pen number	1,32	0.871	0.612	4.134	0.050

Table 2.

Results of Linear Model testing for an association between T-cell mediated immune response and pectoral muscle mass/red blood count before parasite challenge. The model included pheasant body size score.

Response~T-cell mediated immune response	d.f., residuals	Value	s.e.	*F* statistic	*P (F)*
Pheasant body size	1,44	-0.335	0.167	4.049	0.050
Terms dropped					
Pectoral muscle mass	1,42	0.143	0.083	2.985	0.091
Red blood cell count	1,34	-0.002	0.009	0.069	0.795
Pheasant sex	1,43	-0.114	0.853	0.018	0.894
Pheasant age	1,43	0.779	0.579	1.808	0.186
Pen number	1,43	0.476	0.475	1.002	0.322

Table 3.

Results of Linear Model testing for an association between the recovery from T-cell mediated immune response and pectoral muscle mass/red blood count before parasite challenge. The model included pheasant body size score.

Response~Recovery from T-cell mediated immune response	**d.f., residuals**	**Value**	**s.e.**	***F* statistic**	***P (F)***
Pheasant body size	1,44	0.320	0.154	4.283	0.044
Terms dropped					
Pectoral muscle mass	1,42	-0.074	0.079	0.876	0.355
Red blood cell count	1,34	0.007	0.009	0.601	0.444
Pheasant sex	1,43	-0.165	0.791	0.044	0.836
Pheasant age	1,43	-0.672	0.539	1.558	0.219
Pen number	1,43	-0.631	0.435	2.104	0.154

Table 4.

Results of a negative binomial Generalised Linear Model examining the effects of T-cell mediated immunity and pheasant condition upon parasite intensity after challenge with *H. gallinarum* eggs. The model included pheasant body size score, sex, age and a sex*age interaction, and interactions between red blood cell count*pheasant sex and pectoral muscle mass*pheasant age.

	Response~*H. gallinarum* intensity	**d.f., residuals**	**Value**	**s.e.**	**Deviance**	***P* (Chi)**
Minimal model	NULL	36			98.355	
	Pheasant body size	1,30	-0.356	0.150	10.946	<0.001
	Red blood cell count	1,30	-0.009	0.003	7.972	<0.005
	Pectoral muscle mass	1,30	0.089	0.044	0.721	0.396
	Pheasant sex	1,30	-3.126	0.746	11.440	<0.001
	Pheasant age	1,30	3.175	1.301	3.983	0.046
	Recovery from T-cell mediated immune response	1,30	-0.154	0.043	10.054	<0.002
	Red blood cell count x pheasant sex	1,27	0.033	0.011	8.450	<0.004
	Pectoral muscle mass x pheasant age	1,27	-0.196	0.065	8.818	<0.003
	Pheasant sex x age	1,27	1.752	0.604	8.312	<0.004
	Terms dropped excluding interactions					
	T-cell mediated immune response	1,29	0.013	0.061	0.182	0.670
	Pen number	1,29	0.061	0.132	0.366	0.545

Table 5.

Results of Linear Model examining the effects of T-cell mediated immunity and pheasant condition upon female *H. gallinarum* length after challenge with parasite eggs. The model included pheasant body size score and age and parasite intensity.

	Response~*H. gallinarum* intensity	d.f., residuals	Value	s.e.	Deviance	*P* (Chi)
Minimal model	NULL	36		66.654		
	Pheasant body size	1,32	-0.274	0.093	8.296	<0.004
	Red blood cell count	1,32	-0.005	0.003	3.531	0.060
	T-cell mediated immune response	1,32	0.095	0.045	4.398	0.036
	Pheasant sex	1,32	-0.547	0.233	5.445	<0.020
	Terms dropped					
	Pectoral muscle mass	1,31	-0.024	0.026	0.894	0.344
	Pheasant age	1,31	-0.314	0.270	1.356	0.244
	Pen number	1,31	0.094	0.146	0.400	0.527

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Chapter 3

Acquired resistance to infection by the caecal nematode *Heterakis gallinarum* in a ring-necked pheasant host.

Introduction

Upon infection, the innate immune response initially defends against disease. In wild-living species this defence may be the only mechanism of immune response as the risk of reduced survival as a result of predation increases with prolonged disease (Lochmiller and Deerenberg, 2000). But this preliminary reaction may not always have a sufficiently detrimental effect upon the pathogen, and may divert host immune defence away from important functional antigens, immunosuppress the later induced response (if there is one) or may even promote immunopathology. As a result, in some longer-lived species a second line of resistance has evolved to moderate or prohibit further parasite subsistence; the antigenic acquired immune response.

An acquired immune response has been suggested to be the most important physiological immune defence to have evolved against parasites (Roitt et al., 1998). Its expression has been shown to effectively control parasite loads (Baron and Weintraub, 1987; Wakelin and Apanius, 1997). Such a response is characterised by a reduction in parasite intensity or prevalence when a host is re-infected by a parasite species. Acquired immune responses have predominantly been studied in man and domesticated host species with antagonistic parasites or diseases (Anderson and May, 1985a, 1985b, 1991; Baron and

Weintraub, 1987; Crombie and Anderson, 1985; Dobson et al., 1990; Keymer and Tarlton, 1991; Lindenstrom and Buchmann, 2000; May and Anderson, 1983; Wakelin and Apanius, 1997; Wikel, 1996; Woolhouse, 1998; Woolhouse et al., 1991).

The effect of acquired resistance upon *Trichostrongylus tenuis* parasitism in red grouse has been investigated using both population manipulation and observation. Caecal egg counts from adult grouse, treated using anthelmintic drugs to reduce parasite intensities were compared to naive immature and untreated adult grouse (Hudson and Dobson, 1997). There was no detectable difference in parasite re-infection intensity. In seasonally challenged and treated versus untreated grouse, egg counts actually increased between primary and secondary infection, possibly due to a decrease in grouse resistance resulting from the cost of defence during primary infection, or because of differences in infectivity between parasite larvae (Shaw and Moss, 1989). In a simple comparison after quantification of gut samples from 318 individuals, old grouse had much greater parasite intensities than young (Wilson, 1983). These studies suggested little or no effect of acquired immunity upon *T. tenuis* parasitism in the grouse, and it is assumed that the same situation prevails for *Heterakis gallinarum* parasitism in the ring-necked pheasant. This assumption was used in the parameterisation of the apparent parasite-mediated competition model that suggested *H. gallinarum* as one mechanism for grey partridge decline in the UK (Tompkins et al., 1999, 2000a, 2000b).

This work aimed to investigate the possibility of an acquired immune resistance of the pheasant to *H. gallinarum* parasitism.

Testing for acquired resistance

The test of acquired resistance was the comparison of worms burdens and female worm length (indicative of female fecundity) between two groups: one sham-challenged and then challenged once (control), the other challenged twice with *H. gallinarum* eggs (treatment). This was incorporated into a model examining the effects of condition (PCV, RBC and muscle mass) upon the parasite population established.

Hypothesis

The hypothesis was that hosts are capable of mounting an acquired immune response to parasitism. The prediction was therefore that parasite intensity and fecundity would differ between experimental treatment groups.

Clarification of comparable host immune and condition status between experimental treatment groups

To clarify the birds used in both study groups were of equal immune status, variation in CMI response and CMI response recovery after a novel injection of PHA (between the first sham/challenge and before both experimental groups were challenged with parasite eggs) were related to experimental treatment. Host body condition, PCV and RBC throughout the study period were likewise related to experimental treatment and time of blood sampling to confirm equal condition status.

The impact of parasitism upon the actual lean wet breast muscle mass, liver and spleen mass

To observe the impact of parasitism subsequent to challenge upon host immune resistance and condition, breast muscle mass, liver and spleen mass were related to characteristics of the parasite population established, controlling for other condition indicators and experimental treatment.

Materials and methods

32 ring-necked pheasant chicks were hand-reared from 2-days until four months old in sterile conditions to ensure naivety to infection. During this period, all individuals were randomly substituted every two days into one of two identically sized pens (necessary for animal husbandry reasons) to ensure that all had been identically treated. Birds were given water and supplied with chick crumbs, and were then maintained on standard maintenance pellets *ad-libitum.* At four months old they were randomly selected and split into two groups and caecal droppings were collected from their pens to verify the absence of *H. gallinarum* prior to challenge. Both groups were then weighed (to the nearest 25 g) and their tarsal and wing chord lengths were measured and pectoral muscle profiles were traced. They were then blood sampled to examine condition (RBC and PCV).

After blood sampling one group of randomly selected birds was orally challenged using a 2 ml single dose suspension of approximately 100 *H. gallinarum* eggs; the second group was sham-challenged with 2 ml of 0.05% saline solution (containing no nematode eggs). Both groups were then maintained for a 30-day period during which caecal droppings

were again collected to verify the presence or absence of parasite eggs. They were then weighed, measured, pectoral muscle profiles were traced and they were blood sampled again.

Following this period, all birds were treated with an anthelmintic (Flubenvet Intermediate^{TM}) to remove any parasites; caecal droppings were collected and examined for eggs twice a day for two days after, to confirm the efficacy of the treatment. They were then retained for a period of 20 days to ensure there were no residues of the anthelmintic left to interfere with subsequent parasite challenges. Both groups of birds were again then weighed, measured, pectoral muscle profiles traced, blood samples taken and were challenged with phytohaemagglutinin (PHA) to examine their direct T-cell mediated immune response. They were then orally challenged with approximately 100 *H. gallinarum* eggs and were maintained for another 30-day period with identical treatment and subsequent weighing, measurement, pectoral muscle profile tracing and blood sampling.

All birds were then euthanased to quantify the ensuing parasitism. During post-mortem, the actual lean wet breast muscle mass was weighed. For full methodologies please see Chapter 1 (General Methodology). All birds were maintained in sterile conditions throughout the trial period.

Statistical analyses

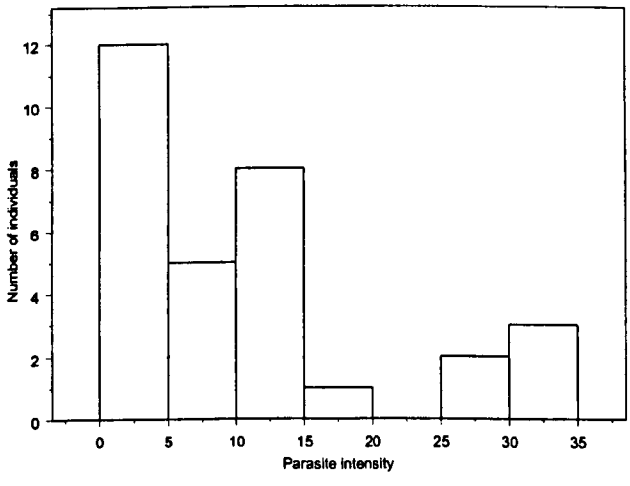
The covariates and factors considered in analyses were packed-cell volume (PCV), red blood cell count (RBC) and a modelled muscle mass index which were used to represent host condition, CMI immune response 6 hours after challenge with PHA and the difference between the response at 12 and 6 hours (indicating the longevity of response) which were used to represent CMI response, and experimental treatment and time of blood sampling. These were examined in relation to the intensity of parasite infection established, female worm length (indicative of female fecundity), actual lean wet breast muscle mass, liver and spleen masses subsequent to challenges. The percentage of adults in the parasite population (indicative of the fitness of the parasite population due to the speed of juvenile maturation) was not examined as juvenile parasites were only found in 16 % (n = 5) of host individuals.

All linear modelling (LM) and generalised linear modelling (GLM) analyses were undertaken using S-PLUS, version 6 for Windows™ Professional Release 2 (Mathsoft Engineering and Education, Cambridge, Massachusetts, USA, © 1988-2001 Insightful Corp.) program, unless otherwise stated. This allowed analyses using Gaussian and negative binomial error distributions.

A statistical test of negative binomial fit (designed and written by Darren Shaw, University of Edinburgh) for the S-PLUS version 6 for Windows™ Professional Release 2 (Mathsoft Engineering and Education, Cambridge, Massachusetts, USA, © 1988-2001 Insightful Corp.) program was used to examine the error distribution of the worm

intensity data. They did not differ significantly from a negative binomial distribution, with a best estimate of the aggregation parameter of (k) = 1.1 and variance/mean ratio of 9.6 (P = 0.27; Figure 1), and were consequently examined using a negative binomial GLM (Wilson and Grenfell, 1997; Wilson et al., 1996). The CMI response data was positively skewed and was consequently $\log_{10}$transformed and analysed using linear modelling with a normal error distribution. Female worm length, CMI response recovery, actual lean wet breast muscle mass, liver and spleen masses were analysed using linear models with normal error distributions.

Figure 1 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 31 birds) after oral sham-/challenge with ~ 100 eggs then further challenge with ~ 100 eggs. 97% of pheasants were infected, and the overall mean ± SD was 10.13 ± 9.84 worms.



As haematological techniques were applied before both challenges and after the 30-day maintenance periods, therefore producing four (1 = before 1st sham-/challenge, 2 = before

anthelmintic usage, 3 = before 2nd parasite challenge, 4 = before euthanasia) blood samples, linear mixed effects models were undertaken to examine the effect of the parasite challenges (treatment), host sex and blood sample upon PCV, RBC and the modelled muscle mass index throughout the trial. This allowed the inclusion of the modelled muscle mass index and RBC/PCV respectively, as random factors within REML (Residual Maximum Likelihood Model) analyses, thereby controlling for variation due to their effects. This statistical technique follows the recent suggestions of Patterson and Lello (in press). REML analyses were undertaken in GenStat programme, version 6 (© 2000 VSN International) by first fitting the maximal model and then using stepwise deletion, calculating significance levels using X^2 tests to create P Wald statistics.

RBC before parasite challenge, female worm length and spleen mass all contained one unusually large outlier (from differing birds in each case) that was excluded from analyses as they were 4.3, 2.7 and 2.9 standard deviations from the mean, respectively.

In all analyses, host sex and a host body size score created using principal component analysis (Chapter 1, General Methodology) were included as control factors, being discarded if non-significant. Minimal models were arrived at using stepwise deletion. Predicted fits were used to display results controlling for other terms remaining the models. The F statistics and deviance values presented are from the minimal models for significant terms, or the minimal model with the non-significant term added onto the model for terms dropped from the maximal model. To compare differences between factor levels, Tukey's honestly significant difference (HSD) was used. To control for type

II statistical errors resulting from a large number of factors being considered in analyses, which meant some factors could falsely show significance due to chance, the method of Benjamin and Hochberg (1995) was used. This technique was chosen over the Bonferroni correction method as it is less conservative (Cotter et al., 2004; Benjamini and Hochberg, 1995).

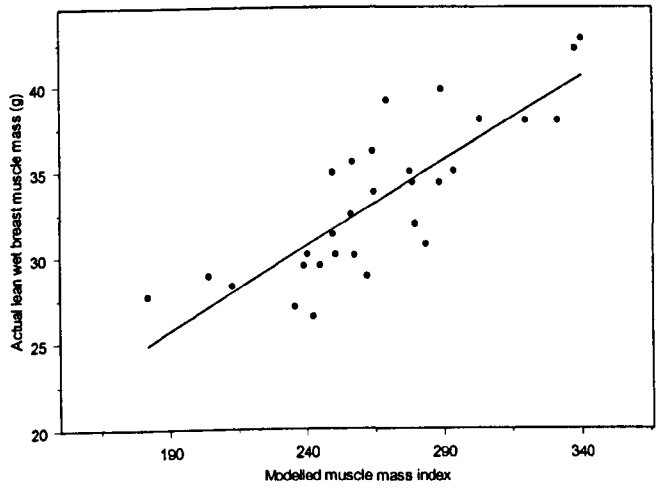
Results

Before the second parasite challenge 1 individual from the control (sham-challenged) group was euthanased due to husbandry factors unrelated to parasite infection.

Accuracy of the modelled muscle mass index

The precision of the technique for measuring body condition on live birds was clarified by undertaking a Pearson's r correlation (bootstrapped with 1000 iterations) on the modelled muscle mass index and actual lean wet breast muscle mass of the birds at the time of euthanasia (after the challenge). The two variables were highly correlated (Figure 2; Pearson's correlation = 0. 823, $P < 0.001$, n = 31), indicating that the modelled muscle mass index was a reliable indicator of actual lean wet breast muscle mass.

Figure 2 Correlation between the modelled muscle mass index and actual lean wet breast muscle mass (n = 31).



Testing for acquired resistance

Parasite intensity

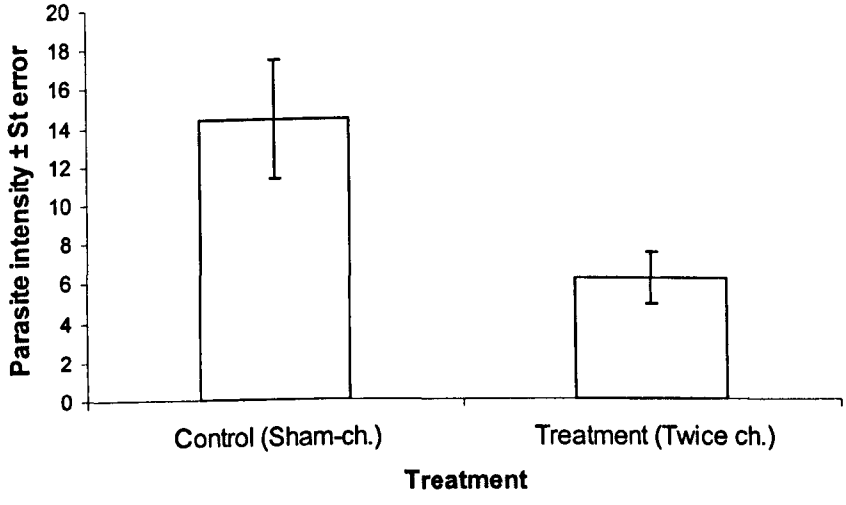
After Benjamin and Hochberg (1995) corrections were undertaken, the intensity of parasite infection after challenge was significantly related to experimental treatment (Figure 3; deviance = 6.673, df = 1,29, $P < 0.010$) with higher parasite burdens in the control (sham-challenged) than treatment (twice challenged) group, using HSD. It was not related to, RBC, host sex, host body size or the modelled muscle mass index (Table 1).

Table 1 Negative binomial model of parasite intensity after challenge.

Response-parasite intensity	d.f.	Value	s.e.	Deviance	*P(Chi)*	Corrected *P*	Significance of corrected *P*
NULL	30			40.709			
Experimental treatment	29	-0.427	0.165	6.673	<0.01**	0.060	**
Terms dropped							
PCV	28	-4.690	4.985	1.099	0.295	0.017	ns
RBC	28	-0.014	0.011	1.438	0.230	0.011	ns
Host sex	28	0.027	0.165	0.027	0.869	0.033	ns
Host body size	28	-0.174	0.233	0.582	0.446	0.022	ns
Modelled muscle mass index	28	0.016	0.037	0.205	0.651	0.028	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 3 Variation in parasite intensity with experimental treatment (n = 31).



Female worm length

After Benjamin and Hochberg (1995) corrections were undertaken female worm length after challenge was non-significantly related to experimental treatment (Figure 4; $F_{1,19}$ = 7.713, P = 0.012) with longer female worm length and therefore higher fecundity in the

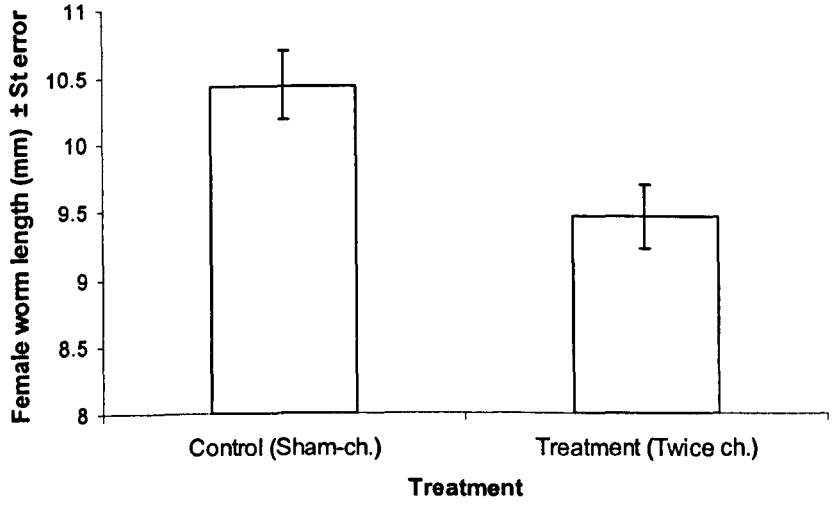
control (sham-challenged) than treatment (twice challenged) group, using HSD. It was not related to parasite intensity, PCV, RBC, host sex, host body size or the modelled muscle mass index (Table 2).

Table 2 Linear model of female worm length after challenge.

Response: female worm length (mm)	d.f., residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Experimental treatment	1,19	-0.491	0.177	7.713	0.012*	0.006	ns
Terms dropped							
$\log_{10}$(parasite intensity)	1,18	0.452	0.629	0.517	0.481	0.022	ns
PCV	1,18	-3.134	5.075	0.381	0.545	0.028	ns
RBC	1,18	0.002	0.010	0.051	0.824	0.033	ns
Host sex	1,18	<0.001	0.182	<0.001	0.999	0.039	ns
Host body size	1,18	-0.248	0.248	1.001	0.330	0.011	ns
Modelled muscle mass index	1,18	-0.035	0.040	0.766	0.393	0.017	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 4 Variation in female worm length with experimental treatment (n = 21).



Clarification of comparable host immune and condition status between experimental treatment groups

CMI response

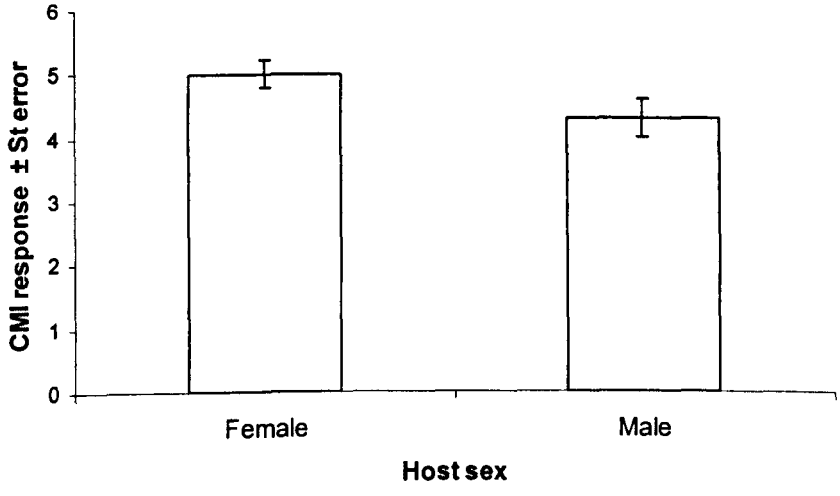
CMI response was not significantly related host sex, host body size or to experimental treatment (Table 3). The trend with host sex was very close to significance after bootstrapping ($F_{1,29} = 4.134$, $P = 0.051$), but factor levels were not significantly different from each other using HSD or after Benjamin and Hochberg (1995) corrections. Within the trend, females had a greater CMI response than male hosts (Figure 5).

Table 3 Linear model of CMI response.

Response $\log_{10}$(CMI response)	df, residuals	Value	se	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Terms dropped							
Host sex	1,29	-0.038	0.019	4.134	0.051	0.006	ns
Host body size	1,29	0.025	0.015	2.711	0.110	0.011	ns
Experimental treatment	1,29	0.022	0.020	1.283	0.267	0.017	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 5 Variation in CMI response with host sex (n = 31).



CMI response recovery

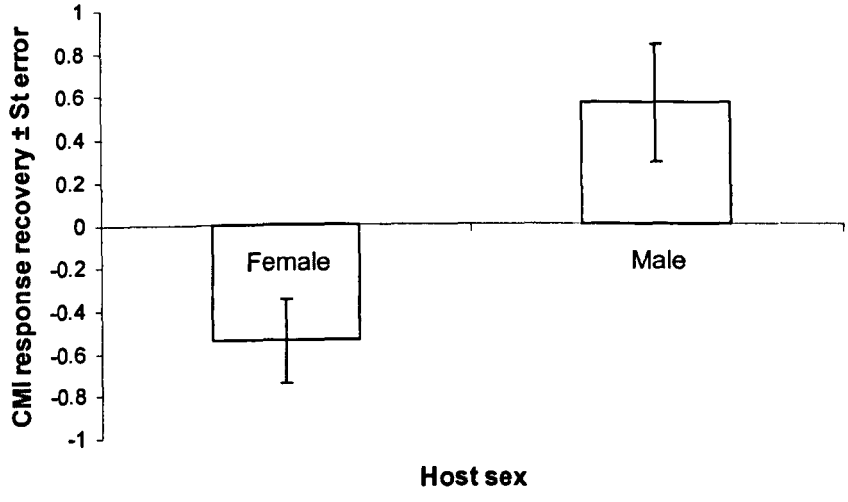
After Benjamin and Hochberg (1995) corrections were undertaken CMI response recovery was significantly related to host sex (Figure 6; $F_{1,29} = 10.605$, $P < 0.003$), with the response elicited for longer in male than female hosts, using HSD. It was not related to host body size or experimental treatment (Table 4).

Table 4 Linear model of CMI response recovery.

Response: CMI response recovery	df residuals	Value	se	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host sex	1,29	0.555	0.170	10.605	<0.003**	0.006	**
Terms dropped							
Host body size	1,28	-0.170	0.140	1.477	0.234	0.017	ns
Experimental treatment	1,28	-0.215	0.169	1.628	0.212	0.011	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 6 Variation in CMI response recovery with host sex (n = 31).



PCV

After Benjamin and Hochberg (1995) corrections were undertaken PCV was related to host sex (Figure 7; Wald statistic = 24.41, $df = 1$, X^2 $P < 0.001$) with higher PCV in male than female hosts. It was not related to host body size, time of blood sampling or experimental treatment (Table 5).

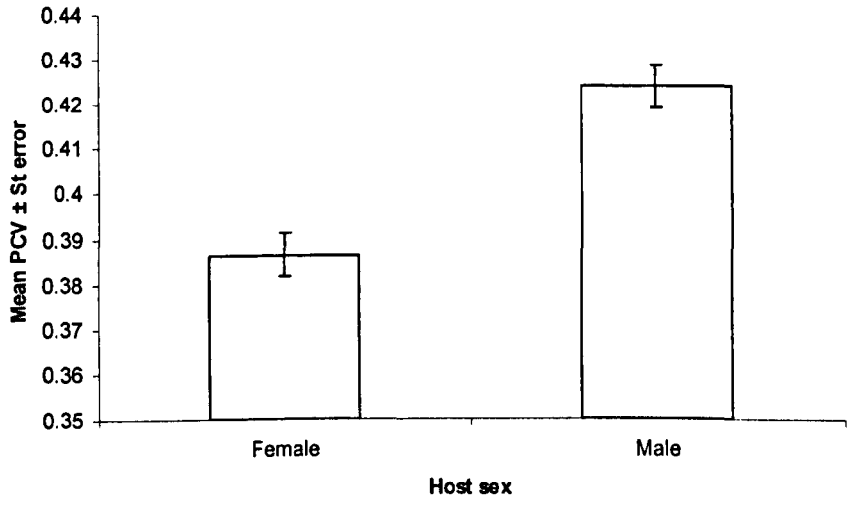
Table 5 REML model of PCV.

Response-PCV	d.f.	Value	s.e.	Wald statistic	*P* (Wald)	Corrected *P*	Significance of corrected *P*
Host sex	1	0.038	0.007	25.93	<0.001***	0.006	***
Blood sampling number	3	#	0.008	2.03	0.566	0.017	ns
Terms dropped							
Host body size	1	0.003	0.002	1.23	0.267	0.011	ns
Experimental treatment	1	<-0.001	0.006	<0.001	<0.001	0.022	ns
Random term = modelled muscle mass index							
	# Values						
	1	0.000					
	2	0.011					
	3	0.060					
	4	0.010					

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 7 Variation in PCV with host sex (n = 124).

The predicted values control for the effects of time of blood sampling.



RBC

After Benjamin and Hochberg (1995) corrections were undertaken RBC was significantly related to an interaction between time of blood sampling and experimental treatment (Figure 8; Wald statistic = 19.78, $df = 3$, X^2 $P < 0.001$). Within this interaction, the control group before the 1st sham-/challenge had significantly higher RBC than all the other groups, the treatment group before the 1st sham-/challenge had lower RBC than at all other times of blood sampling, and the treatment group before euthanasia had higher RBC than the control group. There was a non-significant trend between RBC and host sex (Figure 9; Wald statistic = 4.86, $df = 1$, X^2 $P = 0.028$), with lower RBC in female than male hosts, and RBC was not related to the main effects of time of blood sampling, experimental treatment or host body size (Table 6).

Table 6 REML model of RBC.

Response=RBC	df	Value	s.e.	Wald statistic	$P(X^2)$	Corrected P	Significance of corrected P
Host sex	1.0	4.062	1.844	4.860	0.028*	0.011	ns
Time of blood sampling	3.0	¤	3.744	1.620	0.654	0.022	ns
Experimental treatment	1.0	-14.078	3.686	0.340	0.557	0.017	ns
Time of blood sampling x experimental treatment	3.0	#	5.212	19.780	<0.001***	0.006	***
Terms dropped							
Host body size	1.0	-0.267	1.405	0.040	0.849	0.028	ns
Random term=modelled muscle mass index							

#Values		¤Values	
1	0.000	1	0.000
2	18.907	2	-12.442
3	12.583	3	-6.317
4	21.040	4	-12.392

'Corrected P' and 'Significance of corrected P' are Benjamin and Hochberg (1995) corrections. 'Corrected P' > '*P (F)*' for significance.

Figure 8 Variation in RBC with an interaction between time of blood sampling and experimental treatment (n = 124).

The predicted values control for the effects of host sex (Table 6).

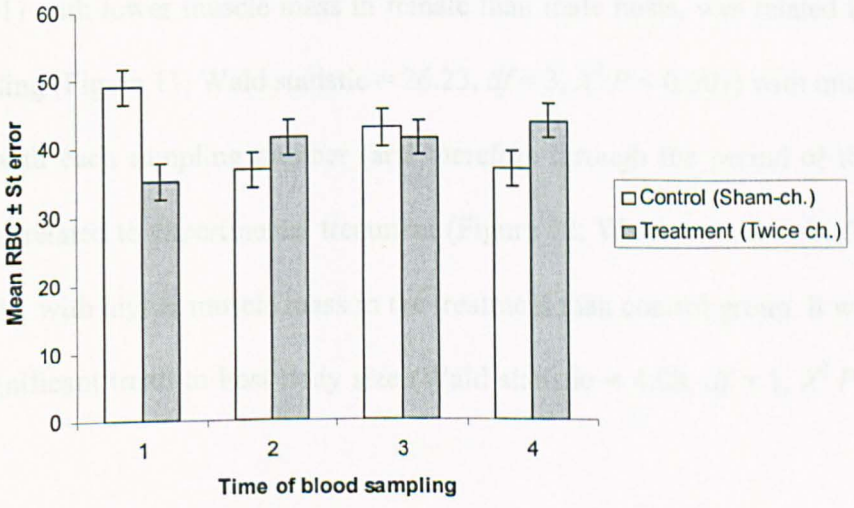
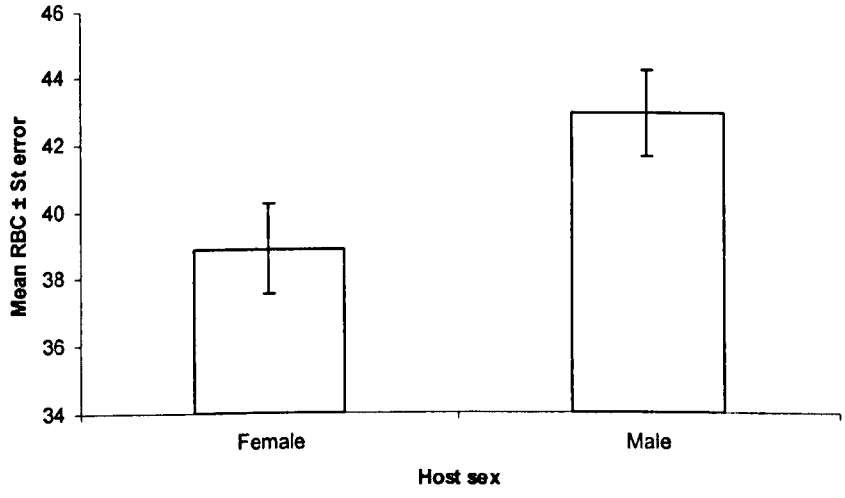


Figure 9 Variation in RBC with host sex (n = 124).

The predicted values control for the main effects of and an interaction of time of blood sampling and experimental treatment (Table 6).



Modelled muscle mass index

After Benjamin and Hochberg (1995) corrections were undertaken the modelled muscle mass index was significantly related to host sex (Figure 10; Wald statistic = 63.77, $df = 1$, X^2 $P < 0.001$) with lower muscle mass in female than male hosts, was related to time of blood sampling (Figure 11; Wald statistic = 26.23, $df = 3$, X^2 $P < 0.001$) with muscle mass increasing with each sampling number (and therefore through the period of the study), and was also related to experimental treatment (Figure 12; Wald statistic = 11.59, $df = 1$, X^2 $P < 0.001$), with higher muscle mass in the treatment than control group. It was related in a non-significant trend to host body size (Wald statistic = 4.08, $df = 1$, X^2 $P = 0.043$) (Table 7).

Table 7 REML model of the modelled muscle mass index.

Response: Modelled muscle mass index	df	Value	se	Wald statistic	*P*(Wald)	Corrected *P*	Significance of corrected *P*
Host sex	1	5.334	0.669	63.77	<0.001***	0.006	***
Host body size	1	-0.512	0.249	4.08	0.043*	0.011	ns
Blood sampling number	3	#	¤	26.23	<0.001***	0.006	***
Experimental treatment	1	2.081	0.611	11.59	<0.001***	0.006	***
Random term=PCV and RBC							

#Values		¤Value	
1	0.000	1	0.000
2	0.921	2	0.790
3	2.227	3	0.787
4	3.801	4	0.792

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 10 Variation in modelled muscle mass with host sex (n = 124).

The predicted values control for the effects of host body size, time of blood sampling and experimental treatment (Table 7).

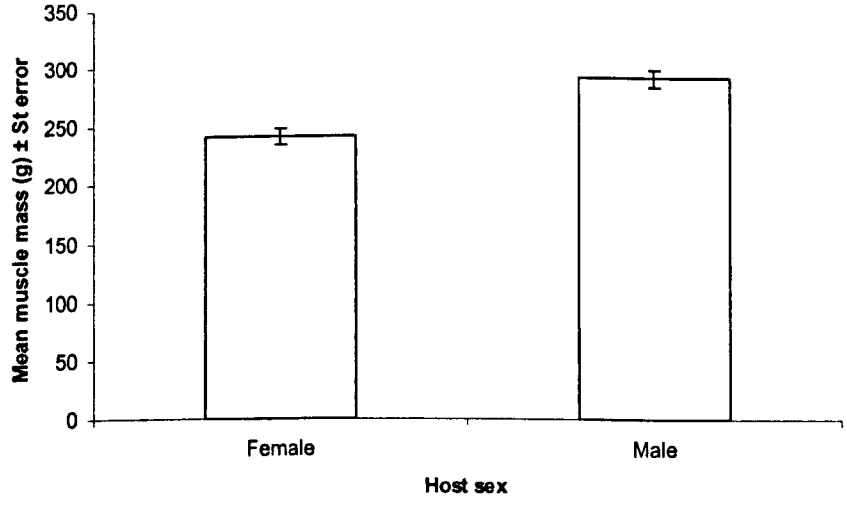


Figure 11 Variation in modelled muscle mass with time of blood sampling (n = 124).

The predicted values control for the effects of host sex, host body size and experimental treatment (Table 7).

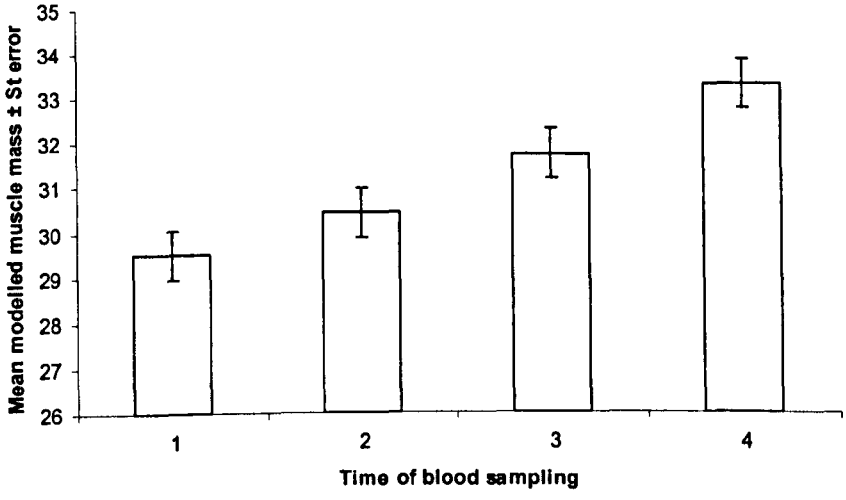
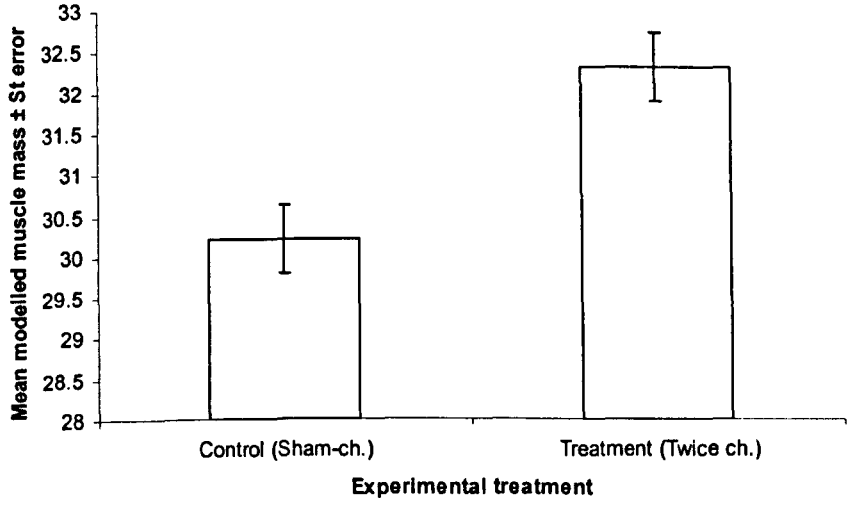


Figure 12 Variation in modelled muscle mass with experimental treatment (n = 124).

The predicted values control for the effects of host sex, host body size and time of blood sampling (Table 7).



The impact of parasitism upon the actual lean wet breast muscle mass, liver and spleen mass

Actual lean wet breast muscle mass

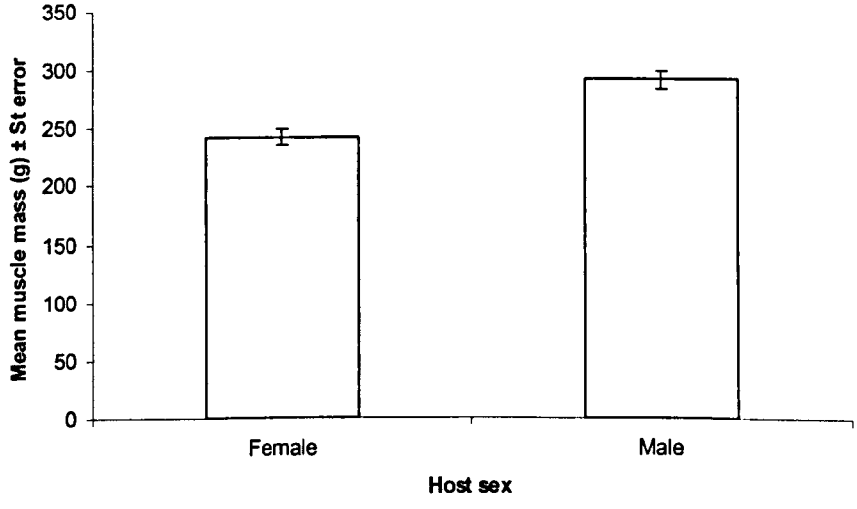
After Benjamin and Hochberg (1995) corrections were undertaken actual lean wet breast muscle mass after the parasite challenges was significantly related to host sex (Figure 13; $F_{1,28} = 23.006$, $P < 0.001$) with higher muscle mass in male than female hosts, but was not related to parasite intensity, female worm length, PCV, RBC, host body size or experimental treatment (Table 8).

Table 8 Linear model of actual lean wet breast muscle mass.

Response: Actual lean wet breast muscle mass (g)	df, residuals	Value	se	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host sex	1,28	24.527	5.114	23.006	<0.001***	0.006	***
Terms dropped							
$\log_{10}$(parasite intensity+1)	1,27	6.338	12.551	0.255	0.618	0.017	ns
$\log_{10}$(female worm length)	1,28	-144.906	164.960	0.772	0.391	0.011	ns
PCV	1,27	44.149	163.019	0.073	0.789	0.003	ns
RBC	1,27	-0.253	0.587	0.185	0.671	0.002	ns
Host body size	1,27	0.819	4.182	0.038	0.846	0.009	ns
Experimental treatment	1,27	-1.927	5.206	0.137	0.714	0.028	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 13 Variation in actual lean wet breast muscle mass with host sex (n = 30).



Liver mass

After Benjamin and Hochberg (1995) corrections were undertaken liver mass was significantly positively related to actual lean wet breast muscle mass at euthanasia ($F_{1,28}$ = 14.955, $P < 0.001$). It was not related to parasite intensity, female worm length, PCV, RBC, CMI response, CMI response recovery, host sex, host body size or experimental treatment (Table 9).

Table 9 Linear model of liver mass.

Response-Liver mass (g)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Actual lean wet breast muscle mass	1,28	0.074	0.019	14.955	<0.001***	0.006	***
Terms dropped							
$\log_{10}$(parasite intensity+1)	1,27	0.619	1.730	0.128	0.723	0.028	ns
$\log_{10}$(female worm length)	1,18	-0.490	24.775	0.001	0.984	0.056	ns
PCV	1,27	-11.434	18.667	0.375	0.545	0.017	ns
RBC	1,27	-0.026	0.074	0.124	0.727	0.033	ns
CMI response	1,27	-0.164	0.734	0.050	0.825	0.039	ns
CMI response recovery	1,27	0.353	0.731	0.233	0.633	0.022	ns
Host sex	1,27	-0.192	0.961	0.040	0.843	0.044	ns
Host body size	1,27	-0.058	0.573	0.010	0.921	0.050	ns
Experimental treatment	1,27	0.691	0.703	0.966	0.335	0.011	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Spleen mass

After Benjamin and Hochberg (1995) corrections were undertaken spleen mass was significantly related to experimental treatment (Figure 14; $F_{1,28} = 14.134$, $P < 0.001$) with higher spleen mass in the treatment than control group. It was positively related within a non-significant trend to CMI response recovery and was not related to parasite intensity, female worm length, PCV, RBC, CMI response, host sex, host body size or actual lean wet breast muscle mass (Table 10).

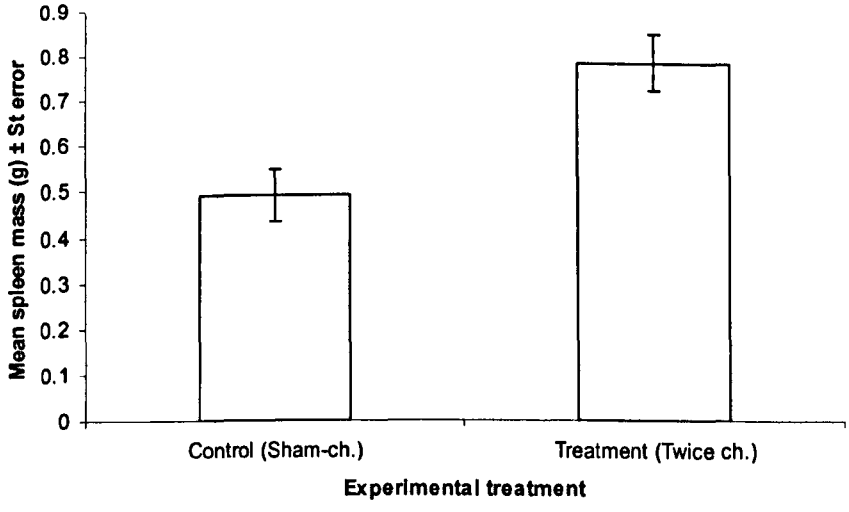
Table 10 Linear model of spleen mass.

Response-Spleen mass (g)	d.f., residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
CMI response recovery	1,28	0.088	0.041	4.539	0.042*	0.011	ns
Experimental treatment	1,28	0.166	0.044	14.134	<0.001***	0.006	***
Terms dropped							
$\log_{10}$(parasite intensity+1)	1,27	-0.101	0.112	0.814	0.375	0.022	ns
$\log_{10}$(female worm length)	1,17	0.111	1.703	0.004	0.949	0.056	ns
PCV	1,27	-0.072	1.032	0.005	0.945	0.050	ns
RBC	1,27	0.001	0.005	0.015	0.903	0.039	ns
CMI response	1,27	0.006	0.056	0.013	0.910	0.044	ns
Host sex	1,27	0.013	0.052	0.059	0.810	0.033	ns
Host body size	1,27	0.023	0.067	0.118	0.734	0.028	ns
Actual lean wet breast muscle mass	1,26	0.002	0.001	1.141	0.295	0.017	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 14 Variation in spleen mass with experimental treatment (n = 31).

The predicted values control for the effects of CMI response recovery (Table 10).



Discussion

This study investigated the hypothesis that hosts are capable of mounting an acquired immune response to parasitism.

The characteristics of the parasite population established were compared to the experimental treatment (sham/oral challenge and then further challenge with ~100 *H. gallinarum* eggs). In order to investigate the effects of host health before the original challenge, the effect of general immune functioning and body condition was also examined in relation to experimental treatment. The consequences of parasitism upon host condition and immune resistance were also examined.

Past work undertaken within the grouse/*T. tenuis* system has indicated that no resistance was acquired after multiple parasite re-infections, and parasitism did not decrease with host age (and therefore with a build up in the exposure of the host to parasitism) (Hudson and Dobson, 1997; Shaw and Moss, 1989; Wilson, 1983). The results of my study on *H. gallinarum* parasitism in the pheasant suggest that the null hypothesis that hosts are not capable of mounting an acquired immune response to parasitism can be rejected. This is because parasite intensity in the control group (sham-challenged) (mean ± SD of 14.4 ± 11.8) was significantly greater (approximately double) than that in the treatment group (twice challenged) (mean ± SD of 6.1 ± 5.3) (Table 1, Figure 3), and there was a non-significant trend for female worm length to be higher in the control group (mean ± SD of 10.43 ± 0.25) than treatment group (mean ± SD of 9.45 ± 0.24) (Table 2, Figure 4). The prediction that parasite intensity and fecundity would differ between experimental

treatment groups was therefore proven to be correct for parasite intensity and was implied for female fecundity. The acquired immune response impacted upon parasitism by reducing parasite intensity and possibly reducing female worm fecundity (although this relationship was non-significant after Benjamin and Hochberg corrections (1995) were undertaken).

Interpretation and the relevance of differences between experimental treatment groups and parasite intensity or female worm length need treating with caution however. This is because the intensity of parasite infection was much lower than expected (from projections from earlier parasite challenge work within this system), although infection rates were low in both experimental treatment groups. The low infection rates may have occurred because of the age of the eggs used within the parasite challenge suspension. Also, the treatment group was not only given double the parasites compared to the control, but the relevance of any results would also be affected by double the impact of parasitism upon the host, (because two full generations of parasites developed within birds in the treatment group, compared to one generation in the control group). But in defence of the results suggesting an acquired immune response, and to propound any unequal effect of parasitism, there was no direct effect of general condition (PCV, RBC or the modelled muscle mass index) and host body size prior to the parasite challenges upon either parasite intensity or female worm length (Tables 1 and 2). There was also no effect of experimental treatment upon PCV or RBC throughout the study period (Tables 5 and 6), CMI response or CMI response recovery (Tables 3 and 4) or the actual lean wet breast muscle mass subsequent to challenges (Table 8). No effect was also found of

parasitism upon actual lean wet breast muscle mass (Table 8). These results suggest that host health prior to infection and the T-cell mediated immune response during infection was similar within experimental treatment groups, and that parasitism did not disproportionately sustain an effect upon host health within the two treatment groups, both during and after parasite infection. They corroborate earlier work undertaken on *H. gallinarum* infection in pheasants, where no relationships were observed between breast muscle mass and parasitism (Tompkins et al., 1999, 2001, 2002), but differ from other nematode/avian systems (Hillgarth, 1991; Hudson and Dobson, 1991; Sage et al., 2002). The difference between these systems may be a function of parasite-host suitability or differences in host immune defence and parasite virulence.

Experimental treatment influenced the modelled muscle mass index, with higher muscle mass in the treatment than control group (Table 7, Figure 12), but this result was for muscle mass throughout the trial period and it's significance could consequently have been driven by either:

- disparity between the control and treatment groups in muscle mass before the initial parasite challenge, or
- the effect of experimental treatment subsequent to parasitism.

It may also have been a result of the immature status of the birds, demonstrated by an increase in muscle mass through the period of study (Table 7, Figure 11). If treatment were to have sustained an effect upon muscle mass, there would also have been a significant interaction of experimental treatment with time of blood sampling upon the modelled muscle mass index; but this was not observed. The effect of differences in the

modelled muscle mass index and therefore condition between treatment and control groups prior to the parasite challenges may also have been affected by another indicator of condition: RBC, with higher levels in the control than treatment group (Table 6), and by an interaction between experimental treatment and time of blood sampling affecting RBC prior to the parasite challenges. Within this relationship RBC was elevated to a level higher than at any other point within the study (Table 6, Figure 8). Although there was no main relationship between RBC and the modelled muscle mass index prior to challenge, these results might:

- point to an immune defence trade-off between muscle mass and RBC, or
- imply negative effects caused by unequal muscle mass between treatment groups may have been reduced by differences between RBC groups, (although RBC was controlled for by its inclusion as a random factor in the analysis of the modelled muscle mass index).

The second point is supported by a reversed relationship prior to euthanasia, as RBC was higher in the treatment than control group at this point (Table 6, Figure 8) and was not related to the actual lean wet breast muscle mass (Table 8).

Significantly lower PCV (Table 5, Figure 7) and a non-significant trend for lower RBC (Table 6, Figure 9) were found in female than male hosts, and CMI response recovery was elicited for longer in male than female hosts (Figure 6). There was a reversed non-significant trend ($P = 0.051$) in CMI response (Table 3, Figure 5), with a higher response in male than female hosts. These results are the opposite to that found in great tits, when PCV, heterophil counts and heterophil/lymphocyte ratios were higher in female than male

hosts (Ots et al., 1998). They do not conform with the Immunocompetence Handicap Hypothesis (ICHH) (Folstad and Karter, 1992), which suggests male body condition and immunity are likely to be compromised and consequently should be relatively low compared to females, due to trade-offs resulting from the costs associated with the creation of secondary sexual display characteristics. They also differ from comparison of mite infestations in pied flycatchers, where no dimorphism between the sexes was found (Potti et al., 1999). Lower PCV and RBC may have occurred in females because red blood cell counts are subject to stress (Ots et al., 1998), to which the sexes may have been disproportionately affected during blood sampling. Females also exhibited lower muscle mass both over the whole trial, (Table 7, Figure 10) and consequent to the parasite challenges (Table 8, Figure 13), and muscle mass increased through the period of study (Table 7, Figure 11) and was negatively related within a non-significant trend to host body size (Table 7), which may suggest the birds had not reached maturity and the ICHH would therefore not be relevant. Were the birds mature, lower PCV, RBC and CMI response would be expected in females, as trade-offs resulting from reproduction have been found to affect both immune functioning and parasitism in a study on female collared flycatchers (Potti et al., 1999).

Liver mass was positively associated with actual lean wet breast muscle mass, but wasn't associated with any other condition indicators or with the factors suggested to be affected by trade-offs in condition (host sex, age and experimental treatment). The relevance of this result is therefore difficult to interpret.

Spleen mass was related to experimental treatment with higher spleen mass in the treatment than control group (Table 10, Figure 14), and there was a non-significant positive trend with CMI response recovery (Table 10). This may suggest the differences in parasite intensity and the non-significant trend in differences in female worm length may have been affected by CMI response and a generalised immune response represented by an increase in spleen mass, as well as an acquired immune response, (although no direct effects of experimental treatment upon CMI response (Table 3) or CMI response recovery (Table 4) were observed). The lack of direct relationships of spleen mass with parasitism, and spleen mass with host sex differ from the results of meta-analysis covering various host/parasite systems (Brown and Brown, 2002; John, 1994; Møller et al., 1998a, 1998b; Morand and Poulin, 2000).

Conclusion

In summary, this study suggests that pheasants acquire an immune resistance against *H. gallinarum* parasitism, which affects parasite intensity and may affect female worm fecundity. This result concurs with similar work on this system undertaken using caecal egg counts (Tompkins *pers. com.*) and differs from past work within the grouse/*T. tenuis* system (Hudson and Dobson, 1997; Shaw and Moss, 1989; Wilson, 1983), possibly because of differences in parasite virulence or host suitability. The effect of an acquired immune response in the pheasant to *H. gallinarum* parasitism could alter the apparent parasite-mediated competition model suggesting *H. gallinarum* as one mechanism for grey partridge decline in the UK (Tompkins et al., 1999, 2000a, 2000b).

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Chapter 4

The effect of food stress upon susceptibility to parasitism: the caecal nematode *Heterakis gallinarum* in the ring-necked pheasant *Phasianus colchicus*.

Introduction

Nutritional stress is one factor known to influence host susceptibility to parasitism because of secondary immunodeficiency (Gershwin et al., 1985; Murray et al., 1997; Wakelin, 1989). For example, diets deficient in certain nutrients have been found to positively influence parasite intensity. This is because they can depress the resistance of the host by affecting nitrogen metabolism and enteric microflora (Ferket, 2003). Along these lines, vitamin B deficiency in chickens has been shown to increase *Ascaridia galli* intensity (Zimmerman et al., 1926). Thus, host resistance can be depressed and parasites encouraged by "impairments in immunity that occur during nutritional deficiencies" (Solomons and Scott, 1994).

Game bird diets are loosely based around research undertaken on broiler chickens and other domesticated bird species. They are designed to promote optimum growth and development conditions: criteria suggested to be insufficient even within the broiler industry (Gous, 1998). These diets have not been developed to ensure long-term health (Dietert and Lamont, 1994) or depress disease and parasitism, which are usually treated when necessary with the use of appropriate drugs. Another method of control could be

through amino acid and protein manipulation in feeds, and the research presented in this chapter sought to investigate this possibility.

Deficiencies in manipulated protein and amino acids have been found to result in reduced individual immune functioning and increased susceptibility to parasitic infection, by decreasing circulating antibodies against challenging organisms (Nilipour, 2001). Comprehensive tables have been compiled which cite examples of protein and specific amino acid deficiencies and their effects upon parasitic infections of host species (Scrimshaw et al., 1948, 1959, 1968), but clear, direct links within relationships are rare. A decreased resistance (*in vitro* T lymphocyte response) to the gastro-intestinal nematode *Thricostrongylus colubriformis* has been observed in young lambs with low compared to standard protein diets, which was also dependent upon age (Kambara et al., 1993). A weak negative relationship with parasitism in food limited snowshoe hares *Lepus americanus* and their parasite infra-communities has been suggested (Murray et al., 1997, 1998) and similarly, rats *Rattus norvegicus* and hill sheep *Ovis aries* on deficient diets have been found to have greater intensities of parasites (Donaldson and Otto, 1946; Paver et al., 1955). Lochmiller investigated immune system functioning in relation to dietary protein quality in northern bobwhite *Colinus virginianus* chicks (Lochmiller et al., 1993). Chicks on the lowest quality protein diet suffered depressed body growth, slower development of the bursa of Fabricius, and depressed spleen and immune system functioning (in the form of lymphoctye yields from dissociated organs and T-cell mediated immune functioning). Pederson noted not only a decrease in parasite intensity and length, but also decreases in the level of parasite aggregation, host body weight gain,

serum albumin, haemoglobin and packed cell volume (PCV) when the protein content of pig diets was lowered (Pedersen et al., 2002). Reidel and Ackert (1951) found a moderate protein diet made chickens *Gallus domesticus* more resistant to ascarids than those receiving a low ration diet, but they also found that compared to this diet, low and high ration diets also retarded the growth rate of chickens. This suggests that if protein levels within diets are set too high, in the same way that decreased protein may cause malnutrition and impact upon immune defence, levels increased above an optimum may also decrease resistance through obesity. Although high dietary protein has been shown to have a positive effect upon T-cell mediated immunity in chickens (Glick et al., 1983) and in wild barn swallow *Hirundo rustica* chicks (Saino et al., 1997), in the swallow chicks this feeding level perhaps brought the protein level to the natural optimum rather than to a level that would cause obesity. Within the research, Saino also commented that 'there is no direct evidence that fat reserves promote survival.' Therefore, in young animals a level of protein which is higher than normal could be used to create accelerated growth and development or an increased immune response and it may consequently not necessarily negatively impact upon host resistance. This is perhaps why both Lochmiller and Birkhead found higher T-cell mediated immune responses in dietary protein supplemented chicks (Birkhead et al., 1999; Lochmiller et al., 1993), and worm egg counts were reduced by feeding protein rich concentrates to young animals such as hogg sheep (White and Cushnie, 1952), lambs (Fraser and Robertson, 1933) and wether hoggs (Naerland, 1949). This research therefore suggests that protein levels increased above an optimum can either immune compromise animals or perhaps increase immune defences, especially in young. In addition, other research on chicken chicks and pheasants

Phasianus colchicus has shown protein levels lowered below the typical may not always impact upon chick or pheasant health, when optimal amino acid levels are maintained (Askelson and Balloun, 1965; Neto et al., 1997). Quail populations selected under low-protein diets have been shown to have no need of high-protein for full expression of their genetic potential, compared to those selected under high-protein diets who continued to require such levels for maximal growth (Marks, 1993). Broiler chickens have been shown to vary their own food intake rates to remain healthy dependent upon energy or protein content within the feed, although as energy intake decreased, or protein intake increased, the birds deposited less carcass fat (Leeson et al., 1996). This research suggests that if optimal amino acid levels are maintained and birds are reared on a low protein diet, the respective level may not limit health but could negatively impact parasitism.

Investigations have shown that the amino acids lysine and methionine are limited in all commercial cottonseed meals and in proteins from corn and soybean meal (Anderson and Warwick, 1966; Baldini and Rosenberg, 1955), which both make up poultry diets. Work undertaken on broiler chickens showed body weight gains were consistently better in birds fed additional amino acids than in control birds (Al-Nasser et al., 1986). Whilst manipulating methionine levels above normal in adult wild northern bobwhite, no adverse effects of high concentrations were observed, but there seemed to be an optimal level for production of viable chicks (Dabbert et al., 1996). In research on chickens Glick showed those fed diets two-thirds deficient in amino acids had lower secondary responses to sheep-red blood cells, than those fed optimal levels (Glick et al., 1981). In later studies investigating T-cell mediated immunity in birds fed the same diets, there was no

reduction in total white blood cells, absolute lymphocytes or absolute heterophils and therefore no change in splenic lymphocytes and the T-cell mediated immune response (Glick et al., 1983). Upon the administration of deficient diets there were also reductions in body weight, but all birds showed equal weight gain after a rest period. This suggests a reduced amino acid diet may have short-lived effects upon certain parts of the immune system, specifically humoral immunity, and that optimal amino acid levels are important in regulating general immune system functioning and body condition.

This study aimed to manipulate crude protein and amino acid (specifically digestible lysine, methionine and cysteine) levels and to observe the effects of such manipulations upon the intensity, aggregation, fecundity of females and development of the juvenile stages of the caecal nematode *Heterakis gallinarum*, within a ring-necked pheasant host. The amino acids lysine and methionine were selected for manipulation as their supplementation in a reduced protein diet (reduced to 90% of the recommendation) has been suggested as the best method of improving amino acid balance, and consequently gut health and individual immune defence in poultry (Ferket, 2003).

Host characteristics both before and after parasite exposure were related to the characteristics of the parasite population established, by quantifying body condition before (modelled muscle mass index) and after (actual lean wet breast muscle mass), and host organ mass (liver and spleen) after exposure, and relating them to parasite burden, female worm length (indicative of female fecundity) and the proportion of adults within the parasite population (indicative of the fitness of the parasite population due to the

speed of juvenile maturation). Using a control diet (with standard crude protein and amino acid levels; diet 1) as a benchmark, parasite populations after the maintenance period were compared against each other. The dietary manipulations were:

1. Low crude protein with lowered amino acid levels (diet 2);
2. Standard crude protein and amino acid level diet with added linseed oil (diet 3);
3. Low crude protein with standard amino acid levels (diet 4).

Hypotheses one

The first hypothesis was that the protein content of the host diet influences the success of parasites in the host. The prediction was therefore that parasite numbers and sizes should differ between hosts maintained on diets that differed in protein content but not amino acid levels. Parasitism and female worm fecundity would be lower in the birds on the low crude protein level (4) than those on the standard crude protein level diet (1). This is because current levels of protein may be influenced by research from the broiler industry and by customer preference. They may consequently be set too high, to drive accelerated growth, which in wild birds such as pheasants could then promote obesity. Obesity may negatively affect individual immune function, allowing parasite intensity to rise and possibly creating a better source of food for parasites.

Hypotheses two

The second hypothesis was that the amino acid content of the host diet influences the condition of the host. The prediction was therefore that body condition should differ between hosts maintained on diets that differed in levels of amino acids. The birds on the

low crude protein, standard amino acid level diet (diet 4) would be in better body condition those on the diet with reduced amino acid levels (diet 2), because amino acid formulations are probably relatively optimal, being set by rigorous industrial research and are therefore less likely to be affected by customer preference than protein levels.

Hypotheses three

The third hypothesis was that differences in the body condition of the host would influence the success of parasites. The prediction was therefore that variation in host body condition would predict variation in the number and size of parasites within the host.

Hypothesis four

The fourth hypothesis was that the linseed oil content of the host diet would influence the success of parasites within the host. The prediction was therefore that the number and size of parasites within the host should differ between hosts maintained on diets containing differing amounts of linseed oil. The linseed oil additive was included as a manipulation because a few cases exist of specific dietary additives being used to depress particular parasitic infections such as the use of omega-3 fatty acids from fish oils, to discourage Coccidiosis (IFOMA, 1999). I therefore tested the effect of a linseed oil additive. Parasite intensity would be reduced in the linseed oil diet (diet 3) compared to the control diet (diet 1).

Materials and methods

Establishment of natural infection

79 pheasant hens of approximately 8 months of age were caught-up together during January 2002. Having been reared and released at the same site the presumption was made that they would have been exposed to similar levels of parasite infection.

Prior to the trial, 15 of the 79 birds were euthanased and their intestinal tracts were examined to quantify the distribution and intensity of pre-trial *H. gallinarum* burdens.

Feed manipulations

The 64 remaining birds were randomly split into 8 groups of 8, were weighed (± 5 g), measured (tarsal length and wing chord), and then maintained (with *ad-libitum* food and water) in 8 outdoor pens (1.8 x 1.8 m) with two pens for each dietary manipulation, for a period of ten weeks. The pens were located on the rearing field of a game bird estate in Stirlingshire, Scotland, which previous sampling had confirmed was heavily contaminated with *H. gallinarum* eggs. Two pens of 8 birds each were randomly allocated one of the dietary manipulations or a standard maintenance control diet. Only two replicates (pens) were possible because of the cost of animal husbandry for the birds within the study.

After manufacture, the crude protein and amino acid levels within the diets were confirmed in laboratory tests.

At intervals all birds were weighed and a profile of the pectoral muscle region taken to estimate changes in breast muscle mass (Bolton et al., 1991). After the maintenance period birds were euthanased to quantify worm burden and female worm length. During post-mortem the actual lean wet breast muscle mass, liver and spleen were weighed. For full methodologies please see Chapter 1 (General Methodology).

Statistical analyses

The covariates and factors considered in analyses were the modelled muscle mass index, host body weight prior to dietary manipulation and dietary manipulation itself. These were examined in relation to parasite intensity, female worm length, the proportion of adults in the parasite population, actual lean wet breast muscle mass, liver and spleen mass subsequent to parasite exposure.

REML (residual maximum likelihood model) analyses were initially used as the statistical methodology rather than generalised linear models because they allowed the inclusion of pen number as a random term, thereby controlling for repeated measures, unbalanced densities within each pen and avoiding pseudoreplication problems (Patterson and Thompson, 1971); (Patterson and Lello, in press). All analyses were consequently initially undertaken in GenStat for windows, version 6 (© 2000 VSN International), by first fitting the maximal model and then using stepwise deletion. Pen was included in analyses as a random factor and then removed and its significance against the dependent variable examined. If non-significant, it was dropped from the model and linear modelling (LM) or generalised linear modelling (GLM) was undertaken instead in S-

PLUS, version 6 for Windows™ Professional Release 2 (Mathsoft Engineering and Education, Cambridge, Massachusetts, USA, © 1988-2001 Insightful Corp.) program. Pen was not included within maximal LM or GLM models as it was also represented within the diet term. If diet was dropped from the model during step-wise deletion however, then pen number was added to avoid pseudoreplication of the replicates.

In all analyses a host body size score created using principal component analysis (Chapter 1, General Methodology) was included as a control factor, being discarded if non-significant. Minimal models were arrived at using stepwise deletion. Predicted fits were used to display results controlling for the other terms remaining in the models. The *F* statistics and deviance values presented are from the minimal models for significant terms, or the minimal model with the non-significant term added on to the model for terms dropped from the maximal model. To compare differences between factor levels Tukey's honestly significant difference (HSD) was used. To control for type II statistical errors resulting from a large number of factors being considered in analyses, which meant some factors could falsely show significance due to chance, the method of Benjamin and Hochberg (1995) was used. This technique was chosen over the Bonferroni correction method as it is less conservative (Cotter et al., 2004; Benjamini and Hochberg, 1995). Worm lengths were averaged per host for analyses, as degrees of freedom were insufficient to include host as a covariate or random factor.

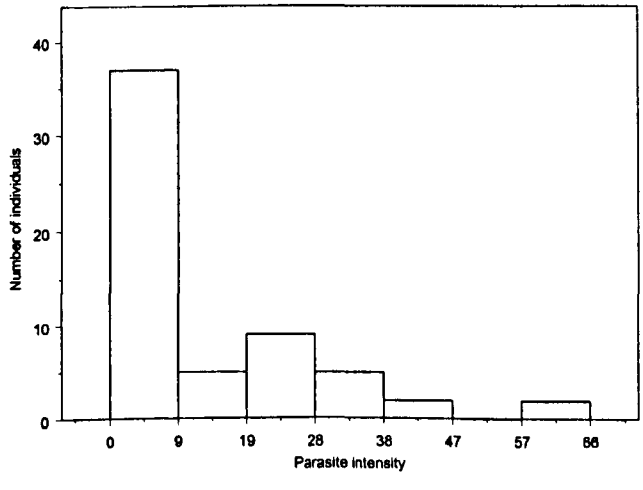
A statistical test of negative binomial fit, designed and written by Darren Shaw, University of Edinburgh for the S-PLUS 6 program was used to examine the error

distribution of the worm intensity data. They did not differ significantly from a negative binomial distribution, with a best estimate of the aggregation parameter of (k) = 0.62, and variance/mean ratio of 18.4 (P = 0.37; Figure 1). As a result the data were $\log_{10}$transformed and analysed initially using REML. As pen did not have a significant effect upon parasite intensity (X^2 = 0.35, df = 1, $P > 0.5$) it was initially dropped from analyses and a GLM with negative binomial error distribution (Wilson and Grenfell, 1997; Wilson et al., 1996) was undertaken instead, using untransformed data. Pen was also initially dropped from the analyses of female worm length, actual lean wet breast muscle mass, liver and spleen mass for the same reason. These were subsequently analysed using LM with normal error distributions. Likewise, pen was initially dropped from the analysis of the proportion of juveniles in the parasite population and a GLM with binomial errors and a 'logit' link function was undertaken instead. The spleen mass data contained two unusually large outliers that were excluded from analyses, as they were 2.9 and 4.1 standard deviations from the mean.

To represent the level of aggregation of the parasites both in the host population and within dietary manipulations, the variance/mean ratio and best estimate of the aggregation parameter (k) were calculated. Both measures were presented, as the variance/mean ratio has been suggested to provide a better measure of the degree of aggregation (the 'length of the tail') and remains more useful when there are a large number of hosts with zero parasites, and k provides more information about the distribution of the data around the mean when there are only a few zeros (Scott, 1987). The best estimate of the aggregation parameter was calculated using the negative binomial fit designed by Darren Shaw, as it

has been suggested to be the most representative measure of parasite aggregation, being calculated using the maximum likelihood estimate of k. This incorporates using all the data for calculation rather than the mean of the data, as with, for instance, the moment estimate of k (Wilson, et al., 2002). As the level of aggregation of parasites is calculated using all individuals within a host population, and because an aggregated distribution means that the variance of the parasite population is greater than the mean number per host, it was not possible to work out an error distribution around the aggregation parameters. As a result, there was no way of calculating whether the level of aggregation differed significantly between dietary manipulations. Interpretations of differences in the level of aggregation were consequently only undertaken if levels differed substantially.

Figure 1 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 60), after 10-weeks of natural exposure to *H. gallinarum*. 100% of pheasants were infected, and the overall mean ± SD was 12.35 ± 15.08 worms.



Results

During the 10-week exposure period, 4 individuals were euthanased due to husbandry factors unrelated to parasite infection. Although the majority of nematodes recovered from the experimental birds were the caecal worm *H. gallinarum,* 88.33% were also infected with the intestinal worm *Capillaria annulata* (with a mean ± SD of 4.31 ± 3.92), but diet, body condition or body size had no effect upon the intensity of this infection.

H. gallinarum infection within the 15 pre-trial birds upon which post-mortem was undertaken corroborated a typically aggregated distribution within the host population.

Testing for the effects of dietary manipulation and parasitism upon lean wet breast muscle mass

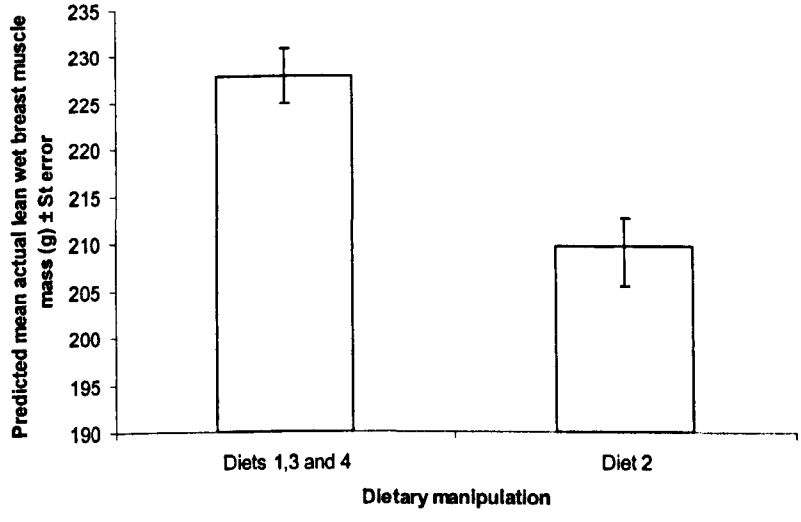
After Benjamin and Hochberg (1995) corrections were undertaken actual lean wet breast muscle mass was significantly related to dietary manipulation (Figure 2; $F_{1,56} = 6.785$, $P = 0.012$) and was positively related to host body size ($F_{1,56} = 39.763$, $P < 0.001$). It was not related to parasite intensity, female worm length or the proportion of adults within the parasite population. HSD showed no significant difference between diet 1 (with standard protein, standard amino acid levels), diet 3 (with standard protein plus linseed) and diet 4 (with low protein, standard amino acid levels), so the manipulations were combined into one category. The model was then re-analysed and significant differences were found between the combined standard amino acid level diets (diets 1, 3 and 4) and diet 2, the low amino acid level diet (Table 1).

Table 1 Linear model of actual lean wet breast muscle mass after dietary manipulation.

Response: Actual lean wet breast muscle mass (g)	d.f., residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host body size	1,56	-12.988	2.060	39.763	<0.001***	0.010	***
Dietary manipulation	1,56	-9.087	3.489	6.785	0.012*	0.020	*
Terms dropped							
$\log_{10}$(parasite intensity+1)	1,55	-3.605	5.544	0.423	0.518	0.040	ns
$\log_{10}$(female worm length)	1,36	5.500	1.236	0.009	0.924	0.050	ns
Proportion of adults in the parasite population	1,46	-1434.110	1290.136	1.236	0.272	0.030	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 2 Variation in actual lean wet breast muscle mass with dietary manipulation (n = 60). The predicted values control for the effects of host body size (Table 1).



Testing for the effects of dietary manipulation and host body condition on susceptibility to parasitism

Parasite intensity

After Benjamin and Hochberg (1995) corrections were undertaken the intensity of parasite infection was significantly related to dietary manipulation interacting with the modelled muscle mass index (Figure 3; deviance = 8.032, $df = 2,51$, $P = 0.018$) and host body weight (Figure 4; deviance = 8.097, $df = 2,51$, $P = 0.017$). It was not significantly related to the main effects of dietary manipulation, the modelled muscle mass index and host body weight or host body size. Within the relationships between parasite intensity and dietary manipulation interacting with both the modelled muscle mass index (Figure 3) and with host body weight (Figure 4), the results for diets 1 and 3 were not significantly different from each other. They were consequently combined to create a category that represented standard protein and amino acid level diets, with/without added linseed (Table 2).

Table 2 Negative binomial model of parasite intensity after dietary manipulation.

Response=Parasite intensity	d.f.	Value	s.e.	Deviance	*P(Chi)*	Corrected *P*	Significance of corrected *P*
NULL	59			81.371			
Modelled muscle mass index	1,55	-6.770	7.031	0.126	0.722	0.000	ns
Host body weight (g)	1,55	0.164	0.170	0.124	0.725	0.025	ns
Dietary manipulation	2,55	#	#	0.532	0.766	0.033	ns
Dietary manipulation x modelled muscle mass inde	2,51	¤	¤	8.032	0.018*	0.042	*
Dietary manipulation x host body weight	2,51	ж	ж	8.097	0.017*	0.017	*
Terms dropped							
Host body size	1,54	-0.113	0.169	0.177	0.674	0.000	ns
		# Values	# s.e.	¤ Values	¤ s.e.	ж Values	ж s.e.
	1	0.000	0.000	0.000	0.000	0.000	0.000
	2	25.612	9.453	23.802	8.082	0.577	0.195
	3	-7.794	6.562	8.664	5.260	-2.110	0.127

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 3 Variation in parasite intensity with dietary manipulation interacting with the modelled muscle mass index (n = 60).

The predicted values control for the effects of host body weight (Table 2).

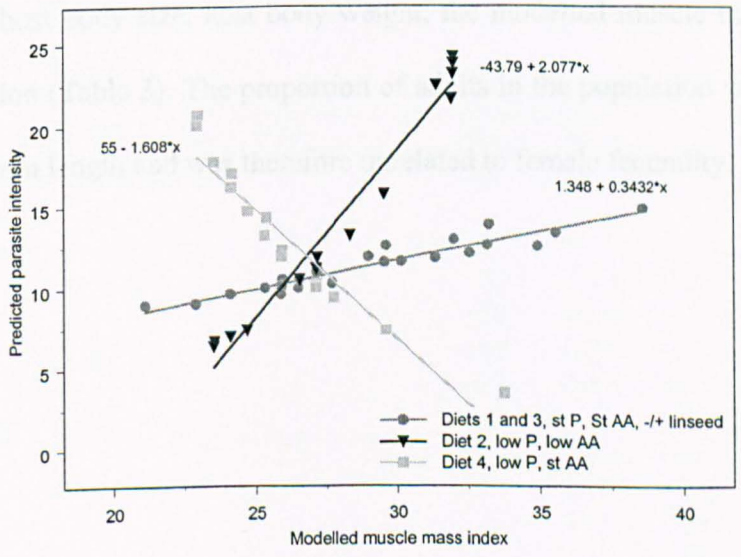
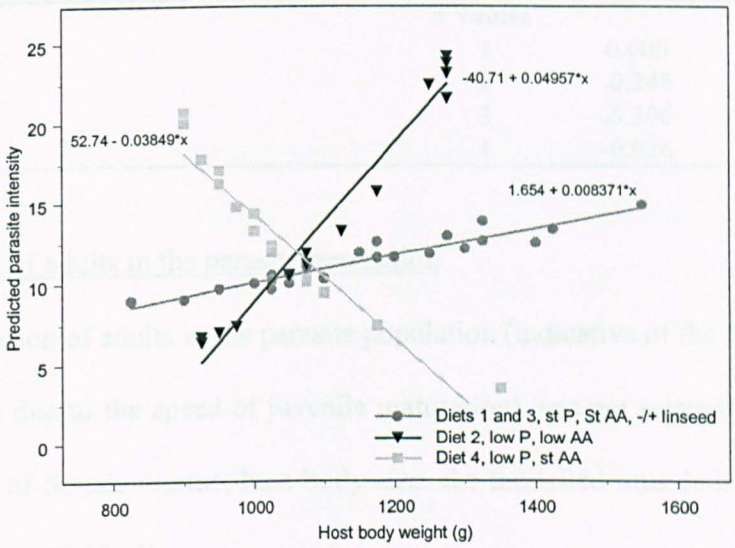


Figure 4 Variation in parasite intensity with dietary manipulation interacting with the host body weight (n = 60).

The predicted values control for the effects of the modelled muscle mass index (Table 2).



Female worm length

The length of female worms and therefore female fecundity was not related to the parasite intensity, host body size, host body weight, the modelled muscle mass index or dietary manipulation (Table 3). The proportion of adults in the population was not related to the female worm length and was therefore unrelated to female fecundity.

Table 3 Linear model of female worm length after dietary manipulation.

Response~Female worm length (mm)	d.f.,residuals	Value	s.e.	*F* statistic
Terms dropped				
$\log_{10}$(parasite intensity + 1)	1,39	0.480	0.523	0.843
Host body size	1,39	-0.232	0.179	0.681
Modelled muscle mass index	1,39	0.077	0.060	1.623
Host body weight (g)	1,39	0.002	0.002	1.624
Dietary manipulation	3,37	#	#	1.274
	# Values		# s.e.	
	1	0.000	1	0.215
	2	-0.246	2	0.293
	3	-0.306	3	0.181
	4	-0.026	4	0.125

Proportion of adults in the parasite population

The proportion of adults in the parasite population (indicative of the fitness of the parasite population due to the speed of juvenile maturation) was not related to parasite intensity, the length of female worms, host body size, the modelled muscle mass index or dietary manipulation (Table 4).

Table 4 Binomial model of the proportion of adults in the parasite population after dietary manipulation.

Response~Proportion of adult worms	d.f.	Value	s.e.	Deviance	*P (Chi)*
NULL	50			0.157	
Terms dropped					
$\log_{10}$(parasite intensity + 1)	1,49	-0.574	6.918	0.007	0.150
$\log_{10}$(female worm length)	1,38	3.440	63.255	0.003	0.093
Host body size	1,49	-0.172	2.354	0.006	0.152
Modelled muscle mass index	1,49	0.069	0.952	0.006	0.152
Host body weight (g)	1,49	0.002	0.023	0.006	0.152
Dietary manipulation	3,47	#	#	0.020	0.137
		# Values	# s.e.		
	1	0.000	3.435		
	2	0.213	5.548		
	3	-0.112	2.941		
	4	-0.211	1.532		

Testing for the effects of parasitism and dietary manipulation upon spleen and liver mass

Spleen mass

Spleen mass was not related to parasite intensity, female worm length, the proportion of adults in the parasite, host body size or dietary manipulation (Table 5).

Table 5 Linear model of spleen mass after dietary manipulation.

Response-Spleen mass (g)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Terms dropped							
$\log_{10}$(parasite intensity+1)	1,56	<-0.001	0.044	<0.001	0.995	0.040	ns
$\log_{10}$(female worm length)	1,38	-0.362	0.549	0.434	0.514	0.050	ns
Proportion of adults in the parasite population	1,47	7.001	10.526	0.443	0.509	0.030	ns
Host body size	1,56	-0.003	0.017	0.024	0.877	0.010	***
Dietary manipulation	3,54	#	#	0.681	0.568	0.020	*
		#Value	#s.e.				
	1	0	0.024				
	2	-0.023	0.034				
	3	-0.011	0.019				
	4	0.015	0.014				

Liver mass

After Benjamin and Hochberg (1995) corrections were undertaken liver mass was positively related to parasite intensity ($F_{1,29}$ = 12.135, P = 0.001), and was related to dietary manipulation (Figure 5; $F_{3,29}$ = 6.938, P = 0.003). It was also related to interactions between female worm length and dietary manipulation (Figure 6; $F_{3,29}$ = 5.733, $P < 0.008$), female worm length and host body size ($F_{1,29}$ = 9.100, P = 0.005) and female worm length and the proportion of adults in the parasite population ($F_{1,29}$ = 7.329, P = 0.011). It was not related to the main effects of the length of female worms, the proportion of adults in the parasite population or host body size. Within the relationship

between liver mass and dietary manipulation interacting with female worm length, the results for diets 1 and 4 were not significantly different from each other and were consequently combined to create a category that represented standard amino acid level diets, without added linseed (Table 6).

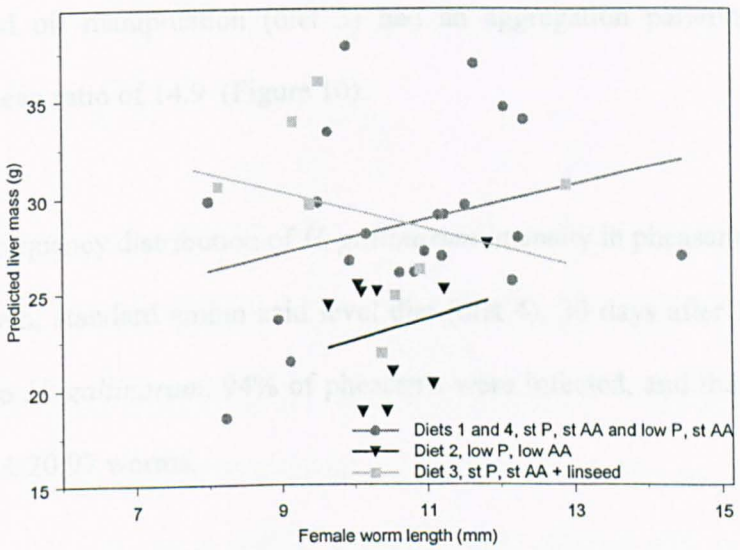
Table 6 Linear model of liver mass after dietary manipulation.

Response-Liver mass (g)	d.f., residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
$\log_{10}$(parasite intensity+1)	1,29	7.071	2.001	12.135	0.001**	0.006	**
$\log_{10}$(female worm length)	1,29	-21220.565	7833.900	0.744	0.395	0.038	ns
Proportion of adults in the parasite population	1,29	-21667.555	7994.211	0.246	0.624	0.044	ns
Host body size	1,29	50.292	16.701	0.046	0.832	0.050	ns
Dietary manipulation	3,29	#	#	6.938	0.003**	0.019	**
$\log_{10}$(female worm length) x dietary manipulation	3,29	¤	¤	5.733	<0.008**	0.025	**
$\log_{10}$(female worm length) x host body size	1,29	-49.009	16.246	9.100	0.005**	0.013	**
$\log_{10}$(female worm length) x proportion of adults in the parasite population	1,29	21243.516	7846.994	7.329	0.011*	0.031	*
		# Value	#s.e.	¤ Value	¤s.e.		
	1	0.000	0.000	0.000	0.000		
	2	15.279	27.453	-16.865	26.760		
	3	50.110	17.022	-49.037	16.852		

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 6 Variation in liver mass with dietary manipulation interacting with female worm length (n = 28).

The predicted values control for the effects of parasite intensity, the proportion of adults in the parasite population, host body size and female worm length interacting with both host body size and the proportion of adults (Table 6).



The effect of dietary manipulation upon parasite aggregation

The aggregation of the parasite population seemed to be affected by the protein level within the diet, with the low crude protein (with standard amino acid levels; diet 4) diet having an aggregation parameter (k) of 0.6182 and variance/mean ratio of 33.2 (Figure 7), and the control diet (with standard crude protein and amino acid levels; diet 1) having an aggregation parameter of 1.1588 and variance/mean ratio of 8.5 (Figure 8).

The aggregation of the parasite population didn't seem to be affected by the amino acid levels in the diet, with the diet with low levels (diet 2) having an aggregation parameter of 0.5052 and variance/mean ratio of 17.5 (Figure 9), and the diet with standard levels (diet 4) having an aggregation parameter of 0.6182, and variance/mean ratio of 33.2 (Figure 7).

The linseed oil manipulation (diet 3) had an aggregation parameter of 0.4582, and variance/mean ratio of 14.9 (Figure 10).

Figure 7 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 16) fed a low crude protein, standard amino acid level diet (diet 4), 30 days after 10-weeks of natural exposure to *H. gallinarum*. 94% of pheasants were infected, and the overall mean ± SD was 13.25 ± 20.97 worms.

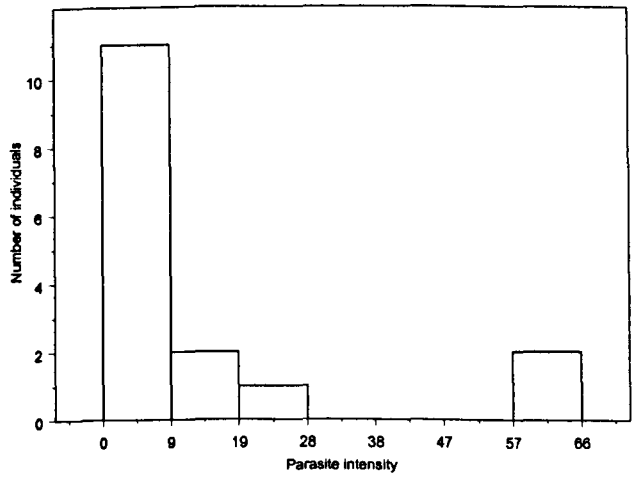


Figure 8 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 15) fed a control diet (with standard crude protein and amino acid levels; diet 1), after 10-weeks of natural exposure to *H. gallinarum*. 93% of pheasants were infected, and the overall mean ± SD was 12.27 ± 10.2 worms.

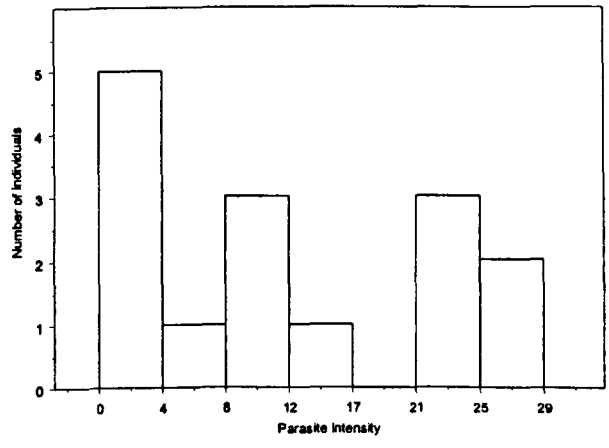


Figure 9 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 14) fed a low crude protein diet with lowered amino acid levels (diet 2), after 10-weeks of natural exposure to *H. gallinarum*. 79% of pheasants were infected, and the overall mean ± SD was 13.79 ± 15.52.

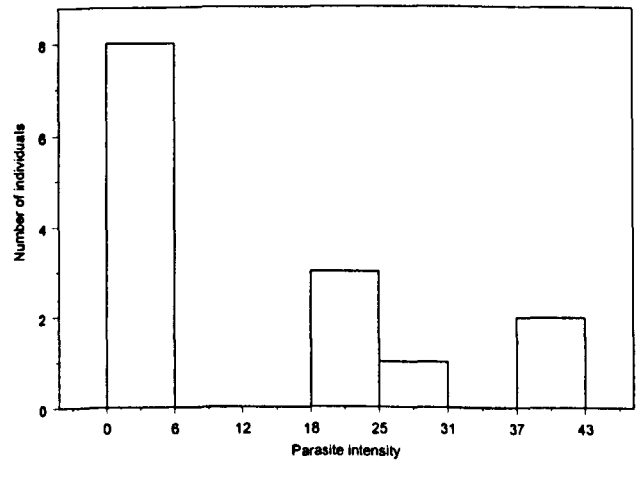
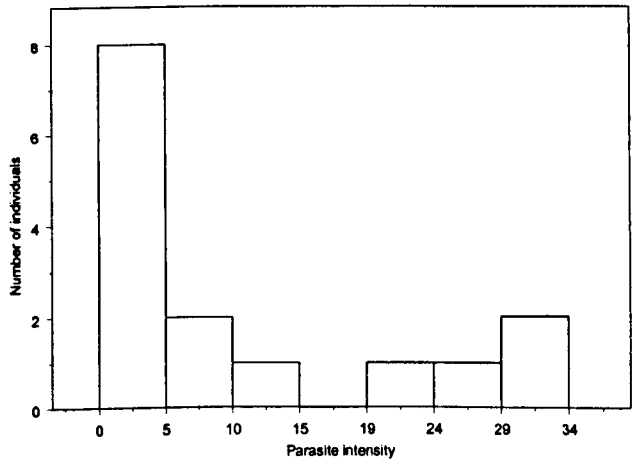


Figure 10 Frequency distribution of *H. gallinarum* intensity in pheasants (n = 15) fed a diet with standard crude protein and amino acid levels with added linseed oil (diet 3), after 10-weeks of natural exposure to *H. gallinarum*. 73% of pheasants were infected, and the overall mean ± SD was 10.13 ± 12.78 worms.



Discussion

This study investigated the hypotheses that:

(1) the protein content of the host diet influences the success of parasites in the host,

(2) the amino acid content of the host diet influences the condition of the host,

(3) differences in the body condition of the host influence the success of parasites, and

(4) the linseed oil content of the host diet influences the success of parasites within the host.

Following controlled exposure, dietary manipulation was significantly related to actual lean wet breast muscle mass (Table 1, Figure 2), with higher muscle mass in birds on the

standard amino acid levels diet (diets 1, 3 and 4) compared to the low amino acid level diet (diet 2). This suggests that the second null hypothesis that the amino acid content of the diet would not influence the condition of the host could be rejected. This is backed up by the effect of dietary amino acid level interacting with the modelled muscle mass index (Table 2, Figure 3) or host body weight (Table 2, Figure 4) upon parasite intensity. Within this relationship, the body condition of the host negatively influenced the success of parasites in the hosts on the standard amino acid level diet, and positively influenced the success of parasites in the hosts on the low amino acid level diet. This is possibly because the birds on the standard amino acid level diet were able to maintain their resistance to parasitism with their condition, whereas the birds in poor condition on the low amino acid level diet, which were probably malnourished, suffered higher parasitism with an increase in condition. This may have occurred because resistance to parasitism did not increase with body condition under amino acid limitation. A healthy or full gut, indicted by good body condition may instead have encouraged parasitism.

As the relationships between the standard crude protein diets (1, 3 and 4) and low crude protein diet (2) were different within the interaction involving the modelled muscle mass index (Figure 3) or host body weight (Figure 4) both affecting parasite intensity, the first null hypothesis that the protein content of the diet would not influence parasite intensity can be rejected. The prediction that parasites numbers and sizes would differ between hosts maintained on diets that differed in protein but not amino acid levels was not completely correct however, because amino acid level did affect parasite intensity in combination with host body condition.

Because variation in host body condition predicted variation in the number of parasites interacting with dietary amino acid level (Figures 3 and 4), this also suggests that the third null hypothesis that differences in body condition would not influence parasite intensity can be rejected. Prior research on many species has found positive links between body condition and resistance to parasitism (Christe et al., 1998; Saino et al., 1997; Soler et al., 1999; Sorci et al., 1997; Tella et al., 2000).

There was no difference in parasite intensity between the standard diet (diet 1) and the diet with added linseed (diet 3), and no effect of dietary manipulation upon female worm length (Table 3) or the proportion of adults in the parasite population (Table 4). This suggests that the fourth null hypothesis that the linseed oil manipulation would not affect the success of parasites within the host can not be rejected. There may have been a weak negative effect of the manipulation upon parasite female fecundity, however. This is suggested by a negative relationship between female worm length and liver mass in the birds on diet 3 (where the relationship was positive in the birds on diets 1, 2 and 4), and a positive relationship between liver mass and parasite intensity (Table 5). This positive relationship suggests an increase in liver mass is representative of a response to parasitism, and the response may therefore have been characterised by a reduction in female worm fecundity within the parasite population.

The level of aggregation does not appear to have been affected by amino acids levels within manipulations (Figures 7 & 9). It does however appear to have been affected by

crude protein levels, with greater aggregation in hosts on the low (Figure 7) than on the standard crude protein level diet (Figure 8). The difference in aggregation with protein level may be a result of individual differences in susceptibility to parasitism between treatment groups, for a number of reasons. For instance, the parasite prevalence of hosts in optimal condition is most likely to be affected by natural susceptibility and acquired immunity, but hosts suffering from reduced immune defence mechanisms due to obesity or malnutrition are likely to be handicapped. Consequently, within these hosts more individuals would suffer higher parasite burdens, therefore decreasing parasite aggregation. The gut of hosts on high protein diets may allow better parasite survival as a result of an improved food source. This would impact upon parasitism by increasing parasite intensity throughout the host population, and possibly lessening the effects of differing host susceptibility and immune response. Also, behavioural differences may be caused by variation in the quality of the diets, whereby some individuals on the low compared to the standard crude protein diets may have attempted to compensate by increasing either the quantity or quality of their intake, foraging to a greater extent and therefore increasing their exposure to the infective stages of the parasite. As has already been suggested, research has indicated moderation of dietary intake for quality is possible in broiler chickens, and therefore it is perhaps also possible in pheasants (Leeson et al., 1996). Whatever the mechanisms driving aggregation, during manipulation of crude protein content in pig diets, a decline in parasite aggregation with protein content reduced from normal (optimal) to low (perhaps malnutritional) levels has been noted (Pedersen et al., 2002).

The weak indication that the linseed oil manipulation (diet 3) may have affected female worm fecundity is backed up by the highly aggregated distribution of the parasites within the birds on this diet (k = 0.4582; Figure 7). Following the suggestion that aggregation decreases as a result of a greater number of handicapped and therefore more susceptible hosts (due to reduced immune defence from obesity or malnutrition), this level of aggregation suggests the individuals were relatively healthy.

Liver mass was significantly positively related to parasite intensity, suggesting an increase in mass may be a response to parasitism, and was also related to dietary manipulation interacting with female worm fecundity (Table 5). Within this relationship, liver mass was positively related to female worm length, increasing with the same magnitude in birds on diets with standard amino acid levels and either standard or low protein levels (diets 1 and 4), or in birds on diets with low protein and low amino acid levels (diet 2). The overall liver mass was however lower in birds on the low protein and low amino acid level diet (diet 2; Figure 6). This may suggest that birds are less able to maintain condition (indicated by liver mass) if both amino acid and crude protein levels are compromised.

Conclusion

In summary, although indirect, there does seem to be a relationship between dietary manipulation, parasitism and host body condition, possibly mediated by host immune function/host suitability to parasitism and/or by behavioural differences between hosts. Although having no effect upon host body condition subsequent to parasite exposure,

when amino acid levels were maintained, low protein seemed to negatively influence parasite intensity, with a greater influence if the hosts were in good body condition. Current amino acid levels within game bird over-wintering feeds therefore seem to be optimal.

Including linseed oil within the standard pheasant over wintering diet may have weakly discouraged female parasite reproduction in conjunction with liver functioning, but this interpretation is negligible.

Implications for game bird management and future work

As Hunter proposed: "the nutritional requirements of the parasite and the relation of this need to the host are not well known, in spite of the fact that many definitions of the term 'parasite' imply a knowledge of how the parasite obtains its food" (Hunter, 1953). The factors connecting parasites to their host are ill understood, but the linkage with body condition inferred within this study suggests that perhaps the general condition and immune status of the host interacting with, and as a result of host diet, may be the key. Negative correlations between *H. gallinarum* intensity in pheasants and muscle mass possibly interacting with nutrition have been found on several other occasions (Sage et al., 2002; M. Woodburn, *pers. com.* in Tompkins et al., 2000), and high numbers of *H. gallinarum* have been found in birds in poor condition (Sage et al., 2002). Future work should therefore further examine the relationship between body condition, immune defence and parasitism, and attempt to separate the interactions seen within this work.

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Chapter 5

The effects of body condition, immune defence and parasitism by the caecal nematode *Heterakis gallinarum* upon secondary sexual ornamentation in a ring-necked pheasant *Phasianus colchicus* host.

Introduction

The susceptibility of hosts and therefore parasite aggregation often differs between the sexes (Chapter 1, General Introduction). When this occurs, parasitism is usually biased towards the male portion of a host population, resulting from environmental and therefore behavioural or individual and therefore immunological differences. This chapter examines the relationships between host immune defence, condition, secondary sexual characteristics and parasite infection, to see whether the expression of ornaments is likely to affect the susceptibility of hosts to *H. gallinarum* through investment in signals.

Sexually dimorphic display characteristics such as combs, wattles and plumage are associated, under Darwin's theory of sexual selection, with increased mating success through either "intrasexual competition or intersexual choice" (Able, 1996). Intrasexual competition using, for instance, the size and colour of the characteristics to decide dominance, avoids resources being wasted in physical confrontation (such as fighting). Mate choice, distinguished in the same way as intrasexual competition (and usually made by the female), is suggested to allow selection for quality (Able, 1996; Balmford and Read, 1991; Bart and Earnst, 1999; Burley and Coopersmith, 1987; Clayton, 1991;

Clayton et al., 1992; Duffy and Ball, 2002; Hamilton and Zuk, 1982; Kirkpatrick, 1987; Møller, 1990b; Møller and Saino, 1994; Nolan et al., 1998; Peters, 2000; Read, 1988; Read and Harvey, 1989; Rintamaki et al., 2000; Saino and Møller, 1994; Weatherhead et al., 1993; Zuk, 1992; Zuk, 1996; Zuk et al., 1995; Zuk et al., 1990b). Exactly how or what display characteristics are advertising has however been, and continues to be, the subject of much debate.

Research within many species has implied that the maintenance of display characteristics causes a cost to the individual (Møller et al., 2000; Møller et al., 1996; Nolan et al., 1998; Saino and Møller, 1996; Saino et al., 1999; Sheldon and Verhulst, 1996; Svensson and Merila, 1996; Verhulst et al., 1999; Zuk, 1996), which needs to be balanced with the possibility of increased reproductive potential. This has led to the development of theories of trade-offs between characteristics and life history traits, which would explain how their advertisement is indicative of fitness. Zahavi first suggested in 'the handicap principle' that an individual with a well-developed sexually selected character had already survived a test in that it was able to actually form the character in the first place. The cost of character formation therefore meant that 'females which selected males with the most developed characters can be sure to have selected from among the best genotypes of the male population' (Zahavi, 1975). Grafen formulated a mathematical model for the until then, theoretical handicap principle, and classified four interpretations from Zahavi's paper as to why costly signals were believable (1990a, b):

- 'Strategic choice.' This suggests that the signaller forms his signal size and quality and therefore handicaps himself according to his own quality and the expected

response of the receiver. Signallers therefore choose to handicap themselves to a greater or lesser extent because the cost and/or benefit to them varies between individuals.

- 'Survival.' The signaller is causing a handicap to himself by forming his signal, and the cost of this formation is lowering his chances of survival. The receiver can tell his quality simply because he has survived, despite this cost.
- 'Revealing.' The effort required by the signaller to create the signal makes it easier for the receiver to judge quality.
- 'Condition-dependent.' Some features are only able to be maintained with, for example, a nutritious diet, low parasite load or low attrition. High-quality signallers are, consequently, the only ones able to maintain high cost characteristics such as iridescent feathers.

But what if a signal was not honest, and an advertiser was able to signal for fitness that was not the reality. Only cheats falsifying their fitness would be likely to decrease their resources as a result of the cost of display, because honest signallers would be investing and gaining from this investment rather than just creating a cost by using their display (Zahavi, 2003). Examples of when signals are unlikely to be falsified have been suggested. For instance, characteristics such as horny protrusions or in mammals, antlers or horns, may be very costly to create and would therefore be difficult to fake. Species which when signalling, make themselves more open to attack through, for example, decreased camouflage are unlikely to fake signalling (Zahavi, 1975). Signals involved in intra-sexual interactions such as 'badges of status' which may not create a direct cost are unlikely to be fake, because the falsifier would make themselves open to competition and

possible punishment from competitors (Møller, 1987). Other signals however may be more easily created and could consequently be used for false advertisement, especially if their production does not lower the chances of survival. The potential gain from falsified signals is likely to be proportional to the cost of the signal (Zahavi and Zahavi, 1997), and the size of the display and therefore cost to the falsifier is likely to be decided by the signal receiver rather than the signaller. As a result, all this literature suggests that the cost of investment in false signals is likely to be proportionally greater for the signaller than the cost of honest signals would be. Signallers are therefore more likely to 'honestly' signal than to falsify signals. When multiple ornaments do and do not honestly signal fitness, Johnston and Grafen (1993) have suggested that for the system to be stable, the collected cost of signalling needs only to be honest 'on average' anyway.

Exactly what display characteristics indicate and how these indications are interpreted within life history has also been much debated. The initial theory proposed was that individuals with the brightest characteristics were able to advertise genetically mediated parasite resistance through their ability to maintain their ornaments in good condition; a trait selected for by females (Hamilton and Zuk, 1982; Møller, 1990a; Møller, 1991). But Able, in the 'Contagion Indicator Hypothesis' suggested that some studies had shown no relationship between parasite intensity and male mating success (Clayton, 1991; Møller, 1990b; Zuk, 1992), and therefore that females may be selecting for a different property (Able, 1996). One such property could be the direct benefit of avoiding parasites, the 'Transmission Avoidance Strategy' (Borgia, 1986; Borgia and Collis, 1990; Freeland, 1976; Møller, 1991), or selecting mates on their likely future ability to provide resources

through parental care (Linville et al., 1998; Read, 1990). Kirkpatrick (1982) however has argued that elaborate characteristics could be used purely for mate attraction.

Some studies of secondary sexual ornaments have suggested that different ornaments reflect different aspects of phenotypic quality (Møller and Petrie, 2002; Møller and Pomiankowski, 1993). Møller and Pomiankowski (1993) argued that in combination, ornaments provide a better estimate of general condition than they would separately, and that ornaments which may not be too costly to maintain may be unreliable signals. Because only some ornaments may be used in mate selection for instance (Omland, 1996; Zuk et al., 1990a,b, 1992), these arguments are upheld.

Regardless of how and what ornaments reflect, all the evidence suggests that generally ornaments reflect quality, and highly ornamented males are preferred by females because of this. Within the topic of host susceptibility to parasitism, the brightest secondary sexual ornamentation is therefore suggested to indicate the highest fitness and reduced susceptibility to parasitism.

Male sexual signalling

General patterns of the qualities indicated by secondary sexual characteristics have been investigated within many species. The main patterns observed have been that the brightness of secondary sexual signalling has been positively linked to testosterone levels in male birds (Evans et al., 2000; Hillgarth and Wingfield, 1997; Lank et al., 1999; Moss et al., 1979; Peters, 2000; Rintamaki et al., 2000; Saino and Møller, 1994; Saino et al.,

1995; Verhulst et al., 1999; Weatherhead et al., 1993; Zuk, 1996; Zuk et al., 1995), the creation of such signals often seems to have incurred a cost and therefore a handicap to either immune defence mechanisms (Buchmann, 1997; Duffy and Ball, 2002; Mougeot, in prep.-b; Saino and Møller, 1996; Verhulst et al., 1999; Zuk, 1996; Zuk et al., 1995) or survivorship (Horak et al., 2001; Nolan et al., 1998; Saino et al., 1995), and the 'showiest' individuals often seem to have been able to ignore this handicap to maintain lower levels of parasitism (Saino and Møller, 1994; Saino et al., 1995; Weatherhead et al., 1993; Zuk, 1991; Zuk et al., 1990c). However, in some cases the signals do not seem to cause a cost to the individual (Getty, 2002; Kurtz and Sauer, 1999; Peters, 2000; Read and Harvey, 1989; Westneat and Birkhead, 1998), and sexual signalling does not seem to be the negatively linked to parasitism (Schall and Staats, 1997).

Female sexual signalling

Female birds are generally considered too 'drab' to be selected as mates by traits similar to those for males. But females of some species show exaggerated secondary sexual characteristics such as crests, prominent beaks, wattles/combs, or extravagant decoration (Amundsen, 2000). In female northern cardinals *Cardinalis cardinalis* and in barn owls *Tyto alba*, positive associations have been found between underwing plumage colour and nestling feeding rate (suggesting increased fitness with 'showiness'; Linville et al., 1998) and the spottiness of plumage and antibody production against a non-pathogenic antigen (Roulin et al., 2000). Brightness has also been negatively linked with haematozoan and chronic blood infection in meta-analyses (Hamilton and Zuk, 1982; Zuk, 1991), but Amundsen said, 'condition-dependence of female ornaments is almost unstudied'

(Amundsen, 2000). To assess the assumptions of honest sexual signalling, with showiness being costly within all individuals and indicating resistance to parasites, female characteristics were also studied.

Sexual signalling of pheasants

Work already undertaken on pheasants has shown that display effort (Hillgarth, 1990), extended wattles (Hillgarth, 1990), fluffed feathers (Hillgarth, 1990), mate calling (Hillgarth, 1990), tail length (Geis and Elbert, 1956; Mateos and Carranza, 1995), length of the ear tufts (Mateos and Carranza, 1995), the presence of black points in the wattle (Mateos and Carranza, 1995) and wattle size (Mateos and Carranza, 1995; Papeschi et al., 2003) appear to act as characteristics of males that are used by female birds as ways of them discriminating between potential partners. Wattle colour and plumage brightness (Mateos and Carranza, 1995) do not seem to be selected for, but assessment of wattle size (Mateos and Carranza, 1997b), plumage brightness (Mateos and Carranza, 1997a; Mateos and Carranza, 1997b) and the length of ear tufts (Mateos and Carranza, 1997b) have been linked with male-male intra-sexual interactions, and wattle size has been linked with larger harems in comparison of treated tick-free and untreated individuals (Whitfield, 2002). Females have also been shown to prefer males with lower levels of *Coccidial* parasitism, higher survival rates (Papeschi and Dessi-Fulgheri, 2003) and higher rates of survival of infection (compared to treated individuals; Hillgarth, 1990), and they therefore seem to be selecting for parasite resistance and quality. Testosterone has been positively linked with wattle size (Briganti et al., 1999; Papeschi et al., 2000), body weight (Briganti et al., 1999), male aggressiveness (Briganti et al., 1999), male rank (Briganti et al., 1999),

the number of male-male interactions (Briganti et al., 1999) and time spent displaying (Mateos and Carranza, 1997b), which may suggest that these characters are testosterone dependent.

Mateos commented that 'the same traits are employed in both inter-male contest and courtship displays...that maintains the honesty of sexual signals' (Mateos, 1998). But male spur length has been linked with some indicators of current quality (harem size, Goransson et al., 1990; male viability, Goransson et al., 1990; von Schantz et al., 1994; von Schantz et al., 1996; phenotypic condition, Goransson et al., 1990; number of hatchlings, Goransson et al., 1990; von Schantz et al., 1994; offspring survival rate, von Schantz et al., 1994; von Schantz et al., 1996; MHC genotype, von Schantz et al., 1996; wattle size, Papeschi et al., 2000; wattle display, Mateos and Carranza, 1996; dominance, Mateos and Carranza, 1996; tail length, Papeschi et al., 2000; body weight, Goransson et al., 1990; Papeschi et al., 2000 and body size, Goransson et al., 1990), but has also been positively linked with age (Mateos and Carranza, 1996; von Schantz et al., 1989), it has only sometimes been linked with mate choice (Grahn and von Schantz, 1994; von Schantz et al., 1989; von Schantz et al., 1990; von Schantz et al., 1994; von Schantz et al., 1996) dependent upon the age of the individual (Mateos and Carranza, 1996). As it has not been linked with testosterone level (Briganti et al., 1999), it is highly repeatable between years (Mateos and Carranza, 1996), it seems unaffected by dietary protein level (where wattle colour and size have been; Ohlsson et al., 2002; Ohlsson et al., 2003) and it has been positively associated with T-cell mediated immune response (a novel measure of resistance to infection; Ohlsson and Smith, 2001) and humoral immune response

(Ohlsson et al., 2003), it perhaps is more indicative of early development than current condition. The links with some indicators of current quality may occur because past development is likely to be reflected by current condition.

Secondary sexual characteristics used within this study

The characteristics recorded within the male birds in this study were the vertical axis (height) of the wattle, the actual traced wattle area, spur length, wattle colouration, ear tuft length and the width of the black points in the wattle. Within female birds the vertical axis of the wattle, the actual traced wattle area and the maximum head crest height were recorded.

Hypothesis one

If secondary sexual characteristics are used in mate sexual selection or male-male intra-sexual interactions, they also need to systematically indicate the same thing. The first hypothesis was therefore that display characteristics would be correlated with each other if they were used in sexual selection or in male-male competitive interactions. Earlier work undertaken on pheasants has demonstrated that the length of the ear tufts, the width of the black points in the wattle and wattle height were positively associated with each other, and these associations were therefore predicted within this study. From earlier work, wattle colour was predicted to not be associated with the length of the ear tufts, the width of the black points in the wattle or wattle height. Spur length was also predicted to be positively associated with wattle height, wattle redness, body weight and body size, but these associations may be because of the size of the individual and their stage of

maturity or may be a result of earlier development rather than due to mate choice or male-male competition. In females, wattle height and the maximum head crest height were predicted to be positively correlated with each other.

Hypothesis two

The second hypothesis is that immune functioning and condition affects male showiness. The size and redness of sexual characteristics were therefore predicted to be positively associated with PCV, RBC, the lean wet breast muscle mass, liver and spleen mass. Work undertaken previously on male pheasants showed that spur length was positively associated with T-cell mediated immune response. Work undertaken on female birds (not pheasants) has shown positive links between the brightness of sexual traits and nestling feeding rate or antibody production against a non-pathogenic antigen, which may suggest a link with female condition.

Hypothesis three

The second part of the assumption that immune functioning and condition affect male showiness is that parasitism also affects male showiness. The third hypothesis was therefore that there would be negative relationships between the brightness of sexual characteristics and parasite intensity, the length (and therefore fecundity) of female worms and the proportion of adults in the parasite population (representing the speed of maturation of juvenile parasites). Previous work on pheasants has suggested a negative correlation between mate choice and *Coccidial* parasitism, and work on other female

birds has negatively linked sexual ornamentation and parasitism/disease. Characteristics were therefore predicted to be negatively associated with the indicators of parasitism.

Materials and methods

Study specific methodology

The data used for this study was obtained from the experiments reported elsewhere in this thesis that examined condition and T-cell mediated immune resistance (Chapter 2, n = 52 birds), acquired immunity (Chapter 3, n = 32 birds) and the effects of nutrition upon parasitism (Chapter 4, n = 14 birds). The individuals used within the first two studies were all naive to parasitism prior to exposure, and were raised in the same animal facility. Those in Chapter 4 were free-living caught-up birds, reared and released at the same site. The differences in experimental protocol were the parasite challenge/exposure regimes and location during the maintenance periods. The trial groups were retained in outdoor enclosures, sterile indoor facilities and outdoor enclosures, respectively. Please see the previous chapters for details of experimental treatments.

For all the birds, at euthanasia a body size score for the individual was created using principle component analysis of tarsal, wing, wing chord and head lengths. Each measurement was undertaken twice and repeatability analysis has shown that they are highly repeatable (repeatability of between 0.99 and 1.0; P <0.0001; Lessells and Boag, 1987). After euthanasia, carcasses were skinned within a day and the skins (including heads with wattles) were immediately frozen (within the same freezer unit) until the time of their examination. The actual lean wet breast muscle mass, body mass, liver and spleen

mass were weighed to the nearest 5 g for body mass, 1 g for muscle mass, 0.1 g for liver mass and 0.0001 g for spleen mass.

For further detail of methodologies, please see the relevant chapter.

Measurement of secondary sexual characteristics in males

The characteristics recorded in males were the vertical axis (height) of the wattle (using Vernier callipers, to the nearest 0.1 mm) which has been shown to be highly correlated with the total wattle area (Briganti et al., 1999; Goransson et al., 1990; Ohlsson et al., 2002; Papeschi et al., 2000) but which is not thought to be chosen as a characteristic by females because subordinate/satellite males can erect their wattles at will (Mateos, 1998; Mateos and Carranza, 1995); the actual traced wattle area; the spur length taken by measuring the length of the spur including the tarsus (Goransson et al., 1990; Grahn and von Schantz, 1994) from which the tarsus width was then subtracted (using Vernier callipers, to the nearest 0.1 mm, this technique has been shown to minimise measurement error; Ohlsson et al., 2002); the ear tuft length (using Vernier callipers, to the nearest 0.1 mm, a preferred trait in females and a characteristic used within male-male intrasexual interactions; Mateos and Carranza, 1995); wattle colouration, which has been shown to be affected by food quality (Ohlsson et al., 2003) and therefore probably acts as a condition indicator, and the width of the black points in the wattle, (their presence a preferred trait in females, to the nearest 0.001 nm; Mateos and Carranza, 1995). Please see below for details of measurement techniques for the last two parameters.

Calculation of wattle colouration

The values for wattle colouration were calculated by taking red, green, blue and hue measurements on Jasc® Paint Shop Pro™ (© 1991-2000 Jasc Software Inc., version 7.02) software, of two grey standards and two samples of the wattle from each (digital) photograph. The values of the two wattle samples were highly repeatable, with a repeatability value of between 0.99 and 1.0; P <0.0001; (Lessells and Boag, 1987), and they were consequently averaged. This wattle value (for red, green, blue and hue separately) was then adjusted to control for differences in photographic conditions, (by subtracting the mean value, from all the photographs together, of the two grey standards). The corrected wattle values were worked out separately for the two grey standards and the results were highly repeatable and therefore reliable; the values were consequently averaged (repeatability value of between 0.99 and 1.0; P <0.0001; Lessells and Boag, 1987). Within the analysis, the score for hue and results of a principle component analysis of the red, green and blue values, (which allowed the examination of the relative amount of red controlling for the knock-on effect of one colour upon another; PCA), were analysed. Hue was defined as 'an attribute of coloured light...depending on its wave length' (Isaacs et al., 1984). Red has the highest wavelength (740-620 nm) and therefore a higher score for hue was interpreted as a greater display of redness.

Calculation of the width of the black points in the wattle

The width of the black points in the wattle was gauged from digital photographs that included a ruler as a measurement control, using ArcView GIS 3.3 (ESRI) software.

Measurements of six point widths were calculated (in mm), and these were then averaged (repeatability analysis was not undertaken as each was independent from the other).

Measurement of secondary sexual characteristics in females

The characteristics recorded in females were the vertical axis of the wattle (using Vernier callipers, to the nearest 0.1 mm); the actual traced wattle area and the maximum head crest height (using Vernier callipers, to the nearest 0.1 mm).

Measurement repeatability

Two measures were made of all the characteristics, for both the left and right side except head crest height, wattle colouration and the width of the black points in the wattle (only the right side was digitally photographed). They were highly repeatable, with repeatability values of between 0.99 and 1.0 (P <0.0001) for vertical wattle height, male spur length including tarsus, male tarsus width, male ear tuft length and female crest height (Lessells and Boag, 1987). High repeatability values were also found in comparison of the left and right side characteristics (vertical wattle height, between 0.99 and 1.0; wattle area, 0.97; male spur length including tarsus, 0.99; male ear tuft length, 0.98; all P <0.0001; Lessells and Boag, 1987) and values were therefore averaged for both sides.

Statistical analyses

Marked sexual dimorphism in the kind and relative size of secondary sexual characteristics within pheasants meant that the data for males and females needed to be examined separately.

Probability distribution plots were used to elucidate the error distributions of the secondary sexual characteristics prior to undertaking correlations, using the Anderson-Darling test in Minitab, version 13.1 (© 2000, Minitab Inc.). The width of the black points in the wattle, wattle colouration (PCA and hue), wattle height, wattle area, spur lengths and head crest height did not differ significantly from a normal error distribution and analyses were consequently undertaken using the Pearson correlation method, and linear modelling (LM) with normal error distributions. The distribution of the ear tuft length data was bimodal, possibly as result of differences in the selective breeding for display traits of the pheasants used within the study. As a result, correlation of this characteristic with the other male characteristics (width of the black points in the wattle, wattle colouration (PCA and hue), wattle height and spur length) was undertaken using the Kendall correlation method.

To assess whether immune functioning and parasitism were correlated with secondary sexual characteristics, LM analyses were employed rather than Pearson or Kendall correlations because they allowed the inclusion of a body size score (described in General Methodology, Chapter 1), host age (which was in effect time of study, and therefore season) and the parasite challenge or exposure regime (dictated by the study) as control factors within analyses. Body size was controlled for in analyses because in earlier work on pheasants, some characters have and others have not been associated with sexual selection and preferential mate choice or parasitism, and this may be a result of differences in character dimensions resulting from selective breeding for display traits within this species. Moore and Wilson suggested the 'factors associated with relative

body mass, growth rate, or size-dependent resource allocation may be important'.... in sex biased parasitism (Moore and Wilson, 2002). The body size score was preferentially selected above the size of the characteristics prior to challenge as some individuals were immature. Within models, host age was included as a continuous variable rather than as a factor as this allowed both age and parasite challenge/exposure regime to be included together within models, thereby controlling for each other, (the degrees of freedom available within analyses were insufficient to include both as factors). If both remained in the minimal model, then the model was re-run with age included as a factor (and without parasite challenge/exposure regime). The comparative power of the two models was then examined and the model that was the best explanation of the dependent variable was used. The results presented for host age and parasite challenge/exposure regime interactions are amalgamations of the results of the two models. Within the analyses examining the factors affecting wattle colouration (PCA and hue) and the width of the black points in the wattle, wattle height was included as a control factor because the physical size of the wattle may act as a dilutant affecting its colour and perhaps also affecting the width of the points. These terms were discarded during model simplification if non-significant. The male ear length data contained one unusually large outlier that was excluded from analysis, as it was 2.9 standard deviations from the mean.

Of the control factors included in analyses, the term was only presented if significant. Minimal models were arrived at using stepwise deletion. Predicted fits were used to display results controlling for the other terms remaining in the models. The F statistics

and deviance values presented are from the minimal models for significant terms, or the minimal model with the non-significant term added on to the model for terms dropped from the maximal model.

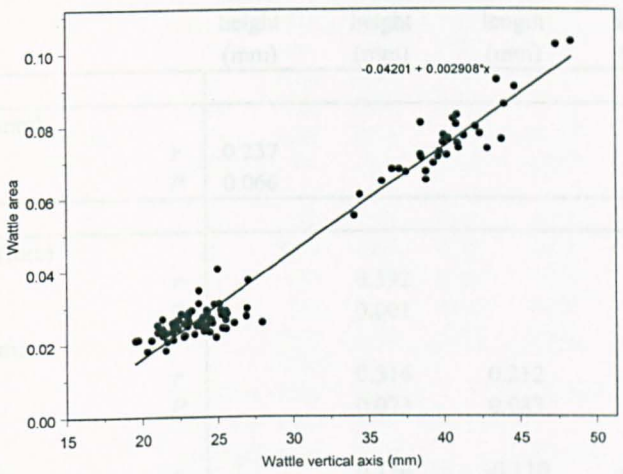
To compare differences between factor levels Tukey's honestly significant difference (HSD) was used. To control for type II statistical errors resulting from a large number of factors being considered in analyses, which meant some factors could falsely show significance due to chance, the method of Benjamin and Hochberg (1995) was used. This technique was chosen over the Bonferroni correction method as it is less conservative (Cotter et al., 2004; Benjamini and Hochberg, 1995).

Results

Relationship between the vertical axis of the wattle and wattle area

Within research on pheasants the vertical axis (height) of the wattle has been found to be highly correlated with, and therefore representative of, the area of the wattle (Briganti et al., 1999; Goransson et al., 1990; Ohlsson, et al., 2002; Papeshi, et al., 2000). This relationship was clarified using the data from this study as the measurement technique for wattle height was felt to be more precise than that of wattle area. The two characteristics were highly correlated (Figure 1; Pearson's $r = 0.984$, $P < 0.001$, $n = 95$), and as a result wattle height was used within analyses.

Figure 1 Correlation between the wattle vertical axis and wattle area (n = 95).



Correlations between male secondary sexual characteristics

Correlations of male secondary sexual characteristics showed a significant positive relationship between wattle height and ear tuft length (Pearson $r = 0.392$, $P = 0.001$) and non-significant trends in relationships between wattle height and spur length (Pearson $r = 0.316$, $P = 0.073$), and ear tuft length and spur length (Kendall $r = 0.212$, $P = 0.083$ (Table 1).

Correlations between female secondary sexual characteristics

Correlations of female secondary sexual characteristics showed a non-significant trend in a relationship between wattle height and head crest height (Pearson $r = 0.237$, $P = 0.066$) (Table 1).

Table 1 Correlations between secondary sexual characteristics

		Head crest height (mm)	Wattle height (mm)	Ear tuft length (mm)	Spur length (mm)	PCA	Hue
Females							
Wattle height (mm)							
	r	0.237					
	P	0.066					
Males							
Ear tuft length (mm)							
	r		0.392				
	P		0.001				
Spur length (mm)							
	r		0.316	0.212			
	P		0.073	0.083			
PCA							
	r		0.134	-0.110	-0.035		
	P		0.457	0.369	0.847		
Hue							
	r		-0.273	-0.169	-0.279	-0.226	
	P		0.124	0.168	0.116	0.205	
Width of the black spots (mm)							
	r		0.087	-0.027	-0.171	0.118	-0.023
	P		0.632	0.828	0.340	0.514	0.901

Secondary sexual characteristics, immune response and condition within males

Male wattle height

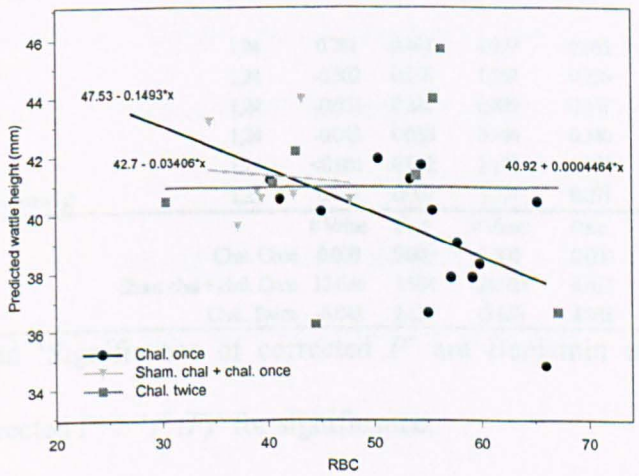
Male wattle height was significantly positively related to PCV ($F_{1,20} = 15.122$, $P < 0.001$) and actual lean wet breast muscle mass ($F_{1,20} = 8.981$, $P = 0.007$). It was also non-significantly related to parasite challenge/exposure regime interacting with RBC (Figure 2; $F_{1,22} = 4.131$, $P = 0.031$). It was not related to the main effects of parasite challenge/exposure regime, RBC, host age, liver mass, spleen mass, host body weight or actual lean wet breast muscle mass (Table 2).

Table 2 Linear model of male wattle height in relation to immune response and condition.

Response=Wattle height (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Challenge/exposure regime	2,23	#	#	0.228	0.798	0.050	ns
PCV	1,23	32.370	12.869	7.945	<0.010**	0.004	**
RBC	1,23	-0.139	0.081	0.428	0.520	0.025	ns
Actual lean wet breast muscle mass (g)	1,23	0.046	0.015	8.625	0.007**	0.008	**
Challenge/exposure regime x RBC	2,23	¤	¤	4.435	0.023*	0.013	ns
Terms dropped							
Host age	1,22	0.230	1.063	0.129	0.723	0.038	ns
Host body size	1,22	0.166	0.699	0.386	0.541	0.029	ns
Liver mass (g)	1,22	-0.026	0.074	0.318	0.578	0.033	ns
Spleen mass (g)	1,22	-1.643	1.213	1.967	0.175	0.017	ns
Host body weight (g)	1,22	0.004	0.006	1.891	0.183	0.021	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	Chal. Once	0.000	0.000		Chal. Once	0.000	0.000
	Sham. chal + chal. Once	6.967	4.860		Sham. chal + chal. Once	-0.184	0.115
	Chal. Twice	-6.185	2.063		Chal. Twice	0.134	0.046

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 2 Variation in male wattle height with RBC interacting with challenge/exposure regime (n = 29). The predicted values control for the effects of PCV and the actual lean wet breast muscle mass (Table 2).



Male spur length

After Benjamin and Hochberg (1995) corrections were undertaken male spur length was significantly related to parasite challenge/exposure regime ($F_{2,25} = 21.098$, $P < 0.001$) and to an interaction between challenge/exposure regime and PCV (Figure 3; $F_{2,25} = 7.726$, $P = 0.002$), with positive relationships in birds challenged once and challenge twice, but larger spur length in the birds challenged twice, and a negative relationship in birds sham-challenged and then challenged once. The spur length of these birds was similar to that of the twice-challenged birds. Spur length was also non-significantly negatively related in a trend to spleen mass ($F_{1,25} = 6.642$, $P = 0.016$) and positively related in a trend to host age ($F_{1,24} = 4.077$, $P = 0.055$). It was not related to the main effects of PCV, host body size, RBC, liver mass, host body weight or actual lean wet breast muscle mass (Table 3).

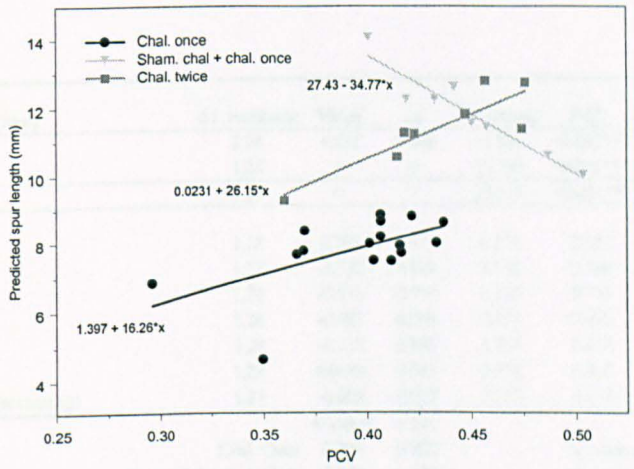
Table 3 Linear model of male spur length in relation to immune response and condition.

Response-Spur length (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Challenge/exposure regime	2,25	#	#	21.098	<0.001***	0.004	***
PCV	1,25	-2.043	6.868	0.307	0.585	0.050	ns
Spleen mass (g)	1,25	-1.930	0.645	6.642	0.016*	0.021	ns
Challenge/exposure regime x PCV	2,25	¤	¤	7.726	0.002**	0.008	**
Terms dropped							
Host age	1,24	0.781	0.467	4.077	0.055	0.013	ns
Host body size	1,24	-0.502	0.338	1.352	0.256	0.025	ns
RBC	1,24	-0.002	0.026	0.405	0.531	0.046	ns
Liver mass (g)	1,24	-0.043	0.038	0.946	0.340	0.042	ns
Host body weight (g)	1,24	<0.001	0.002	1.175	0.289	0.038	ns
Actual lean wet breast muscle mass (g)	1,23	0.003	0.007	1.271	0.271	0.029	ns
		# Value	# s.e.	¤ Value	¤ s.e.		
	Chal. Once	0.000	0.000	0.000	0.000		
	Sham chal + chal. Once	12-646	3.504	-24.923	8.053		
	Chal. Twice	-6.048	2.137	15.053	4.938		

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 3 Variation in male spur length with PCV interacting with challenge/ exposure regime (n = 32).

The predicted values control for the effects of spleen mass (Table 3).



Male ear tuft length

After Benjamin and Hochberg (1995) corrections were undertaken male ear tuft length was related to host age ($F_{2,29}$ = 28.616, $P < 0.001$) and to parasite challenge/exposure regime ($F_{2,28}$ = 17.385, $P < 0.001$) when age was included as a covariate within the analysis ($F_{1,28}$ = 55.896, $P < 0.001$). The two terms could not both be included in the same analysis of variance as factors, due to insufficient degrees of freedom. As a result HSD was undertaken on both factors and the results were combined and are presented in Figure 4. The statistics for non-significant model parameters were obtained by re-adding them into the model including parasite challenge/exposure regime, (as with the inclusion of this factor the parameters were more related to ear tuft length than they were with host

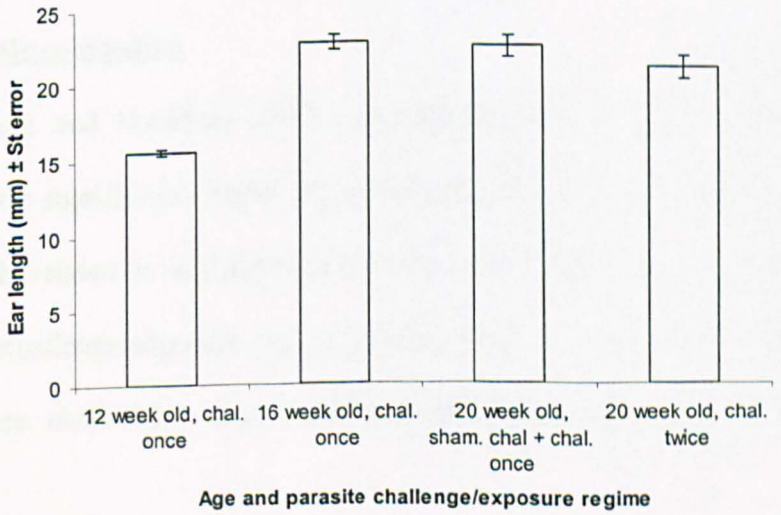
age). Ear tuft length was not related to host body size, PCV, RBC, liver mass, spleen mass, host body weight or actual lean wet breast muscle (Table 4).

Table 4 Linear model of male ear tuft length in relation to immune response and condition.

Response~Ear tuft length (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Host age (as a covariate)	2,28	4.832	0.646	55.896	<0.001***	0.014	***
Challenge/exposure regime	1,28	#	#	17.385	<0.001***	0.021	***
Host age (as a factor)	2,29	¤	¤	28.616	<0.001***	0.007	***
Terms dropped							
Host body size	1,28	0.209	0.570	0.570	0.457	0.025	ns
PCV	1,27	-3.732	9.899	0.142	0.709	0.038	ns
RBC	1,27	-0.013	0.034	0.135	0.716	0.042	ns
Liver mass (g)	1,28	-0.021	0.050	0.178	0.676	0.033	ns
Spleen mass (g)	1,28	-1.128	0.895	1.587	0.218	0.017	ns
Host body weight (g)	1,28	<-0.001	0.003	0.011	0.916	0.050	ns
Actual lean wet breast muscle mass (g)	1,27	-0.009	0.013	0.517	0.478	0.021	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	Chal. Once	0.000	0.000		12 weeks	0.000	0.000
	Sham. chal + chal. Once	-3.549	0.320		16 weeks	3.624	0.495
	Chal. Twice	-1.636	0.646		20 weeks	0.870	0.225

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 4 Variation in male ear tuft length with host age interacting with parasite challenge/exposure regime (n = 32).



Male PCA wattle colouration

Male PCA wattle colouration was not related to host age, host body size, parasite challenge/exposure regime, PCV, RBC, liver mass, spleen mass, host body weight, actual lean wet breast muscle mass or wattle height (Table 5).

Table 5 Linear model of male PCA wattle colouration in relation to immune response and condition.

Response~Wattle colouration (PCA)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*
Terms dropped						
Host age	1,31	-0.031	0.258	0.015	0.904	0.000
Host body size	1,31	-0.123	0.186	0.440	0.512	0.000
Challenge/exposure regime	2,30	#	#	0.006	0.994	0.000
PCV	1,30	-1.641	6.711	0.060	0.808	0.000
RBC	1,30	-0.030	0.027	1.216	0.279	0.000
Liver mass (g)	1,31	0.036	0.029	1.522	0.227	0.000
Spleen mass (g)	1,31	0.290	0.165	0.165	0.687	0.000
Host body weight (g)	1,31	<-0.001	0.002	0.041	0.841	0.000
Actual lean wet breast muscle mass (g)	1,30	<-0.001	0.009	0.041	0.953	0.000
Wattle height (mm)	1,31	0.067	0.089	0.567	0.457	
		# Value	# s.e.			
	Chal. Once	0.000	0.000			
	Sham. chal + chal. Once	0.035	0.353			
	Chal. Twice	0.006	0.227			

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Male hue wattle colouration

After Benjamin and Hochberg (1995) corrections were undertaken male hue wattle colouration was significantly negatively related to host age ($F_{1,29} = 18.165$, $P < 0.001$). It was negatively related in non-significant trends to liver mass ($F_{1,29} = 6.892$, $P = 0.014$) and parasite challenge/exposure regime (Figure 5; $F_{1,29} = 5.254$, $P = 0.029$). The 16-week-old once challenged, sham challenged/once challenged and twice challenged

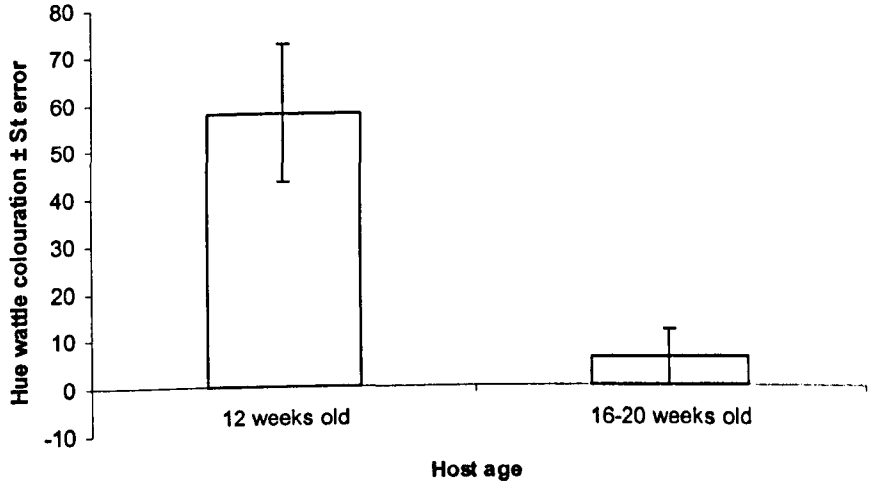
categories were not significantly different from each other and they were consequently combined. As a combined category they were representative of host age, with brighter hue wattle colouration in the 12 than 16 or 20 week old hosts. Hue wattle colouration was also non-significantly negatively related in a trend to PCV ($F_{1,27} = 3.856$, $P = 0.060$), and was not related host body size, RBC, spleen mass, host body weight, actual lean wet breast muscle mass or wattle height (Table 6).

Table 6 Linear model of male hue wattle colouration in relation to immune response and condition.

Response-Wattle colouration (hue)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host age	1,29	-47.801	11.216	18.165	<0.001***	0.004	***
Challenge/exposure regime	1,29	24.446	10.665	5.254	0.029*	0.013	ns
Liver mass (g)	1,29	-1.937	0.738	6.892	0.014*	0.008	ns
Terms dropped							
Host body size	1,28	-4.315	4.274	1.019	0.321	0.042	ns
PCV	1,27	-314.014	159.912	3.856	0.060	0.017	ns
RBC	1,27	0.855	0.666	1.647	0.210	0.025	ns
Spleen mass (g)	1,28	7.536	13.810	0.278	0.590	0.046	ns
Host body weight (g)	1,28	0.052	0.039	1.803	0.190	0.021	ns
Actual lean wet breast muscle mass (g)	1,27	0.213	0.206	1.073	0.310	0.038	ns
Wattle height (mm)	1,28	0.927	1.863	0.248	0.623	0.050	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 5 Variation in male hue wattle colouration with host age (from parasite challenge/exposure regime) (n = 33). The predicted values control the effects of challenge/exposure regime and liver mass (Table 6).



Width of the black points in the wattle within males

After Benjamin and Hochberg (1995) corrections were undertaken the width of the black points in the wattle within males was positively related in a non-significant trend to spleen mass ($F_{1,31} = 4.875$, $P = 0.035$). They were not related to host age, host body size, parasite challenge/exposure regime, PCV, RBC, liver mass, host body weight, actual lean wet breast muscle mass or wattle height (Table 7).

Table 7 Linear model of the width of the black points in the wattle of males in relation to immune response and condition.

Response–Width of the black points in the wattle (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Spleen mass (g)	1,31	0.229	0.104	4.875	0.035*	0.004	ns
Terms dropped							
Host age	1,30	-0.033	0.040	0.697	0.410	0.025	ns
Host body size	1,30	0.008	0.030	0.070	0.793	0.042	ns
Challenge/exposure regime	2,29	#	#	1.048	0.363	0.021	ns
PCV	1,29	-0.397	1.089	0.133	0.718	0.038	ns
RBC	1,29	-0.001	0.004	0.065	0.801	0.042	ns
Liver mass (g)	1,30	0.004	0.005	0.908	0.348	0.017	ns
Host body weight (g)	1,30	<-0.001	<0.001	0.549	0.465	0.029	ns
Actual lean wet breast muscle mass (g)	1,29	<-0.001	0.01	0.046	0.832	0.046	ns
Wattle height (mm)	1,30	0.014	0.013	1.163	0.289	0.013	ns
		# Value	# s.e.				
	Chal. Once	0.000	0.000				
	Sham chal + chal. Once	0.006	0.054				
	Chal. Twice	-0.045	0.033				

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Secondary sexual characteristics, immune response and condition within females

Female wattle height

After Benjamin and Hochberg (1995) corrections were undertaken female wattle height was related in non-significant trends, positively to PCV ($F_{1,38}$ = 4.538, P = 0.040), host body weight ($F_{1,37}$ = 3.025, P = 0.090) and actual lean wet breast muscle mass ($F_{1,37}$ = 6.914, P = 0.012) and negatively to host body size ($F_{1,36}$ = -0.535, P = 0.053). Wattle height was not related to host age, parasite challenge/exposure regime, RBC, liver mass or spleen mass (Table 8).

Table 8 Linear model of female wattle height in relation to immune response and condition.

Response-Wattle height (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
PCV	1,38	17.397	8.167	4.538	0.04*	0.006	ns
Terms dropped							
Host age	2,36	#	#	1.477	0.242	0.039	ns
Host body size	1,36	-0.535	0.267	4.017	0.053	0.017	ns
Challenge/exposure regime	2,36	¤	¤	1.343	0.274	0.044	ns
RBC	1,37	-0.029	0.021	1.951	0.171	0.028	ns
Liver mass (g)	1,37	-0.025	0.071	0.129	0.721	0.050	ns
Spleen mass (g)	1,37	1.400	1.030	1.850	0.182	0.033	ns
Host body weight (g)	1,37	0.002	0.001	3.025	0.090	0.022	ns
Actual lean wet breast muscle mass (g)	1,37	0.015	0.006	6.914	0.012	0.011	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	Chal. Once	0.000	0.000		Chal. Once	0.000	0.000
	Sham chal + chal. Once	0.262	0.315		Sham chal + chal. Once	0.238	0.332
	Chal. Twice	0.249	0.169		Chal. Twice	0.257	0.214

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Female crest height

After Benjamin and Hochberg (1995) corrections were undertaken female crest height was significantly positively related to host body weight ($F_{1,52} = 8.350$, $P < 0.006$) and to parasite challenge/exposure regime (including the main effects; $F_{3,52} = 6.138$, $P = 0.001$) interacting with actual lean wet breast muscle mass (Figure 6; $F_{3,52} = 4.421$, $P< 0.008$). It was not related to the main effects of actual lean wet breast muscle mass, host age, host body size, PCV, RBC, liver mass or spleen mass (Table 9).

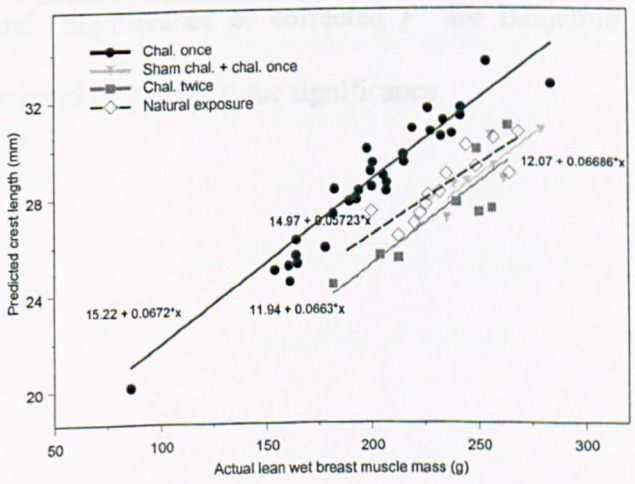
Table 9 Linear model of female crest height in relation to immune response and condition.

Response~Crest height (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Challenge/exposure regime	3,52	#	#	6.138	0.001**	0.005	**
Host body weight (g)	1,52	0.014	0.005	8.35	<0.006**	0.010	**
Actual lean wet breast muscle mass (g)	1,52	0.070	0.028	1.505	0.225	0.020	ns
Actual lean wet breast muscle mass (g) x challenge/exposure regime	3,52	¤	¤	4.421	<0.008**	0.015	**
Terms dropped							
Host age	1,51	0.319	0.887	0.030	0.863	0.045	ns
Host body size	1,50	-0.491	0.721	0.004	0.953	0.050	ns
PCV	1,32	-1.690	16.950	0.061	0.806	0.040	ns
RBC	1,32	-0.054	0.060	0.321	0.575	0.025	ns
Liver mass (g)	1,51	-0.083	0.120	0.180	0.673	0.030	ns
Spleen mass (g)	1,51	0.175	1.636	0.130	0.720	0.035	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	Chal. Once	0.000	0.000		Chal. Once	0.000	0.000
	Sham. chal + chal. Once	-28.931	10.148		Sham. chal + chal. Once	0.106	0.040
	Chal. Twice	13.310	4.518		Chal. Twice	-0.060	0.019
	Natural exposure	-0.561	3.071		Natural exposure	0.005	0.013

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 6 Variation in female crest height with parasite challenge/exposure regime interacting with actual lean wt breast muscle mass (n = 61).

The predicted values control for the effects of host body weight (Table 9).



Secondary sexual characteristics and parasitism within males

Male wattle height

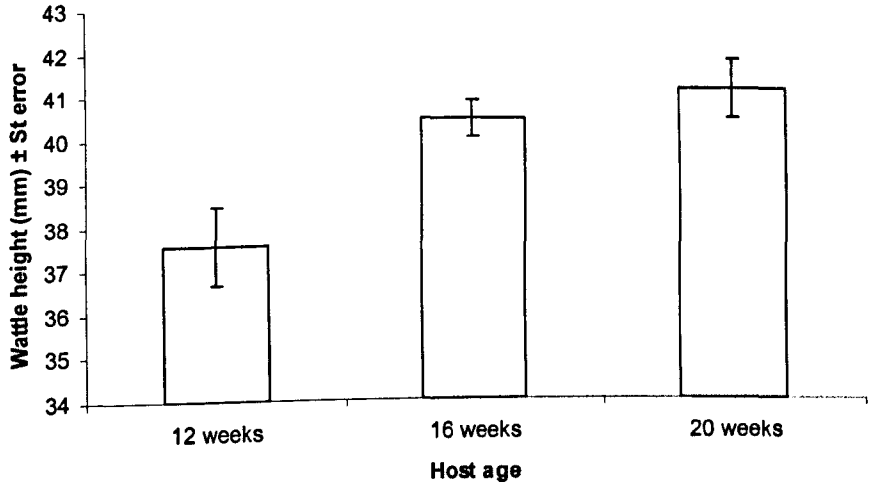
After Benjamin and Hochberg (1995) corrections were undertaken male wattle height was related in a non-significant trend to host age (Figure 7; $F_{2,30} = 4.681$, $P = 0.017$). Within this relationship wattle height was smaller in 12-week-old hosts than those 16 or 20 weeks old. It was not related to parasite intensity, female worm length, the proportion of adults in the parasite population, host body size or parasite challenge/exposure regime (Table 10).

Table 10 Linear model of the male wattle height in relation to parasitism.

Response-Wattle height(mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Host age	2,30	#	#	4.681	0.017*	0.008	ns
Terms dropped						0.000	
$\log_{10}$(parasite intensity + 1)	11,29	1.529	1.543	0.982	0.330	0.025	ns
$\log_{10}$(female worm length)	1,24	-7.871	11.528	0.466	0.501	0.050	ns
Proportion of adults	1,29	0.233	0.304	0.589	0.449	0.033	ns
Host body size	1,29	0.316	0.434	0.529	0.473	0.042	ns
Challenge/exposure regime	2,29	¤	¤	1.533	0.233	0.017	ns
		# Value	# s.e.	¤ Value	¤ s.e.		
	12 weeks	0.000	0.000	0.000	0.000		
	16 weeks	2.005	0.733	-1.070	1.145		
	20 weeks	0.657	0.341	-0.710	0.516		

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 7 Variation in male wattle height with host age (n = 33).



Male spur length

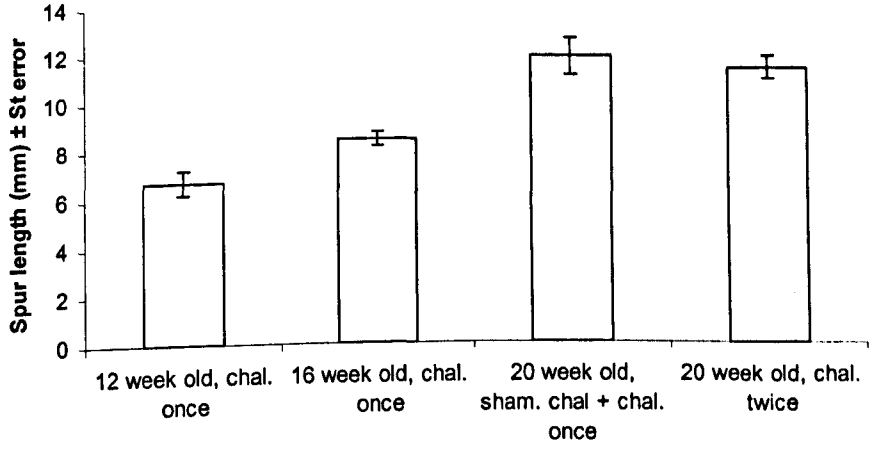
After Benjamin and Hochberg (1995) corrections were undertaken male spur length was significantly related to host age ($F_{2,30}$ = 31.347, P < 0.001) and to parasite challenge/exposure regime ($F_{2,30}$ = 24.730, P < 0.001). The two terms could not both be included within the same analysis of variance as factors, due to insufficient degrees of freedom. As a result, HSD was undertaken on both factors and the results were combined and are presented in Figure 8. The statistics for non-significant model parameters were obtained by re-adding them into the model including parasite challenge/exposure regime, as with the inclusion of this factor rather than host age the parameters were more related to spur length. Using either factor however, spur length was not related to parasite intensity, female worm length, the proportion of adults in the parasite population or host body size (Table 11).

Table 11 Linear model of the male spur length in relation to parasitism.

Response-Spur length (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Host age	2,30	#	#	31.340	<0.001***	0.008	***
Challenge/exposure regime	2,30	¤	¤	24.730	<0.001***	0.017	***
Terms dropped							
$\log_{10}$(parasite intensity + 1)	1,29	1.192	0.826	4.825	0.160	0.025	ns
$\log_{10}$(female worm length)	1,24	2.819	6.300	0.200	0.658	0.050	ns
Proportion of adults	1,29	0.151	0.151	0.995	0.327	0.033	ns
Host body size	1,29	-0.199	0.375	0.282	0.600	0.042	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	12 weeks	0.000	0.000		Chal. Once	0.000	0.000
	16 weeks	0.047	0.364		Sham. chal + chal. Once	2.001	0.332
	20 weeks	1.344	0.170		Chal. Twice	0.515	0.214

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 8 Variation in male spur length with host age interacting with parasite challenge/exposure regime (n = 33).



Male ear tuft length

After Benjamin and Hochberg (1995) corrections were undertaken male ear tuft length was related to host age ($F_{2,29}$ = 28.616, P < 0.001) and to parasite challenge/exposure

regime ($F_{2,28}$ = 17.385, $P < 0.001$) as with the analysis examining immune response and condition (Table 12 and Figure 4).

Table 12 Linear model of the male ear tuft length in relation to parasitism.

Response=Ear length (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host age (as a covariate)	2,28	4.832	0.646	55.896	<0.001***	0.014	***
Challenge/exposure regime	1,28	#	#	17.385	<0.001***	0.021	***
Host age (as a factor)	2,29	¤	¤	28.616	<0.001***	0.007	***
Terms dropped							
$\log_{10}$(parasite intensity + 1)	1,27	2.089	0.950	4.841	0.037	0.029	ns
$\log_{10}$(female worm length)	1,22	6.815	6.915	0.971	0.335	0.036	ns
Proportion of adults	1,27	0.042	0.209	0.040	0.843	0.050	ns
Host body size	1,27	-0.282	0.436	0.420	0.523	0.043	ns
		# Value	# s.e.			¤ Value	¤ s.e.
	Chal. Once	0.000	0.000		12 weeks	0.000	0.000
	Sham chal + chal. Once	-3.549	0.320		16 weeks	3.624	0.495
	Chal. Twice	-1.636	0.646		20 weeks	0.870	0.225

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Male PCA wattle colouration

Male PCA wattle colouration was not related to host age, parasite challenge/exposure regime, parasite intensity, female worm length, the proportion of adults in the parasite population or host body size (Table 13).

Table 13 Linear model of male PCA wattle colouration in relation to parasitism.

Response~Wattle colouration (PCA)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*
Terms dropped					
Host age	1,31	-0.031	0.258	0.015	0.904
Host body size	1,31	-0.123	0.186	0.440	0.512
Challenge/exposure regime	2,30	#	#	0.006	0.994
$\log_{10}$(parasite intensity + 1)	1,31	-0.665	0.495	1.804	0.189
$\log_{10}$(female worm length)	1,26	-4.673	5.587	0.700	0.410
Proportion of adults	1,31	-0.068	0.145	0.217	0.645
		# Value	# s.e.		
	Chal. Once	0.000	0.000		
	Sham. chal + chal. Once	0.035	0.353		
	Chal. Twice	0.006	0.227		

Male hue wattle colouration

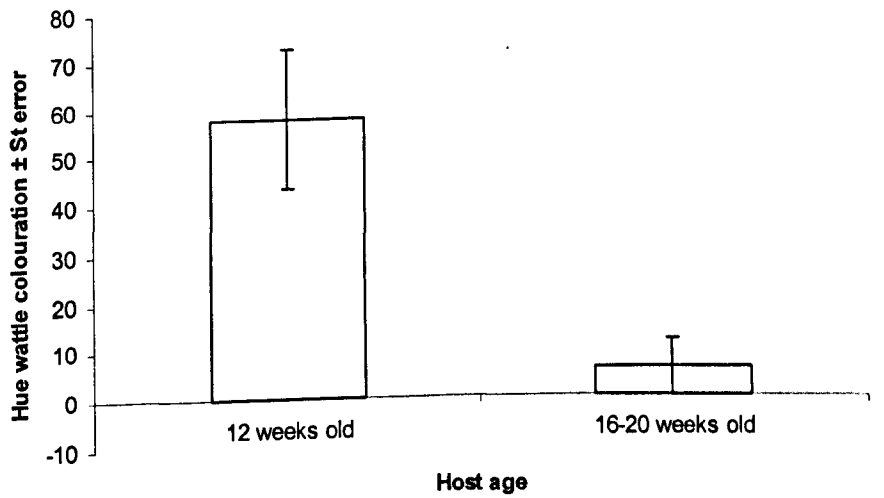
After Benjamin and Hochberg (1995) corrections were undertaken male hue wattle colouration was significantly related to host age (Figure 9; $F_{2,39} = 6.903$, $P = 0.003$) with 12 week old hosts having brighter wattles than those 16 or 20 weeks old. It was not related to parasite challenge/exposure regime, parasite intensity, female worm length, the proportion of adults in the parasite population or host body size (Table 14).

Table 14 Linear model of male hue wattle colouration in relation to parasitism.

Response-Wattle colouration (hue)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Host age	2,30	#	#	6.903	0.003**	0.008	**
Terms dropped						0.000	
Host body size	1,29	-3.317	4.698	0.498	0.486	0.042	ns
Challenge/exposure regime	2,29	¤	¤	2.320	0.116	0.017	ns
$\log_{10}$(parasite intensity + 1)	1,29	23.485	16.392	2.053	0.163	0.033	ns
$\log_{10}$(female worm length)	1,24	-60.045	117.859	0.260	0.615	0.050	ns
Proportion of adults	1,29	-4.712	3.199	2.169	0.152	0.025	ns
		# Value	# s.e.			¤	¤
	12 weeks	0.000	0.000		Chal. Once	0.000	0.000
	16 weeks	-24.636	7.926		Sham chal + chal. Once	16.005	12.114
	20 weeks	-9.800	3.709		Chal. Twice	11.481	5.447

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 9 Variation in male hue wattle colouration with host age (n = 33).



Width of the black points in the wattle within males

After Benjamin and Hochberg (1995) corrections were undertaken the width of the black points in the wattle within males was significantly related to parasite challenge/exposure

regime interacting with parasite intensity (Figure 10; $F_{2,25} = 13.494$, $P < 0.001$), with a negative relationship in hosts challenged once or twice, and a positive relationship in hosts sham challenged and then challenged once. The black points in the wattle were much wider and parasite intensity was much higher in hosts challenged once than in those challenged twice, and parasite intensity remained much higher with a decrease in the width of the black points. The width of the black points was also related in a non-significant trend to parasite intensity interacting with host body size ($F_{1,25} = 5.007$, $P = 0.034$), and was not related to the main effects of host body size, parasite challenge/exposure regime, parasite intensity, female worm length or the proportion of adults in the parasite population (Table 15).

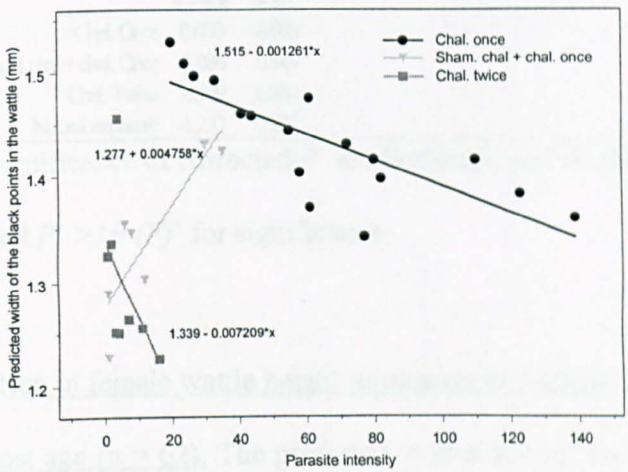
Table 15 Linear model of the width of the black points in the wattle within males in relation to parasitism.

Response-Width of the black points in the wattle (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Host body size	1,25	0.288	0.139	0.031	0.861	0.031	ns
Challenge/exposure regime	2,25	#	#	0.602	0.555	0.050	ns
$\log_{10}$(parasite intensity + 1)	1,25	-0.230	0.124	0.124	0.978	0.006	ns
Challenge/exposure regime x $\log_{10}$(parasite intensity + 1)	2,25	¤	¤	13.494	<0.001***	0.013	***
$\log_{10}$(parasite intensity + 1) x host body size	1,25	-0.215	0.096	5.007	0.034*	0.000	ns
Terms dropped							
Host age	1,24	0.057	0.068	0.767	0.390	0.019	ns
log10(female worm length)	1,19	0.282	0.700	2.592	0.124	0.038	ns
Proportion of adults	1,24	0.002	0.018	0.196	0.662	0.000	ns

	# Value	# s.e.		¤ Value	¤ s.e.
Chal. Once	0.000	0.000	Chal. Once	0.000	0.000
Sham chal + chal. Once	-1.345	0.273	Sham chal + chal. Once	0.884	0.087
Chal. Twice	-0.185	0.093	Chal. Twice	0.093	0.006

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 10 Variation in the width of the black points in the wattle within males in relation to parasite challenge/exposure regime interacting with parasite intensity (n = 33). The predicted values control for the effects of parasite intensity interacting with host body size.



Secondary sexual characteristics and parasitism within females

Female wattle height

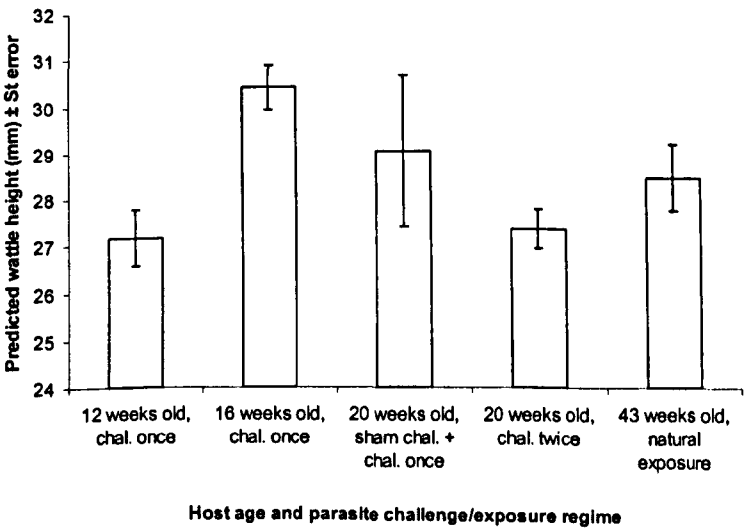
After Benjamin and Hochberg (1995) corrections were undertaken female wattle height was significantly related to parasite challenge regime (Figure 11; $F_{3,56} = 9.978$, $P < 0.001$). It was also non-significantly related in a positive trend to host age ($F_{1,56} = 5.912$, $P = 0.018$) and in a negative trend to parasite intensity ($F_{1,56} = 5.080$, $P = 0.028$), and was not related to host body size, female worm length and the proportion of adults in the parasite population (Table 16).

Table 16 Linear model of female wattle height in relation to parasitism.

Response-Wattle height (mm)	d.f., residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host age	1,56	0.824	0.339	5.912	0.018*	0.017	ns
Challenge/exposure regime	3,56	#	#	9.978	<0.001***	0.008	***
log10(parasite intensity + 1)	1,56	-1.042	0.462	5.08	0.028*	0.025	ns
Terms dropped							
log10(female worm length)	1,54	-0.097	0.268	0.131	0.719	0.033	ns
Proportion of adults	1,45	-1.007	5.539	0.033	0.857	0.050	ns
Host body size	1,54	-0.004	0.011	0.106	0.746	0.042	ns
		# Value	# s.e.				
	Chal. Once	0.000	0.000				
	Sham chal + chal. Once	-1.034	0.519				
	Chal. Twice	-0.299	0.264				
	Natural exposure	-1.233	0.251				

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 11 Variation in female wattle height in relation to parasite challenge/exposure regime interacting host age (n = 62). The predicted values control for the effects of host age and parasite intensity (Table 16).



Female crest height

Female crest height and was not related to host age, host body size, parasite challenge/exposure regime, parasite intensity, female worm length or the proportion of adults in the parasite population (Table 17).

Table 16 Linear model of female crest height in relation to parasitism.

Response~Crest length (mm)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	
Terms dropped						
Host age	3,57	#	#	2.436	0.074	
Host body size	1,58	-0.569	0.545	1.091	0.301	
Challenge/exposure regime	3,57	¤	¤	0.277	0.842	
log10(parasite intensity + 1)	1,59	1.637	0.915	3.202	0.079	
log10(female worm length)	1,49	13.497	12.982	1.081	0.304	
Proportion of adults	1,58	0.028	0.021	1.731	0.193	
	# Value	# s.e.			¤ Value	¤ s.e.
12 weeks old	0.000	0.000		Chal. Once	0.000	0.000
16 weeks old	1.753	0.657		Sham. chal + chal. Once	0.195	0.824
20 weeks old	-0.220	0.390		Chal. Twice	-0.492	0.542
43 weeks old	-0.031	0.294		Natural exposure	0.028	0.329

Discussion

This study aimed to investigate the effects of *H. gallinarum* parasitism, body condition and spleenic response upon secondary sexual characteristics in the pheasant. The ornaments were related to these indicators of quality to see whether their expression was likely to affect the susceptibility of hosts to *H. gallinarum* through investment in signals. The work was undertaken using the data and pheasant skins available from the other studies discussed within this thesis. Within an ideal situation, the use of birds from the same stock, bred for the same display characteristics, of the same age may have led to a more controlled study than this. However, I feel that because the data is an amalgamation of the characteristics of mixed stock, multiple ages and both sexes then any patterns

which emerge and point to either immunocompetence trade-offs due to the creation of characteristics, or honest sexual signalling, will be all the more applicable to general patterns within the pheasant species.

The hypotheses examined within the study were that:

(1) characteristics would to be correlated with each other if they were used in mate sexual selection or male-male intra-sexual interactions,

(2) immune functioning and condition would affect male showiness,

(3) parasitism would also affect male showiness.

Correlations between secondary sexual characteristics

The data demonstrated a significant positive association between male wattle height and ear tuft length, and positive trends between male wattle height and spur length, male ear tuft length and spur length, and female wattle height and head crest height. These results suggest that the first null hypothesis can be rejected, as there were correlations between characteristics likely to be used within sexual selection and intrasexual interactions. Within females, they may also suggest that wattle height and head crest height are used within sexual selection, although as the pheasant is a highly polygamous species (Scribner et al., 1989) with males having large harems, wattle height relations in females could be a residual effect of strong mate selection characteristics in males. Head crest height may indicate condition through increased predator avoidance, as when startled, females raise their crests possibly as a warning of danger to others.

Secondary sexual characteristics, immune response and condition

Positive relationships were predicted between the size and redness of sexual characteristics and PCV, RBC, the lean wet breast muscle mass, liver and spleen mass.

The results of my study showed significant positive relationships within males between wattle height (and therefore size) and PCV, and wattle height and actual lean wet breast muscle mass, and a non-significant trend of wattle size and parasite challenge/exposure regime interacting with RBC (Table 2, Figure 2). Within this trend, wattle height was negatively related to RBC in young individuals (12 and 16 weeks old) who were naive to infection, whereas there seemed no relationship in older individuals (20 weeks old) with prior infection. Within females, wattle height was positively related in a non-significant trend to PCV (Table 8). These relationships suggest wattle size is condition dependent, and that if pheasants are responding to a primary infection of *H. gallinarum* they may trade-off maintenance of wattle size for continuance of general condition. Within earlier work undertaken on pheasants, wattle height has also been positively correlated with condition (although the indicator was body weight rather than muscle mass; Papeschi et al, 2000).

Male spur length was significantly related to parasite challenge/exposure regime and to an interaction of challenge/exposure regime with PCV (Figure 3), and was negatively related in a non-significant trend to spleen mass and related in a positive trend to host age (Table 3). The relationships with challenge/exposure regime and host age are very similar (Figure 4), suggesting that spur length was mainly influenced by host age, something

which has been found before in pheasants (Mateos and Carranza, 1996; von Schantz et al., 1989) but also suggesting condition and parasitism can influence spur length; once pheasants have reached a certain age, upon primary *H. gallinarum* infection (whereby an acquired immune resistance to parasitism has not been established) spur length may be involved in a trade-off with condition. If individuals have already been exposed to the parasite and have therefore perhaps developed an acquired immune resistance, this trade-off does not occur. The influence of condition upon spur length is supported by earlier work undertaken on pheasants when it was positively associated with body weight (Papeschi et al, 2000). The negative trend with spleen mass (Table 3) may also suggest a trade-off overall between spur length and the ability to response to *H. gallinarum* infection with a spleenic reaction.

Male tuft ear length was related to both host age and parasite challenge/exposure regime (Table 4, Figure 4). Within this relationship, ear length was smaller in 12 than 16-week-old hosts, but there was no difference in ear length between hosts 16 and 20 weeks old. This suggests that the development of the ear as a sexual ornament occurs during adolescence. There was no difference in ear length between pheasants that had been challenged once and were 16 or 20 weeks old, but there was a significant difference between those challenged once at 16 weeks old, and those challenged twice at 20 weeks old. Those challenged once had larger ears, which suggests that in adulthood ear tuft length is negatively influenced by parasitism.

Male PCA wattle colouration was not affected by immune response and condition (Table 5), but hue was affected by host age (Table 6, Figure 5) with wattles of a greater hue in adolescent 12-week-old hosts than 16 or 20 weeks old. Hue wattle colouration was also negatively related in trends to liver mass and PCV, suggesting that it's development may be a trade-off with general condition. Such a trade-off is contradictory to the general theory of wattle colouration as a fitness signal, although colouration was not found to be a trait used for mate selection within earlier research undertaken on pheasants (Mateos and Carranza, 1995).

The width of the black points in the wattle within males was positively related to spleen mass (Table 7), however this became non-significant after Benjamin and Hochberg corrections (1995). This may suggest that larger black points indicate an increased immune response.

Female crest height was significantly positively related to host body weight and to actual lean wet breast muscle mass interacting with parasite challenge/exposure regime. Crest height was larger with lower muscle mass in the once challenged treatment group (aged 12 and 16 weeks old), and did not differ significantly between the sham challenged then once challenged (20 weeks old), twice challenged (20 weeks old) and natural exposure (43 weeks old) groups (Table 9, Figure 6). This is possibly as a result of dimorphism in body size, although the body size score was not related to crest height.

These results suggest that the second null hypothesis that immune function and condition would not affect secondary sexual characteristics can be rejected for wattle size, male spur length and hue wattle colouration, can not be rejected for male ear tuft length, PCA wattle colouration and female crest height, and can only negligibly be rejected for the width of the black points in the wattle.

Secondary sexual characteristics and parasitism

Negative relationships were predicted between the brightness of sexual characteristics and parasite intensity, the length (and therefore fecundity) of female worms and the proportion of adults in the parasite population (representing the speed of maturation of juvenile parasites).

Although there was no direct effect of parasitism upon male wattle height and therefore size (Table 10), an effect of parasite challenge/exposure regime was found during examination of the immune response and condition. Within this relationship there seemed to be a non-significant trade-off between maintenance of wattle size and continuance of general condition within young birds (12 and 16 weeks old) in individuals responding to a primary infection of *H. gallinarum* (Table 2, Figure 2). As there seemed little difference within this interaction between the sham challenged then once challenged and twice challenged groups, and both were 20 weeks old, and as there was an effect of age in the model examining parasitism within which wattle size increased with age, these results suggest the main factors influencing an increase in male wattle size are age (rather than parasite challenge/exposure regime) and general condition (PCV and actual lean wet

breast muscle mass). This however is not upheld in the results from examination of female wattle height (Table 16). Within these results, age had a non-significant effect, but when combined with the significant effect of parasite challenge/exposure regime, wattle height increased with age through adolescence (larger wattle size in 16 than 12-week-old birds), and then seemed to be negatively influenced by prior exposure to parasitism (because wattle height in adult birds (43 weeks old) with natural exposure to parasitism was smaller than in 16-week-old birds which had been challenged once with parasites, bearing in mind that these relationships were not influenced by host body size which was controlled for within the analysis; Table 16). Within these results parasite intensity was also negatively related, within a non-significant trend, to wattle height. The suggested greater influence of immune functioning than parasitism upon male wattle height concurs with a recent study examining comb size, *Trichostrongylus tenuis* infection intensity and immune functioning in male red grouse, which concluded that comb size was more likely to be influenced by immune functioning than parasitism (Mougeot, in prep.-a). The differences between the sexes may also occur because male wattle height is more likely to be influenced by trade-offs with condition, as testosterone also affects wattle size (Briganti et al., 1999; Mougeot, in prep.-b; Papeschi et al., 2000) and males with bigger wattles are likely to be of higher phenotypic quality and may therefore be better able to cope with the effects of parasitism (Hamilton and Zuk, 1982; Møller, 1990a; Møller, 1991; Mougeot, in prep.-a), whereas in females these trade-offs are less likely to occur and wattle size may consequently be influenced more by parasitism.

As described within the section examining secondary sexual characteristics, immune response and condition, male spur length (Table 11) and ear tuft length (Table 12) seem mainly influenced by host age, but may also have been influenced by parasitism. Male PCA and hue wattle colouration were not affected by parasitism (Tables 13 and 14, respectively).

Although also not affected directly by parasitism, the width of the black points in the wattle in male pheasants was affected by an interaction between parasite intensity and parasite challenge/exposure regime (Table 15, Figure 10). A negative trend between spleen mass and the black points in the wattle may suggest wider points are a sign of an increased immune response (Table 7), which is also suggested by negative relationships between parasite intensity and the width of the black points in hosts challenged once or challenged twice (Table 15, Figure 10). This relationship however was not upheld within the sham challenged then challenged hosts, perhaps because of differences in host age and trade-offs in immune defence mechanisms where an acquired immune resistance has not already been developed; because during adolescence when a primary infection occurs and immune defence mechanisms are elevated (Merceroltjen and Woodard, 1987) the relationship is positive, whereas it becomes negative in older males exposed to primary infection.

These results suggest that the third null hypothesis that characteristics would not be related to parasitism can be rejected for female wattle size and the width of the black points in the wattle within males as they were negatively correlated with parasitism, and

can only negligibly be rejected for make spur and ear tuft length. They also suggest that the third null hypothesis can not be rejected for male wattle size, PCA and hue wattle colouration and female crest height, as they seemed to be unrelated to parasitism.

Conclusion

The results of this study suggested that the brightness of secondary sexual ornamentation is mediated through host condition and immune response and also as a result of parasitism, but different characteristics can indicate immune resistance and fitness, resistance against parasitism or perhaps both. Male wattle size seemed to be condition dependent (PCV and actual lean wet breast muscle mass), possibly due to a trade-off resulting from the influence of testosterone upon the immune system. In females the size seemed more dependent upon parasitism (parasite intensity). Larger wattles however were associated with increased condition or immune response, and with decreased parasitism, which suggests that wattle size honestly signals fitness and resistance to parasitism. Male spur and ear tuft length were mainly influenced by age, but also seemed to have been affected by, and may therefore indicate, condition in adulthood (PCV) if an acquired immune resistance to parasitism had not been gained. Hue wattle colouration seemed to be negatively influenced by condition (PCV and liver) and may therefore indicate fitness. The width of the black points in the wattle seemed to be influenced by both the immune response (spleen) and parasite intensity, larger points indicating both increased parasitism and the elicitation of a spleenic response. They may therefore honestly signal both fitness and resistance to parasites, just like wattle size.

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Chapter 6

The effects of *Heterakis gallinarum* infection on the condition and spleenic response of the ring-necked pheasant *Phasianus colchicus.*

Introduction

That one organism; the parasite, obtains its food directly from another; the host, implies that the parasite must in some way negatively affect the host. Some parasitic organisms such as wasps kill the host, for instance by laying their eggs inside the hosts' body cavity and creating a fresh food source for their young. Other species, usually endoparasites, may compromise host fitness much less as to do so would also compromise their own survival.

Tompkins investigated the effects of the caecal nematode *Heterakis gallinarum*, which is able to parasitise both the grey partridge and the ring-necked pheasant in the UK, and is implicated in creating apparent parasite-mediated competition between the species. He concluded that the partridge suffered greater morbidity as a result of *H. gallinarum* than the pheasant, although the pheasant had a higher parasite carrying capacity (Draycott *pers. com.*), a higher parasite egg ingestion rate (Tompkins et al., 2000), higher parasite establishment (Tompkins et al., 2000) and higher parasite fecundity (Tompkins et al., 2000), the partridge had greater host mortality (Robertson and Dowell, 1990) and seemed to suffer an increase in mortality as a result of *H. gallinarum* parasitism (Tompkins et al, 1999). The pheasant suffered very little morbidity as a result of this parasitism, other than

a slight reduction in caecal activity (Tompkins et al., 2001) and a reduction in fecundity (M. Woodburn, *pers. com.*). Possible reasons for the greater effects of *H. gallinarum* parasitism upon the morbidity of one host compared to the other are host suitability, which may be affected by host parasite co-evolution (Tompkins *pers. com.*), or differences in host immune defence and parasite virulence.

The work undertaken within this chapter aimed to examine the effects of *H. gallinarum* parasitism upon pheasant morbidity.

Hypothesis one

The first hypothesis was that *H. gallinarum* parasitism has an impact upon pheasant fecundity. The prediction was that variation in the intensity of *H. gallinarum* parasitism between hosts should predict variation in host body condition, PCV and RBC. However, in a previous study on *Trichostrongylus tenuis* parasitism of red grouse there was no association between egg count and body weight (although there was a positive association between egg count and host body condition) or egg count and red blood cell count (RBC), and there was an inverse association between egg count and packed cell volume (PCV) pooled over the course of parasite challenge (Wilson and Wilson, 1978).

Hypothesis two

The liver and spleen are suggested to play a role in the immunological response of birds, although their specific functions are not altogether understood (John, 1994a, 1994b).

Hypothesis two was that host liver size was dependent on host condition in the absence of parasitism. The prediction was therefore that variation in host liver mass should be predicted by variation in the measures of host body condition.

Hypothesis three

Spleen mass has been positively linked to the T-cell mediated immune response (due to the conceivable lymphocyte production; John, 1994a) and negatively linked to parasitism (Brown and Brown, 2002; John, 1994a, Møller et al., 1998; Møller and Erritzoe, 2002; Morand and Poulin, 2000) in colonially living species (which are likely to encounter greater infection and parasitism; Brown and Brown, 2002; Møller, 1998; Møller and Erritzoe, 1998). The third hypothesis was that the spleen was involved in the immune response to parasitism. Therefore I predicted that variation in spleen mass would predict variation in the intensity of *H. gallinarum* infection between hosts.

Materials and methods

52 ring-necked pheasant chicks were hand-reared from 2-days old in sterile conditions to ensure naivety to infection; half were retained until 12 weeks of age and half to 16 weeks. During this period, all individuals were randomly substituted every couple of days into one of two identically sized pens (two pens were necessary for animal husbandry reasons) to ensure that all had been identically treated. Birds were given water, supplied with chick crumbs and were then retained on standard maintenance pellets *ad-libitum.* At 12 weeks, half were randomly selected, and caecal droppings were collected from their pen to verify the absence of *H. gallinarum* prior to challenge. The selected group of birds were then

weighed (to the nearest 25 g) and had their tarsus and wing chord lengths measured and pectoral muscle profiles traced, and were then blood sampled to examine general condition (PCV and RBC).

Immediately after blood sampling, all birds were orally challenged using a 2ml single dose suspension of approximately 100 *H. gallinarum* eggs. Birds were then randomly split into one of two groups (for animal husbandry reasons), moved into exterior enclosures and maintained over a 30-day period. Caecal droppings were collected and examined for eggs to ascertain the success of the challenge.

After this period all birds were again weighed and blood sampled and were then euthanased to quantify female worm length, the intensity of parasite infection and the ratio of juvenile to adult worms. Within the same procedure as the blood sampling, pectoral muscle profiles were once again traced, the actual lean wet breast muscle mass, liver mass and spleen mass were weighed. At 16 weeks the remaining 26 poults were treated identically to the first group. For full methodologies please see Chapter 1 (General Methodology).

Statistical analyses

All analyses were undertaken using linear modelling (LM) in S-PLUS (Version 6 for Windows™ Professional Release 2, Mathsoft Engineering and Education, Cambridge, Massachusetts, USA, © 1988-2001 Insightful Corp.) program, unless otherwise stated.

When examining the effects of parasitism upon PCV, RBC and muscle mass subsequent to parasite challenge, their status before challenge was included to control for condition. In all analyses host sex and age, pen number and a body size score created using principal component analysis (Chapter 1, General Methodology) were included as control factors, being discarded if non-significant. Minimal models were arrived at using stepwise deletion. Predicted fits were used to display results controlling for the other terms remaining in the models. The F statistics presented are from the minimal models for significant terms, or the minimal model with the non-significant term added on to the model for terms dropped from the maximal model.

To compare differences between factor levels Tukey's honestly significant difference (HSD) was used. To control for type II statistical errors resulting from a large number of factors being considered in analyses, which meant some factors could falsely show significance due to chance, the method of Benjamin and Hochberg (1995) was used. This technique was chosen over the Bonferroni correction method as it is less conservative (Cotter et al., 2004; Benjamini and Hochberg, 1995).

Liver and spleen weight data both contained one unusually large outlier (from differing birds in each case) that was excluded from analyses, as they were 5.4 and 4.6 standard deviations from the mean, respectively.

Results

Accuracy of the modelled muscle mass index

The precision of the technique for measuring body condition on live birds was clarified in chapter 2 (Condition, the T-cell mediated immune response and parasitism). The modelled muscle mass index was a reliable indicator of actual lean wet breast muscle mass and therefore of muscle mass at the time of measurement.

During the 30-day maintenance period one individual was euthanased due to husbandry factors unrelated to parasite infection.

Parasites and host condition

Actual lean wet breast muscle mass

After Benjamin and Hochberg (1995) corrections were undertaken, actual lean wet breast muscle mass subsequent to parasite challenge was significantly negatively related to host body weight ($F_{1,31} = 9.316$, $P < 0.005$) and RBC ($F_{1,31} = 7.318$, $P = 0.011$), and positively related to the modelled muscle mass index before parasite challenge ($F_{1,31} = 9.560$, $P = 0.004$). It was related to host age with higher muscle mass in 16 than 12 weeks old hosts ($F_{1,31} = 9.147$, $P < 0.005$), and was related to interactions between female worm length and host sex (Figure 1; $F_{1,31} = 9.486$, $P = 0.004$) and host body weight and host age (Figure 2; $F_{1,31} = 6.272$, $P = 0.018$). It was also related in a non-significant trend to pen number (Figure 3; $F_{1,31} = 5.077$, $P = 0.031$) with higher muscle mass in the birds from

pen 1 than pen 2, and was not related to parasite intensity, the proportion of adults in the parasite population, PCV before parasite challenge and host body size (Table 1).

Table 1 Linear model of actual lean wet breast muscle mass after parasite challenge.

Response~Actual lean wet breast muscle mass (g)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
$\log_{10}$(female worm length)	1,31	91.404	73.803	1.030	0.318	0.031	ns
RBC before parasite challenge	1,31	-0.214	0.112	7.318	0.011*	0.019	*
Modelled muscle mass index	1,31	283.349	225.677	9.560	0.004**	0.004	**
Host sex	1,31	220.855	71.430	0.708	0.407	0.035	ns
Host age	1,31	11.325	16.162	9.147	<0.005**	0.015	**
Pen number	1,31	-8.483	2.898	5.077	0.031*	0.027	ns
Host body weight (g)	1,31	-6.586	5.429	9.316	<0.005**	0.012	**
Host sex x $\log_{10}$(female worm length)	1,31	-216.688	70.355	9.486	0.004**	0.008	**
Host body weight (g) x host age	1,31	-0.042	0.017	6.272	0.018*	0.023	*
Terms dropped							
$\log_{10}$(parasite intensity + 1)	1,30	-3.958	12.349	0.552	0.463	0.042	ns
Proportion of adults	1,30	0.065	0.761	0.323	0.574	0.046	ns
PCV before parasite challenge	1,30	-9.937	61.916	0.053	0.819	0.050	ns
Host body size	1,29	7.436	5.750	0.654	0.425	0.038	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 1 Variation in actual lean wet breast muscle mass with female worm length interacting with host sex (n = 41). The predicted values control for the effects of RBC, the modelled muscle mass index, pen number, and the main effects and an interaction of host age and host body weight (Table 1).

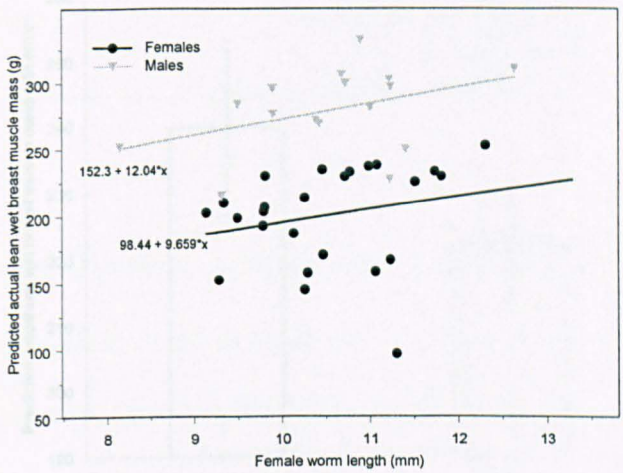


Figure 2 Variation in actual lean wet breast muscle mass with host body weight interacting with host age (n = 41). The predicted values control the effects of RBC, the modelled muscle mass index, pen number, and the main effects and an interaction of female worm length and host sex (Table 1).

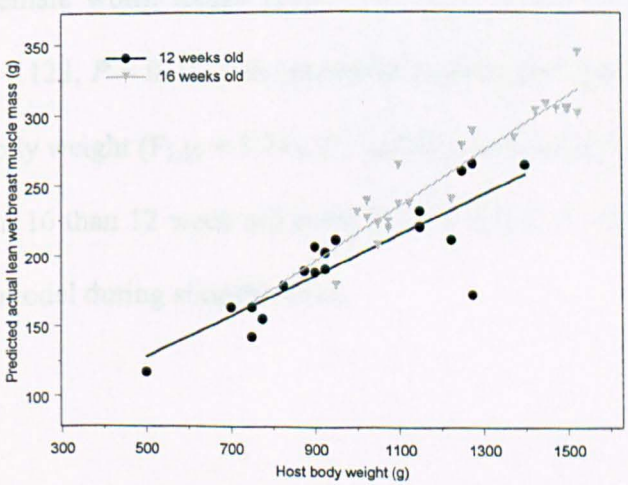
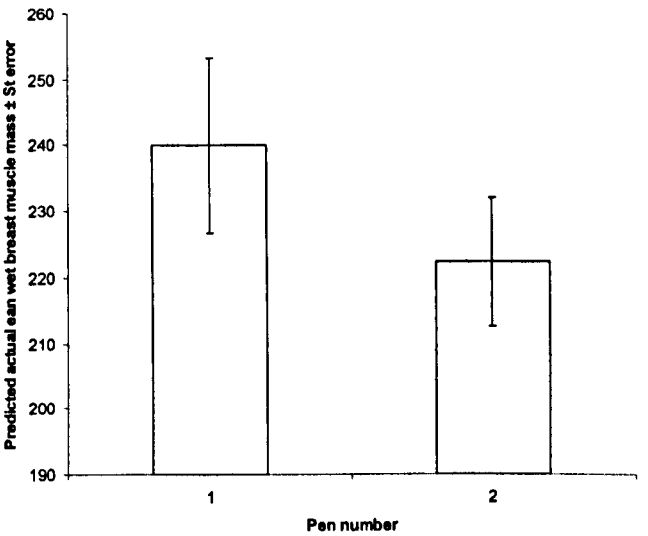


Figure 3 Variation in actual lean wet breast muscle mass with pen number (n = 41).

The predicted values control for the effects of female worm length, RBC, the modelled muscle mass index, host sex, host age, host body weight and interactions between host sex and female worm length and host body weight and host age (Table 1).



PCV

Host PCV subsequent to parasite challenge was not related to parasite intensity, the proportion of adults in the parasite population, host sex, RBC before parasite challenge, pen number or host body size (Table 2). It was related in non-significant positive relationships to female worm length ($F_{1,39}$ = 3.131, P = 0.085), PCV before parasite challenge ($F_{1,39}$ = 7.123, P = 0.011), the modelled muscle mass index ($F_{1,39}$ = 3.735, P = 0.061) and host body weight ($F_{1,39}$ = 3.748, P = 0.060), and to a trend with host age where PCV was higher in 16 than 12 week old hosts ($F_{1,39}$ = 5.214, P = 0.028), but these terms dropped from the model during simplification.

Table 2 Linear model of PCV after parasite challenge.

Response~PCV after parasite challenge	**d.f.,residuals**	**Value**	**s.e.**	***F* statistic**	***P (F)***
Terms dropped					
$\log_{10}$(parasite intensity + 1)	1,39	0.027	0.019	1.900	0.176
$\log_{10}$(female worm length)	1,39	0.235	0.133	3.131	0.085
Proportion of adults	1,39	<0.001	0.002	0.002	0.965
Host sex	1,39	0.006	1.150	1.150	0.290
Host age	1,39	0.011	0.005	5.214	0.028
PCV before parasite challenge	1,39	0.239	0.090	7.123	0.011
Pen number	1,39	-0.007	0.005	1.892	0.177
Modelled muscle mass index	1,39	0.002	0.001	3.735	0.061
Host body weight (g)	1,39	<0.001	<0.001	3.748	0.060
Host body size	1,38	-0.006	0.004	2.364	0.132

RBC

Host RBC subsequent to parasite challenge was not related to parasite intensity, female worm length, the proportion of adults in the parasite population, host sex, host age, RBC before parasite challenge, pen number, the modelled muscle mass index, host body weight or host body size (Table 3).

Table 3 Linear model of RBC after parasite challenge.

Response~RBC after parasite challenge	**d.f.,residuals**	**Value**	**s.e.**	***F* statistic**	***P (F)***
Terms dropped					
$\log_{10}$(parasite intensity + 1)	1,39	-3.153	5.587	0.319	0.576
$\log_{10}$(female worm length)	1,39	32.044	0.692	0.692	0.411
Proportion of adults	1,39	32.044	38.518	1.282	0.264
Host sex	1,39	-0.420	1.516	0.088	0.768
Host age	1,39	-0.451	1.480	0.213	0.647
RBC before parasite challenge	1,39	-0.683	0.044	0.235	0.631
Pen number	1,39	-0.369	1.480	0.062	0.804
Modelled muscle mass index	1,39	-0.296	0.225	1.731	0.196
Host body weight (g)	1,39	-0.007	0.005	1.730	0.196
Host body size	1,38	1.392	1.034	1.813	0.186

Parasites and host organ characteristics

Liver mass

After Benjamin and Hochberg (1995) corrections were undertaken liver mass was significantly related to host age (Figure 4; $F_{1,45} = 40.403$, $P < 0.001$) and negatively related to host body size ($F_{1,45} = 64.140$, $P < 0.001$). There was also a near significant trend with host sex ($F_{1,44} = 3.810$, $P = 0.057$), with heavier livers in males than females, and liver mass was not related to parasite intensity, female worm length, the proportion of adults in the parasite population, PCV and RBC before parasite challenge, the modelled muscle mass index, pen number, host body weight or host body size (Table 4).

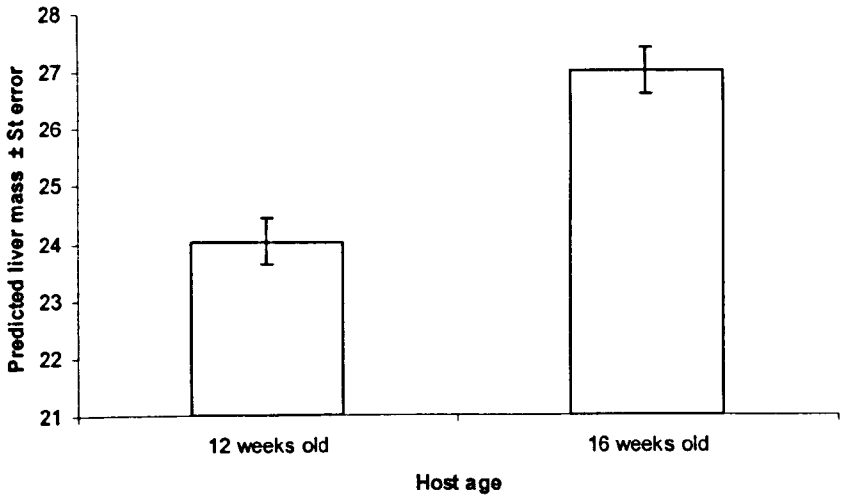
Table 4 Linear model of liver mass after parasite challenge.

Response-Liver mass (g)	d.f.,residuals	Value	s.e.	*F* statistic	*P(F)*	Corrected *P*	Significance of corrected *P*
Host age	1,45	-3.775	0.594	40.403	<0.001***	0.009	***
Host body size	1,45	-3.299	0.412	64.140	<0.001***	0.005	***
Terms dropped							
$\log_{10}$(parasite intensity + 1)	1,30	1,44	1.835	2.132	0.740	0.036	ns
$\log_{10}$(female worm length)	1,31	10.378	12.945	0.643	0.427	0.027	ns
Proportion of adults	1,30	-0.001	0.108	<0.001	0.993	0.050	ns
PCV before parasite challenge	1,30	-6.752	11.618	0.338	0.565	0.032	ns
RBC before parasite challenge	1,31	-0.032	0.019	2.728	0.108	0.018	ns
Modelled muscle mass index	1,31	-0.012	0.203	0.003	0.954	0.041	ns
Host sex	1,31	1.859	0.952	3.810	0.057	0.014	ns
Pen number	1,31	-0.515	0.471	1.199	0.280	0.023	ns
Host body weight (g)	1,31	<0.001	0.005	<0.001	0.985	0.045	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 4 Variation in liver mass with host age (n = 48).

The predicted values control for the effects of host body size (Table 4).



Spleen mass

After Benjamin and Hochberg (1995) corrections were undertaken spleen mass was significantly related to host age (Figure 5; $F_{1,45} = 19.993$, $P < 0.001$) and negatively related to actual lean wet breast muscle ($F_{1,45} = 25.973$, $P < 0.001$). There was also an effect of pen, with heavier spleens within the birds in pen 2 than pen 1 but the term dropped from the model during simplification ($F_{1,44} = 7.248$, $P = 0.010$). It was not related to parasite intensity, female worm length, the proportion of adults in the parasite population, PCV and RBC before parasite challenge, host sex, host body weight or host body size (Table 5).

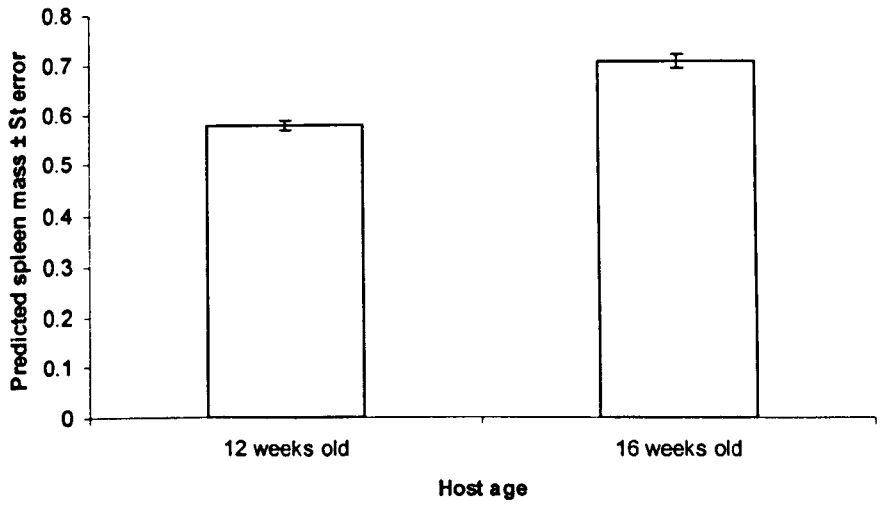
Table 5 Linear model of spleen mass after parasite challenge.

Response–Spleen mass (g)	d.f.,residuals	Value	s.e.	*F* statistic	*P (F)*	Corrected *P*	Significance of corrected *P*
Modelled muscle mass index	1,45	-0.215	0.048	25.973	<0.001***	0.005	***
Host age	1,45	0.038	0.008	19.993	<0.001***	0.009	***
Terms dropped							
$\log_{10}$(parasite intensity + 1)	1,44	0.057	0.143	0.157	0.694	0.050	ns
$\log_{10}$(female worm length)	1,43	0.915	0.880	1.080	0.304	0.018	ns
Proportion of adults	1,44	0.007	0.007	0.924	0.342	0.023	ns
PCV before parasite challenge	1,36	0.351	0.661	0.282	0.599	0.036	ns
RBC before parasite challenge	1,36	<-0.001	0.001	0.202	0.656	0.045	ns
Host sex	1,44	-0.027	0.055	0.235	0.630	0.041	ns
Pen number	1,44	-0.081	0.030	7.248	0.010	0.014	*
Host body weight (g)	1,44	0.034	0.062	0.299	0.587	0.032	ns
Host body size	1,43	-0.042	0.050	0.689	0.411	0.027	ns

'Corrected *P*' and 'Significance of corrected *P*' are Benjamin and Hochberg (1995) corrections. 'Corrected *P*' > '*P (F)*' for significance.

Figure 5 Variation in spleen mass with host age (n = 48).

The predicted values control for the effects of the modelled muscle mass index (Table 5).



Discussion

This study investigated the hypotheses that:

(1) *H. gallinarum* parasitism has an impact upon pheasant fecundity,

(2) host liver size depended on host condition in the absence of parasitism, and

(3) the spleen was involved in the immune response to parasitism.

The results of this study have been compared with those from a challenge of *T. tenuis* in red grouse (Wilson and Wilson, 1978) to identify evidence of a generalised immune response to endoparasitic infection in avian species. If similar patterns were observed for *H. gallinarum* parasitism of pheasants, this would point to general effects of endoparasitic infection upon the condition and spleenic response of avian species.

If H. gallinarum parasitism were to impact upon pheasant fecundity, it was suggested to predict variation in host body condition, PCV and RBC. Within my work examining *H. gallinarum* parasitism in the pheasant, the main effects of parasite intensity, female worm length and the proportion of adults in the parasite population did not affect actual lean wet breast muscle mass (Table 1), PCV (Table 2) or RBC (Table 3) and therefore body condition subsequent to parasite challenge. Female worm length interacting with host sex did significantly affect actual lean wet breast muscle mass, with positive relationships for both sexes but lower muscle mass in female than male hosts. This may be because individuals may gain muscle mass due to better gut health, which could have favourable knock-on effects upon the fecundity of female worms. As muscle mass increased with female worm fecundity, and because RBC and the modelled muscle mass index were both

also significantly positively associated with actual lean wet breast muscle mass, this suggests that the interaction with female worm fecundity did not negatively affect body condition (Table 1). There were also non-significant positive trends in relationships between PCV and female worm length, the modelled muscle mass index or host body weight. These relationships may also suggest that as with actual lean wet breast muscle, individuals may gain muscle mass due to better gut health, which could have favourable knock-on effects upon the fecundity of female worms. The first null hypothesis that *H. gallinarum* parasitism would not affect pheasant fecundity can not therefore be refuted: pheasant female fecundity must be affected by *H. gallinarum* parasitism in some other way. Earlier work within this system also found no relationship between *H. gallinarum* intensity and either pheasant body mass before or body condition subsequent to parasite challenge (Tompkins et al., 1999, 2001, 2002), however previous work undertaken on anthelmintic dosed wild pheasants has suggested that there may be an impact upon body condition, possibly interacting with host nutrition (M. Woodburn, *pers. com.* in Tompkins et al. 2002) and *H. gallinarum* has been negatively correlated with cloacal fat in pheasants (Hillgarth, 1991). Within grey partridge, a contrasting positive relationship has been found, with a link between the intensity of *H. gallinarum* and partridge muscle mass (Sage et al., 2002). Within the study of red grouse, inverse associations were found between *T. tenuis* egg count and PCV. Negative effects of parasitism upon PCV have also been observed in fledgling pied flycatchers (Potti et al., 1999), and PCV decreased throughout a season in the house martin (Christe et al., 2002), possibly with development of ectoparasitic infection.

Hypothesis two

The liver and spleen are suggested to play a role in the immunological response of birds, although their specific functions are not altogether understood (John, 1994a, 1994b).

As no relationship was found between liver mass and any indicators of condition prior to parasite challenge (actual lean wet breast muscle mass, PCV or RBC; Table 4), the second null hypothesis could not be rejected. As an indicator of state, liver mass may reflect condition during parasitism, but is unaffected by condition in the absence of parasitism.

Hypothesis three

Spleen mass has been positively linked to T-cell mediated immune response (due to the conceivable lymphocyte production; John, 1994a) and negatively linked to parasitism (Brown and Brown, 2002; John, 1994a, Møller et al., 1998; Møller and Erritzoe, 2002; Morand and Poulin, 2000) in colonially living species (which are likely to encounter greater infection and parasitism; Brown and Brown, 2002; Møller, 1998; Møller and Erritzoe, 1998). The third hypothesis was that spleen mass would not be related to parasitism. As there were no significant relationships between spleen mass and parasite intensity, female worm length or the proportion of adults in the parasite population, this could not be rejected.

Conclusion

As with past research undertaken on *H. gallinarum* parasitism in the pheasant, the results of this study suggest this parasite has little impact upon pheasant morbidity as it did not

significantly affect muscle mass and therefore condition, PCV, RBC or liver and spleen mass subsequent to parasite challenge. Pheasant female fecundity must be affected by something other than differences in body condition (mediated by *H. gallinarum* parasitism). These results differ from those found by studying *T. tenuis* parasitism in red grouse (Wilson, and Wilson, 1978), *H. gallinarum* parasitism in grey partridge (Sage et al., 2002), parasitised fledgling pied flycatchers (Potti et al., 1999) and from patterns described using meta-analyses of spleen mass and parasitism. Differences in host responses probably occur as a result of host suitability to parasite species, which may be affected by host parasite co-evolution (Tompkins *pers. com.*), or differences in host immune defence and parasite virulence. The virulence of *H. gallinarum* was low in the highly susceptible pheasant host, indicated by a lack of morbidity. As a result, parasite virulence may be important in affecting host susceptibility, as susceptibility has been shown to be important in influencing parasite virulence (Bull, 1994; May and Anderson, 1983, 1990).

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Chapter 7

General discussion and conclusions

General discussion

The aim of this thesis was to identify and parameterise the factors affecting host susceptibility to parasitism.

Host susceptibility can be affected by innate phenotype, genetic background, body condition, immune functioning, sex, age and stresses caused by social interactions, host density, the environment and habitat. The work undertaken within this thesis examined the effects of condition and the T-cell mediated immune response (Chapter 2), acquired immunity (Chapter 3) and nutritional stress (Chapter 4) upon the resistance of the pheasant to parasitism by the caecal nematode *H. gallinarum.* An examination was also made of the effect of condition, spleenic response and parasitism by *H. gallinarum* upon sexual signalling in the pheasant (via secondary sexual ornamentation; Chapter 5). The final chapter examined the effects of *H. gallinarum* parasitism upon the morbidity of the pheasant host, comparing the results to those of similar parasite/host relationships to identify evidence of a generalised immune response to endoparasitic infection in avian species (Chapter 6).

Within this concluding chapter, discussion is made of the factors determining host susceptibility to parasitism and therefore parasite aggregation, how food stress impacts upon parasite aggregation within a host population, and how parasite challenge compared to natural exposure to parasitism affects parasite aggregation within the host population.

It has become increasingly important to examine the characteristics within hosts that affect susceptibility to parasitism, as current control methods are becoming more restricted. Non-invasive parasite control procedures for game birds could solve many of the problems associated with large-scale anthelmintic usage and resultant resistance (Wakelin et al., 2002).

The factors determining host susceptibility to parasitism

Within the second chapter, the relationships between host condition, the T-cell mediated immune response and parasitism in naive individuals were investigated. The correlation between pectoral muscle mass and red blood cell count followed the hypothesis that their maintenance is accountable and may be limited by resources, and suggested that further trade-offs with life-history characteristics could occur. The lack of association between pectoral muscle mass and T-cell mediated immune response suggested that perhaps this immune response was not linked with these particular indicators of condition, except that there did seem to be a relationship which was complicated by the effects of pheasant body size. The implication of resource limitation was that T-cell mediated immunity must be limited more directly, and compromised by, other life-history characteristics. This was implied by the negative relationship between immunity and parasitism.

The conclusion of the research discussed within this chapter was that the difference in the energetic costs likely to be used for reproduction, to respond immunologically to parasites and for the display of secondary sexual characteristics compared to the

maintenance of general condition, may perhaps explain why more basic underlying condition constraints could not be identified within this study.

Within the third chapter the possibility of an acquired immune resistance to *H. gallinarum* parasitism within a pheasant host was investigated. Pheasants were found to be able to mount an acquired immune response to *H. gallinarum* parasitism. The results within this chapter suggested that the null hypothesis could be rejected because parasite intensity in the control group, which received one parasite challenge, was greater than that of the treatment group, which had received a previous parasite challenge and hence had a pre-existing acquired immune response. Female worm length was also non-significantly higher in the once versus twice-challenged treatment group: the effect of acquired resistance was therefore characterised by a reduction in parasite intensity and possibly by a reduction in female worm fecundity.

The conclusion of the research discussed within this chapter was that prior exposure to parasites lowered host susceptibility to parasitism. Within the model suggesting *H. gallinarum* as one mechanism for grey partridge decline in the UK because of apparent parasite-mediated competition, an assumption of little or no effect of acquired immunity was used (Tompkins et al., 1999, 2000a, 2000b). The result of an acquired immune response observed within this research could change the model equilibrium to one of pheasant and partridge coexistence (Tompkins *pers. com*).

Within the fourth chapter the effects of crude protein and amino acid limitations and a linseed oil additive were examined in relation to pheasant body condition and *H. gallinarum* parasitism. The protein content and a linseed oil additive within the host

diet, and host body condition were suggested to influence the success of parasites in the host, and the amino acid content within the host diet was suggested to influence the condition of the host. The first null hypothesis that protein would not influence parasitism was rejected because there was a positive relationship between body condition and parasite intensity for the birds on the standard crude protein level diets, compared to (a stronger) positive or negative relationship for the birds on the low crude protein level diets. Host body condition negatively influenced the success of parasites within hosts on the standard amino acid level diet, and positively influenced the success of parasites in hosts on the low amino acid level diets. As a result the second null hypothesis that the amino acid content of the diet would not influence the condition of the host was rejected. As variation in host body condition predicted variation in the number of parasites interacting with dietary amino acid level, this also suggested that the third null hypothesis that differences in body condition would not influence parasite intensity could be rejected. There was no difference in parasite intensity between the standard diet and the diet with added linseed oil, and no effect of this dietary manipulation upon female worm length or the proportion of adults in the parasite population. This suggested that the fourth null hypothesis that the linseed oil manipulation would not affect the success of parasites within the host could not be rejected.

The conclusions of the research discussed within this chapter were that crude protein and amino acid levels within over-wintering maintenance diets can affect pheasant susceptibility to *H. gallinarum* parasitism in co-ordination with the body condition of the host, and a linseed oil additive did not affect host susceptibility.

Within the fifth chapter the relationships between host immune defence, condition, secondary sexual characteristics and parasite infection were examined to see whether their expression was likely to affect the susceptibility of pheasants to *H. gallinarum*, through investment in signals. The characteristics used in ornamentation were found to be correlated with each other, and were related to host immune function, body condition and *H. gallinarum* parasitism. The first null hypothesis was rejected because there were positive significant or non-significant correlations between male wattle height and ear tuft length, male wattle height and spur length, male ear tuft length and spur length, and female wattle height and head crest height. The second null hypothesis that there would be no effect of immune functioning and condition upon secondary sexual ornaments was rejected because male wattle size, spur length, hue wattle colouration and the width of the black points within male wattles were all correlated with body condition. The third null hypothesis that there would be no effect of parasitism upon secondary sexual ornaments was also rejected because female wattle size, male ear tuft length and the width of the black points within male wattles seemed to indicate resistance to parasitism.

The conclusion of the research discussed within this chapter was that the brightness of secondary sexual ornamentation is mediated through host condition and immune response and also as a result of parasitism, but different characteristics can indicate immune resistance and fitness, resistance against parasitism or perhaps both. Host susceptibility to parasitism may therefore be affected by secondary sexual ornamentation, which may also be affected by parasitism.

H. gallinarum parasitism affects morbidity and mortality in the grey partridge to a much greater extent than in the pheasant (Robertson and Dowell, 1990; Tompkins et al, 1999) but pheasants are more highly susceptible (Tompkins et al., 2000) to the parasite and exhibit a higher parasite carry capacity (Draycott *pers. com.*). To investigate the reasons behind this high susceptibility the sixth chapter examined the effects of *H. gallinarum* parasitism on pheasant morbidity, in terms of condition and spleenic response. *H. gallinarum* parasitism was suggested to affect pheasant condition. As an indicator of condition, the mass of the liver was suggested to depend on host condition in the absence of parasitism and would therefore be correlated with condition. A spleenic response was expected to result from parasitism, and spleen mass would therefore be correlated with parasitism. The first null hypothesis that *H. gallinarum* parasitism would have no impact upon pheasant condition was not refuted (parasitism was not related to actual lean wet breast muscle mass, PCV or RBC). The second and third null hypotheses could not be rejected either, as no relationship was found between liver mass and any indicators of condition prior to parasite challenge, and spleen mass was not related to parasitism.

The results of this chapter suggest that the virulence of *H. gallinarum* was low in the highly susceptible pheasant host, indicated by a lack of morbidity. The conclusion was that pheasant female fecundity must be affected by something other than differences in body condition (mediated by *H. gallinarum* parasitism), and parasite virulence may be important in affecting host susceptibility, as susceptibility has been shown to be important in influencing parasite virulence (Bull, 1994; May and Anderson, 1983, 1990).

From all the research undertaken within this thesis, a general pattern of male hosts having higher condition and immune response has emerged. Males had higher modelled muscle mass, actual lean wet breast muscle mass, PCV, RBC and liver mass than female hosts (chapters 3 and 6). However, when examining RBC prior to parasite challenge, females had higher levels and males had higher *H. gallinarum* intensities. The effect of host age upon susceptibility was not as clear-cut however. Before parasite challenge, condition (PCV and RBC) seemed to be higher in younger (12-week-old) than older (16-week-old) hosts, whereas muscle mass was higher in older than younger hosts (chapter 2). After challenge, condition (PCV and liver mass) was higher in older (16-week-old) than younger (12-week-old) hosts. This suggests that parasitism may have a greater effect upon the morbidity of younger than older hosts, possibly because more resources are used in growth and development in younger adolescents than older adult hosts. Differences in muscle mass probably occurred as a result of differences in body size.

The impact of nutritional susceptibility upon parasite aggregation within a host population

Within the chapter examining nutritional stress using dietary manipulation (chapter 4), the level of aggregation seems to have been affected by crude protein but not by amino acid levels. The level of aggregation was higher in the hosts on the low rather than standard crude protein level diets, for a number of possible reasons. For example, the parasite prevalence of hosts in optimal condition is most likely to be affected by natural susceptibility and acquired immunity, but hosts suffering from reduced immune defence mechanisms due to obesity or malnutrition are likely to be

handicapped. Consequently, within these hosts more individuals would suffer higher parasite burdens, therefore decreasing parasite aggregation. The gut of hosts on high protein diets may allow better parasite survival, as a result of an improved food source. This would impact upon parasitism by increasing parasite intensity throughout the host population, and possibly lessening the effects of differing host susceptibility and immune response. Also, behavioural differences may be caused by variation in the quality of the diets, whereby some individuals on the low compared to the standard crude protein diets may have attempted to compensate by increasing either the quantity or quality of their intake, foraging to a greater extent and therefore increasing their exposure to the infective stages of the parasite. As has already been suggested, research has indicated moderation of dietary intake for quality is possible in broiler chickens, and therefore is perhaps also possible in pheasants (Leeson et al., 1996). If parasite aggregation decreases as a result of a greater number of handicapped and therefore more susceptible hosts (due to reduced immune defence from obesity or malnutrition), the highly aggregated distribution of the parasites within the birds on the diet with added linseed oil may suggest these individuals were relatively healthy.

The impact of natural exposure to parasitism compared to parasite challenge techniques upon parasite aggregation within the host population

Within the research undertaken in this thesis, three different groups of pheasants have been orally challenged with *H. gallinarum* parasites, and two groups were naturally exposed to parasitism. In the work previously undertaken by Tompkins, another two groups were orally challenged with parasites and three groups were naturally exposed to parasitism. A statistical test of negative binomial fit (designed and written by

Darren Shaw, University of Edinburgh) for the S-PLUS 6 program was used to examine the error distribution of the worm intensity data for all these groups. The level of aggregation (k is an inverse measure) was generally much higher in host populations that were orally challenged compared to those which were naturally exposed to parasitism (Table 1).

Table 1 The aggregation of *H. gallinarum* parasitism in orally challenged versus naturally exposed pheasant hosts.

Type of infection	Sample size (n)	Best estimate of the aggregation parameter (k)	Reference
Challenge	24	6.76	Chaper 2, aged 12 weeks, this thesis
Challenge	26	4.81	Chaper 2, aged 16 weeks, this thesis
Challenge	31	1.10	Chapter 3, this thesis
Challenge	6	3.64	Tompkins et al., 2000a
Challenge	176	3.94	Tompkins and Hudson, 1999
Exposure	11	0.41	Prep. work, this thesis
Exposure	60	0.62	Chapter 4, this thesis
Exposure	12	1.25	Tompkins et al., 1999
Exposure	68	1.05	Tompkins et al., 2002
Exposure	56	1.14	Tompkins et al., 2000b

This suggests that differences in parasite aggregation may be caused in part by behavioural differences between hosts, which could affect parasite infection rates. For example, some hosts may be more heavily parasitised than others because they forage to a greater extent and therefore expose themselves more to the reproductive stages of the parasite. Documented examples of host behaviour influencing the level and therefore aggregation of parasitism within hosts have been widely reported (for example Altizer et al., 2000; Bundy, 1988; Florez-Duquet et al., 1998; Folstad et al., 1991; Gilbert, 1997; Hart, 1994, 1997; Holmes and Zohar, 1990; Hutchings et al., 2002a, 1998, 1999, 2002b; Jaenike and Anderson, 1992; Karban, 1998; Monagas and Gatten, 1983; Moore and Gotelli, 1990, 1996; Mooring and Hart, 1992; Pfennig and

Tinsley, 2000; Poulin, 1994a, 1994b; Rubenstein and Hohmann, 1989; Tinsley, 1989; Thompson, 1990; Thompson and Kavaliers, 1994).

General conclusions

The susceptibility of the pheasant host to parasitism and therefore parasite aggregation is affected by the T-cell mediated immune response of the pheasant host, possible acquired immune resistance of the pheasant to *H. gallinarum* parasitism, nutritional stress interacting with body condition, and possible trade-offs between condition, spleenic response, secondary sexual ornaments and *H. gallinarum* parasitism.

Therefore, in general the work undertaken within this thesis suggests that differences in the susceptibility of hosts to parasitism do affect the aggregation of parasite populations. However, the effects of host immune function, condition and acquired immune responses to parasitism seem relatively system specific when my results are examined within the context of other studies, but trends in positive relationships between condition and host immune function and negative relationships between host immune function and parasitism do seem relevant within all systems. From comparison of parasite challenged hosts versus those naturally exposed to parasitism, the level of aggregation seems to be affected by behavioural differences between hosts, which could affect parasite infection rates.

The study of the factors affecting parasite aggregation within host populations is important within the context of general science because differences in aggregation could have ramifications for the evolutionary and population dynamics of

parasite/host systems (Anderson and May, 1978; May and Anderson, 1978; Poulin, 1993). The regulatory and selective pressures of the parasite are much greater in host populations in which the parasite is more randomly distributed (compared to an aggregated parasite population). This is because the effects of macroparasitism upon hosts are generally dose-dependent. As a result, the susceptible hosts in the 'tail' of the distribution suffer the greatest morbidity and mortality. As the level of parasite aggregation decreases and parasitism becomes more randomly distributed, more susceptible hosts will suffer greater parasite-mediated morbidity, thereby making parasitism a greater regulatory (Anderson and May, 1978; May and Anderson, 1978) and selective (Poulin, 1993) mechanism for host populations (Wilson et al., 2002).

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