

# Trade-offs in evolutionary immunology: just what is the cost of immunity?

Robert L. Lochmiller and Charlotte Deerenberg

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

It has become increasingly clear that life-history patterns among the vertebrates have been shaped by the plethora and variety of immunological risks associated with parasitic faunas in their environments. Immunological competence could very well be the most important determinant of life-time reproductive success and fitness for many species. It is generally assumed by evolutionary ecologists that providing immunological defences to minimise such risks to the host is costly in terms of necessitating trade-offs with other nutrient-demanding processes such as growth, reproduction, and thermoregulation. Studies devoted to providing assessments of such costs and how they may force evolutionary trade-offs among life-history characters are few, especially for wild vertebrate species, and their results are widely scattered throughout the literature. In this paper we attempt to review this literature to obtain a better understanding of energetic and nutritional costs for maintaining a normal immune system and examine how costly it might be for a host who is forced to up-regulate its immunological defence mechanisms. The significance of these various costs to ecology and life history trade-offs among the vertebrates is explored. It is concluded that sufficient evidence exists to support the primary assumption that immunological defences are costly to the vertebrate host.

*R. L. Lochmiller, Dept of Zoology, Oklahoma State Univ., Stillwater, OK 74078, USA (rllzool@okway.okstate.edu). – C. Deerenberg, Research Center for Ornithology of the Max-Planck-Society, D-82346 Andechs, Germany.*

In recent decades, interest of ecologists and evolutionary biologists in the relevance of parasitism to numerous facets of a host's life history has provided exciting new frontiers of investigation into vertebrate life history (e.g., Hamilton and Zuk 1982, Loye and Zuk 1991, Clayton and Moore 1997). While the study of specific host-parasite relationships have proven insightful, they reflect only a small part of the wealth of parasites and pathogens in an animal's internal and external environment. The immune system is the animal's defense mechanism to fight or control any parasitic or pathogenic infection, and as such, warrants our interest as a focus of study (Lochmiller 1996, Sheldon and Verhulst 1996, Zuk 1996). In addition, immunity is one of the major physiological mechanisms regulating host survival. Survival has long been the most neglected attribute of fitness in part because of the difficulty ecologists have

had in quantifying it in the wild. In contrast to this void, reproduction has been the cornerstone of research efforts into life history theory for decades. Reproduction is a clearly defined physiological event both in the field and laboratory, is characterised by having a defined starting and ending point, and can be easily evaluated relative to success, effort, and costs. Ultimately, fitness does not only depend on reproductive success, but on longevity as well.

It has become increasingly clear, however, that mechanisms regulating survival and reproduction are far from mutually exclusive. **Apart from survival, mate selection, breeding performance, fecundity, growth, and possibly other life-history traits appear to be associated in various ways with immunity.** Mounting an immune response, and even maintaining a competent immune system, is thought by many ecologists to be a nutrition-

Accepted 26 April 1999

Copyright © OIKOS 2000

ISSN 0030-1299

Printed in Ireland – all rights reserved

ally demanding process that necessitates trade-off decisions among competing nutrient demands for growth, reproduction, temperature, work, and immunity (Sheldon and Verhulst 1996). In a recent review on nutritional modulation of immunity, however, Klasing (1998) postulates that the nutritional cost of maintaining a normal immune system is minimal relative to tissue growth or egg production. He estimates that the cumulative mass of immune products (cells and molecules) in a chicken does not exceed 5% of total body mass, and that leukopoiesis, even during an immune challenge, accounts for less than 1% of the daily increase in body mass of a growing chick. While such estimates would appear to contradict the assumption that immunity is a costly endeavour that potentially competes with other life-history events for limited nutrients, we suggest that metabolic requirements of immune cells and indirect consequences of mounting an antigen-induced immune response (e.g. acute inflammatory response) are also integral components of the cost equation. Factoring in these additional costs into the equation should provide a more realistic perception of costs of immunity in terms of trade-off decisions, because only a costly immune defence system can provide a relevant explanation of observed parasitism–immunity–reproduction patterns in the wild (Festa-Bianchet 1989, Norris et al. 1994, Richner et al. 1995, Oppliger et al. 1996, Siikamäki et al. 1997, Nordling et al. 1998).

Actual attempts to demonstrate the magnitude of this apparent “cost of immunity” have been limited and no comprehensive ecological review of the subject has been completed, which was the primary motivation for preparing this manuscript. In this paper, the literature is reviewed in an attempt to elucidate the various nutritional costs associated with simply maintaining a normally functioning immune system and to evaluate the costs associated with actually using it. These two physiological traits are not easily addressed quantitatively, particularly with respect to the former, because of the integrated and organisational characteristics of the immune system with other physiological systems. However, enough literature now exists from both the biomedical and animal science arenas to provide some quantitative estimates to these costs. Collectively, these studies demonstrate that considerable nutritional costs (protein and energy) are associated with up-regulation of the immune system. The consequences of such up-regulation to vertebrate survival in the wild appears so significant, that selection should have favoured a level of first-line immunological defence that minimises risk of developing disease (innate immunity) over acquired mechanisms of immunity. Nutritional trade-offs between immunity and other nutrient-demanding processes (e.g., reproduction, growth, thermoregulation) have been an important focus of recent investigations into evolutionary immunology (Sheldon and Verhulst 1996). Trade-offs such as these may be most pro-

nounced within the acquired arm of the immune system as opposed to the more important innate arm (Fearon and Locksley 1996).

## **Innate immunity and up-regulation**

Infection elicits a complete shift in metabolic priorities within the host to those associated with immunity, including related necessities as the repair of wounded tissues. Because immune challenges are effective at suppressing food intake, the body has evolved a complex mechanism for mobilising reserves of protein and energy to support the initial acute-phase response, characterised by hypermetabolism and protein malnutrition. In fact, immunologic challenges are capable of instilling protein malnutrition in a few days that would take several weeks to develop during simple starvation (Long 1977). So although many of the apparent metabolic adjustments a host undergoes during an infection would at first appear quite counterproductive, overall survival and fitness is undoubtedly enhanced (Kyriazakis et al. 1998). The initial acute response to infection is probably the most important to the fitness and life history of an animal in its natural habitat where one might posit that prolonged disease essentially does not occur because the weakened state of the animal would dramatically increase its risks of predation or starvation. Thus, one might hypothesise that survival in an infectious environment would depend more upon the competence of the innate arm of the immune system, a first-line surveillance and defence mechanism that also involves the acute-phase inflammatory response, than it would depend on acquired facets of immunity. Further, the innate compartment of the immune system would be most important among short-lived prey species under intense selection pressure from predation. In all likelihood, an infection, even a rather mild immune challenge, would not persist for more than a few hours or days before an animal would succumb to predation or starvation. Survival contributes more to fitness in long-lived species relative to short-lived species, because maximum fitness is only attained when a long-lived animal survives through many reproductive seasons. Thus, development of strong innate and acquired arms of immunity would seem an appropriate strategy for long-lived species, while a short-lived species may gamble that it survives until successful (early) reproduction with a less well-developed defence mechanism.

The generalised response to an infection is for the body to redistribute nutritional and energy resources away from anabolic and maintenance processes at the extremities to the vitally important metabolic processes driving immunity and disease resistance. This sequence is orchestrated by a complex cascade of events that

begin with the mononuclear phagocytic system and their release of an array of secondary messengers. While extremely complex, a classic response leads to anorexia, increased degradation and decreased synthesis of protein in skeletal muscle, elevated rates of metabolism, increased glucose utilisation, hepatic acute-phase protein synthesis, increased de-amination of gluconeogenic amino acids, alterations in levels of trace minerals, decreased skeletal development, and increased hepatic lipogenesis. The monokines interleukin-1 (IL-1), IL-6, and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) are the primary cellular messengers triggering the cascade of metabolic alterations following an immune challenge (Klasing and Johnstone 1991, Tsigos et al. 1997). Several excellent reviews have been published in recent years that provide detailed insights into the physiological mechanisms and pathways involved in the monokine-induced inflammatory response to infection (Klasing and Johnstone 1991, Taylor and Piantadosi 1995, Michie 1996).

Macrophages, like other immune cells, are nutrient-demanding cells as evidenced by their hypermetabolic state and significant rates of glucose and glutamine utilisation relative to other cells in the host. Based on *in vitro* O<sub>2</sub>-consumption rates, elicited macrophages turn over ATP in the cell 10 times per minute, almost comparable to maximally functioning heart muscle (Newsholme and Newsholme 1989). Thus, immunity demands fuel in terms of nutrients and energy at remarkable levels, which reflects the evolutionary importance that has been placed on ensuring host survival. Accelerated lipolysis, proteolysis, and glycolysis supply the fuel necessary for mounting the initial immune responses to infection, resulting in substantial loss of body weight if infections are prolonged and severe.

### Nutritional constraints

An unfortunate consequence of fighting an infection in nearly all species examined is a reduction in feed intake. This sepsis-induced anorexia is striking in the sense that it occurs at a time when the body needs an influx of nutrients to support the increased demands of mounting an immune response (Scrimshaw 1991). Reduced feed intake appears even with rather mild immune challenges such as those associated with simple vaccination (Gandra and Scrimshaw 1961). Consequently, catabolic processes must activate to support the additional fuel requirements of immune cells and protein synthesis (acute-phase proteins, antibodies). Kyriazakis et al. (1998) forward two hypotheses to explain the occurrence of anorexia during sepsis that are consistent with observations during infection. One hypothesis states that the reduced food intake promotes an effective immune response, while the other (non-exclusive?)

one asserts that anorexia allows the animal to be more selective in its diet to reduce the risk of further infection. The obligatory reduction in intake of food during infection could be especially important to wild animals during winter when the thermogenic benefits of digestion are critical to maintaining homeostasis. Winter mortality is a common phenomenon for many species in nature, possibly reflecting the futility of trying to satisfy both the excessive nutritional demands of infection and thermoregulation in a food-limiting environment (White 1993, Schetter et al. 1998). Such risks to wild animals are evident from the observation that the combined stress of shivering thermogenesis and illness in human patients can elevate resting metabolism rapidly to nearly 300% of normal (Chiolero et al. 1997).

Malabsorption also can accompany infections leading to additional nutritional constraints on the host. A combination of factors contribute to this syndrome, including physical blockage of absorption sites by bacterial overgrowth in the gut, alterations in villus structure, reductions in transit time such as with diarrhoea, and reductions in blood flow to the gut. During the acute-phase immune response, the metabolism of many nutrients is altered and the requirement for some is actually elevated due to increased catabolic and excretion processes. For example, vitamin A requirements appear to be significantly elevated during infections as a result of urinary excretion during fever (Campos et al. 1987, Stephensen et al. 1994).

### Metabolic costs: energy

Metabolic costs of maintaining a competent immune system are extremely difficult, if not impossible, to adequately measure for a variety of reasons. In fact, we know of no study that comes close to truly addressing the question about how costly it is to maintain a competent immune system for any vertebrate species. However, we have gleaned some important insight into this question by observing physiological changes that accompany the down-regulation or up-regulation of the immune system. It is well known that energy restriction in the diet, if prolonged, can lead to suppression of the immune system and increase the risks of infection, especially with opportunistic pathogens (Klurfeld 1993, Lochmiller and Dabbert 1993). The importance of energy in maintaining the immune system has also become clear from the space programme where the minimal energy demands and production manifested by zero-gravity environments can result in declines of 90% in cytotoxic T-lymphocyte function in astronauts (Taner 1992).

Unlike starvation where the host eventually adjusts physiologically to the reduction of nutrient inputs by decreasing metabolism, infection leads to a hyperme-

catabolic state to support the up-regulation of the immune system (Table 1). Isolated mitochondria of laboratory rats stimulated *in vivo* with TNF- $\alpha$  or IL-1 can undergo a 30% increase in respiration rate (Jin et al. 1995). Infusions of IL-6 into healthy volunteers will increase resting metabolic rates by 25% (Tsigos et al. 1997). To fuel this up-regulation, immune cells require glucose and glutamine at high levels (Crouser and Dorinsky 1996), which leads to the rapid breakdown of the body's reserves of protein (to provide glutamine), carbohydrates, and lipids (Michie 1996). Moderate infections can easily lead to 150–200% increases in rates of gluconeogenesis in the host, often leading to severe wasting of lean tissue if such infections chronically persist. Animals typically become insulin-resistant as an adaptation to insure glucose concentrations in circulation remain high for the insulin-independent immune cells involved in wound healing and combating infection (Chiolero et al. 1997).

General statements about the metabolic costs of immunity to the whole organism are difficult to assess during an immune challenge. Severity, type, and duration of infection, ambient temperature, and gender, age, and nutritional status of the host all influence the cost of mounting an immune response. However, it is clear from selected studies with humans and laboratory animals that even mild up-regulation of the immune system can be costly metabolically to the host. In general, neonates are buffered from many of the costs that become evident in a fully matured immune system of adults (McIntyre and Hull 1996). In humans, severe infections routinely lead to losses of 15–30% body weight, with 25–55% increases in resting metabolic rates during sepsis compared to healthy subjects (Long 1977, Kreymann et al. 1993). Hypermetabolism approaching two-fold increases in the resting metabolic rate has been observed in some severely ill patients (Frankenfield et al. 1994). About 20% of the protein mobilised from skeletal muscle is used to satisfy these energetic demands (Duke et al. 1970), the remainder is fuelled by carbohydrates and lipids. Borel et al. (1998) estimated that ca 50% of the elevation in metabolic rate

during an infection can be attributed to the elevated energetic cost of protein synthesis (acute-phase inflammatory proteins, antibodies). Whole organism use of glucose can increase 68% during the acute-phase immune response (Klasing 1988). Fever, a hallmark of infection, usually elicits a 10–15% elevation in basal metabolic rate for each 1°C rise in body temperature in humans (Roe and Kinney 1965).

Mild immune challenges such as those associated with vaccination with protein antigen (e.g., keyhole limpet hemocyanin; Demas et al. 1997, Svensson et al. 1998) or avirulent organisms (typhoid vaccine; Cooper A. L. et al. 1992) can result in 15–30% increases in the metabolic rate of a host. Additionally during the recovery period, an animal may be faced with substantial nutritional costs associated with replenishing body tissue reserves that were depleted during the catabolic processes accompanying a typical immune challenge. The energy cost of synthesising new proteins to replace those lost in skeletal muscle must be added to the overall cost of mounting an immune response. An estimated 24 kcal is required by the host to deposit a single gram of protein (Scrimshaw 1991). It may be that the negative nitrogen balance persisting during an immune challenge becomes a more critical factor than elevated energy requirements to survival of animals in natural populations where protein resources in the available food supply fall short of meeting requirements (White 1993, Schetter et al. 1998).

### Metabolic costs: protein

Negative nitrogen balance is a classic response during an immune reaction and varies in proportion to severity of infection (Table 2). Inflammatory cytokines and glucocorticoids mediate this energy-dependent proteolytic response in skeletal muscle (myofibril proteins). So, not only does this lead to a negative nitrogen balance, but the accelerated breakdown of proteins places additional energetic demands on the host (Hasselgren and Fischer 1998). The obligatory reduction in

Table 1. Estimated energetic costs (percentage increase in resting metabolic rate as compared to controls) to the vertebrate host when mounting an immune response during vaccination or sepsis.

Species	Immune challenge	Cost	Reference
Human	Sepsis	30%	Kreymann et al. (1993)
	Sepsis	30%	Carlson et al. (1997)
	Sepsis and injury	57%	Clark et al. (1996)
	Typhoid vaccination	16%	Cooper A. L. et al. (1992)
	Sickle cell disease	15%	Borel et al. (1998)
Laboratory rat	IL-1 infusion	18%	Tocco-Bradley et al. (1987)
	Inflammation	28%	Cooper et al. (1994)
Laboratory mouse	KLH challenge <sup>a</sup>	30%	Demas et al. (1997)
Sheep	Endotoxin	28%	Fewell et al. (1991)
	Endotoxin	10–49%	Baracos et al. (1987)

<sup>a</sup> Keyhole limpet hemocyanin injection.

Table 2. Estimated alterations (percentage change compared to controls) in feed intake, body weight (BW) gain, urinary nitrogen excretion, rates of protein catabolism (RPC), rates of protein synthesis (RPS), and total body protein (TBP) levels in vertebrate hosts with an up-regulated immune system.

Host	Reference	Condition	Parameter	Percent change
Pig	Spurlock et al. (1997)	PRRS vaccine <sup>a</sup>	BW gain	−21%
Chicken	Lee and Reid (1977)	HVT vaccine <sup>b</sup>	Feed intake	−15%
			Feed intake	−3%
	Henken and Brandsma (1982)	SRBC <sup>c</sup>	Feed intake	0%
			BW gain	0%
Human	Klasing et al. (1987)	SRBC	BW gain	−13%
			BW gain	−18%
	Borel et al. (1998)	Sickle cell disease	RPC	+32%
			RPS	+38%
			N excretion	+160%
			TBP	−12%
Laboratory rat	Carlson et al. (1997)	Sepsis	RPC	+40%
	Hobler et al. (1998)	Sepsis and injury		

<sup>a</sup> Porcine respiratory and reproductive syndrome.

<sup>b</sup> Herpes virus of turkeys.

<sup>c</sup> Challenge with sheep red blood cells.

cell mass that occurs from this wasting syndrome, if prolonged, can result in tremendous reductions in body mass. The demands for amino acids to support protein synthesis, elevated metabolic rates, and increased gluconeogenesis are so great that the host has evolved a radical physiological mechanism for responding to an immunological challenge from infecting organisms. Nearly every defensive mechanism available in the immunological arsenal of the host requires significant supplies of amino acids for production of proteins (Beisel 1977).

Quantitative estimates of protein costs to the host for mounting an immune response are difficult to derive and so few exist in the literature. Even less is known of the protein costs for just simply maintaining baseline immune function. Nitrogen excretion in the form of urea can elevate to 160% above normal during sepsis in humans (Carlson et al. 1997). Although synthesis of selected acute-phase inflammatory proteins and antibodies can be elevated, synthesis of other less-vital proteins are reduced during an immune challenge (Biolo et al. 1997). Protein synthesis does not keep pace with accelerated rates of protein loss from skeletal muscle, resulting in losses in total body protein of 20% in septic patients (Biolo et al. 1997). Even intensive intervention by supplying amino acids and glucose to an ill patient is generally unsuccessful at obviating the loss of lean body mass. Septic laboratory rodents have rates of muscle protein breakdown approaching > 40% and significant reductions in rates of protein synthesis (Hobler et al. 1998). Even mild challenges of the immune system such as those induced by vaccinations can reduce nitrogen retention by as much as 30% in the vertebrate host (Gandra and Scrimshaw 1961, Hentges et al. 1984).

It appears that even rather mild, subclinical challenges of the immune system can result in negative nitrogen balance persisting for several days or weeks

and requiring nearly four times as long to replenish body reserves (Scrimshaw 1977). The consequences to animals in the wild appear obvious, and one would predict that selection has favoured an immune strategy that minimises the risk of developing a full-blown, clinical infection. Survival for most diseased animals in the wild would quickly approach zero under the natural forces of predation and nutritional stress (Wobeser and Wobeser 1992).

### Trade-offs between production and immunity

Energy and nutrients required for growth (cellular proliferation leading to an increase in body size) are often expensive because of inherent inefficiencies in the process as well as dietary imbalances. These “overhead costs” can make the process of growth particularly sensitive to alterations in immune status. In general, stimulation of a host’s immune system equates to proportional declines in growth as endogenous strategies of resource allocation shift towards survival and away from nonessential processes such as growth. Even mild up-regulation of the maternal immune system can have suppressive effects on foetal growth and development as a consequence of direct and indirect mechanisms (Rivera et al. 1998). This reallocation and ultimate growth suppression that results demonstrates the costly nature to the host of maintaining a competent immune system and mounting an immune response against infectious agents. This reallocation is another reflection of the major metabolic and nutritional adjustments that can occur following up-regulation of the immune system.

Some of the most interesting insights into the trade-offs between growth and immunity have come from studies in germ-free and antigen-free environments (Tables 3, 4) and with the use of antibiotics in meat-animal

production systems (Table 5). The antigenicity of environments varies greatly and includes everything from food antigens to microbes and collectively calls upon the immune system to work. Considerable research has focused on the consequences of a germ-free existence, primarily in an attempt to ascertain the role of gut microflora on nutrient dynamics in the host (Muramatsu et al. 1994). It is difficult to separate out the positive effects that gut microbes have on nutrient digestion and metabolism from those negative effects on nutrient balance because their presence in the gut effectively stimulates the immune system as well. Despite these difficulties, numerous studies of this type have provided interesting clues and insights into how exposure to normal aseptic environments challenges the physiology of vertebrate hosts (Table 3). Concentrations of monokines such as IL-1 are greater in laboratory animals raised under unsanitary conditions even when clinical symptoms of infection are not present (Klasing et al. 1987, Klasing 1988, Roura et al. 1991). Similarly, IgG and IgA-secreting lymphocytes in immune organs are substantially increased (ca. 100-fold) in conventional husbandry conditions when compared to germ-free, antigen-free, or specific pathogen-free practices (Hooijkaas et al. 1984, Pereira et al. 1986, Bos et al. 1988, Bakker et al. 1995). Bacteria, especially the normal streptococci inhabitants of the gut, inoculated into germ-free chicks cause a 5–15% depression in growth, which can be ameliorated with antibiotics (Huhtanen and Pensack 1965, Fuller et al. 1979). Germ-free chicks fed diets adequate in energy have lower metabolisable energy intakes, greater rates of protein and energy retention, lower maintenance energy requirements, and ultimately greater rates of growth (5–30%) when compared to conventionally reared chicks (Table 3). Greater efficiency of converting

metabolised energy and protein into production in germ-free chicks and maintenance energy requirements that are 20% greater in conventional chicks both contribute to observed differences in body growth (Furuse and Yokota 1984a, 1985).

The consequences of a germ-free existence appear to differ between rodents and poultry, as well as being strain-dependent (Table 4). Under optimum dietary conditions, the benefits derived from microbial populations in the gut can result in greater body size in conventional rats compared to those reared under germ-free conditions, but an opposite response develops when animals are raised with restrictions in dietary intake (Levenson 1978, Snyder and Wostmann 1987). Most studies have revealed that germ-free rodents have metabolic demands for energy that are 10–30% below levels required by conventional animals which appears to be linked somehow to caecal enlargement (Levenson et al. 1966, Sewell et al. 1975, Yamanaka et al. 1977). However, other studies have demonstrated no such relationship under ad libitum feed conditions (Wostmann et al. 1983).

Negative effects of gut bacteria on animal growth become most remarkable when diet quality is less than optimum (Levenson 1978, Furuse and Yokota 1984a, b). This suggests that maintenance of immunity in the gut has considerable priority over body growth when nutrients, especially protein, are limited in the diet. Such nutrient limitations probably come closer to reflecting the dietary conditions that exist in the habitat of natural vertebrate populations (White 1993). The magnitude of growth suppression in birds under nutritional stress can be dramatic as evidenced by a 78% greater rate of growth in germ-free chicks compared to conventional-reared chicks fed a low-protein diet (Furuse and Yokota 1984a).

Table 3. Metabolic and developmental characteristics of chickens reared in germ-free (GF) or conventional (CV) environments and fed nutritionally adequate or inadequate diets. Differences in body weight (BW) gain, food intake, metabolizable energy (ME), and nutrient retention rates between these two models were obtained from published literature.

Reference and experimental conditions	Parameter	Response measured
Furuse and Yokota (1985) Chicks fed adequate protein diets	BW gain Food intake Protein required for growth Energy required for growth	27% > in GF similar 23% > efficiency in GF 27% > efficiency in GF
Furuse et al. (1985) Chicks fed adequate energy in diets	ME intake BW gain Protein retention	CV > GF 7% > in GF 7% > in GF
Furuse and Yokota (1984a) Chicks fed diets that differed in protein	ME retention Maintenance energy costs	78% > in GF on low protein diets 20% > in CV
Furuse et al. (1991) Chicks fed complete diets with sorbose	Protein retention ME retention BW gain	15% > in GF 17% > in GF 31% > in GF
Fuller et al. (1979) GF chicks were inoculated with bacteria	BW gain	5–15% decline in gnotobiotic
Freeman et al. (1975) Chicks fed a complete basal diet	BW gain	17% > in GF
Huhtanen and Pensack (1965)	BW gain	CV gain was 62–68% of GF

Table 4. Metabolic and developmental characteristics of mammals reared in germ-free (GF) or conventional (CV) environments and fed nutritionally adequate or inadequate diets. Differences in body weight (BW) gain, maintenance energy (ME<sub>m</sub>), efficiency of gain (BW/ME intake), and metabolizable energy (ME) intake and requirement between these two models were obtained from published literature.

Reference and experimental conditions	Parameter	Response measured
Snyder and Wostmann (1987) Wistar rats fed ad lib or 70% ad lib for 2 yr	BW growth (ad lib) BW growth (70%)	CV and GF similar GF 12% > than CV rats at 2 yr
Eggum and Chwalibog (1983) Gut microflora reduced 90% in Wistar rats using antibiotic (GF <sub>r</sub> )	BW gain and ME <sub>m</sub> Efficiency of gain	No difference with control. GF <sub>r</sub> 19–22% > CV rats
Wostmann et al. (1983) Adult Wistar rats in a food balance trial	ME utilization Energy absorption	GF and CV rats similar CV > GF rats
Yamanaka et al. (1977) ICR strain mouse in energy balance trials	ME requirement ME intake	44% more in CV mice GF mice inoculated with <i>Staphylococcus</i> had > intake
Sewell et al. (1975) Wistar rats fed a complete diet	Metabolic rate	20–30% lower in GF rats
Levenson et al. (1966) Fischer rats compared by indirect calorimetry	Rectal temperature Metabolic rate	Lower in GF rats 15–20% lower in GF rats

Livestock and chickens fed selected antibiotics in their feed, especially those residing in unsanitary environments, often show improved growth performance, feed efficiency, and reduced oxygen consumption (Coates et al. 1952, Lev and Forbes 1959, Huhtanen and Pensack 1965, Freeman et al. 1975, Yen et al. 1985). Germ-free chickens do not respond to orally administered antibiotics, while conventional-reared birds can demonstrate a remarkable increase in body mass gain (Freeman et al. 1975). Autoimmune disorders are also capable of impairing growth performance in animals for many of the same reasons (Halliday 1980). Livestock producers have long noted the benefits to body mass gain that can be realised if animals are reared in an environment where microbial challenges are minimised through the use of disinfectants, even when clinical diseases or pathologies do not exist (Klasing and Barnes 1988). Klasing and Barnes (1988) have referred to this continual activation of the immune system as “chronic immunologic stress”. Although the exact mechanisms responsible for the observed antibiotic-induced improvements in growth performance are speculative and have included alterations in microbial metabolism and urease production, it has also been proposed that antibiotic-induced reductions in antigenic challenges allows protein and energy destined for immune surveillance and response (prevention of the catabolic action of immune cytokines) to be reallocated into host production (Roura et al. 1991). It has been suggested that selection for greater body size in poultry may have been achieved in part through trade-offs with the immune system, as heavier breeds respond less to some immune challenges (Cook et al. 1993).

The pathophysiological consequences of infectious disease and parasitism in a vertebrate host can be readily seen with a quick examination of the literature. Several excellent reviews of the subject can be found as it pertains to human health and livestock production (Cooper et al. 1992, Fox 1997). These studies reveal that one of the most widely reported effects of disease (to include parasitism) is suppressed growth, which can lead to reductions in reproductive fitness (Goossens et al. 1997). Catabolic wastage via activation of the acute phase immune response, malabsorption and digestive problems, and nutrient competition with gastrointestinal parasites interact to create a growth-suppressing environment in the host (Elsasser et al. 1988, Nesheim 1993, Solomons 1993). Parasite control in cattle, especially those harbouring substantial loads of parasites, can lead to vast improvements in feed efficiency, weight gain, immunity, carcass quality, reproductive performance, and survival (Hawkins 1993). Similar observations have been made in children from impoverished areas when they are provided antihelminthic treatments (Stephenson 1994).

It is apparent that immune system challenges do not have to be remarkable to alter nutrient dynamics in the host because even rather mild immune reactions like those associated with vaccination can suppress feed intake and development. Pigs vaccinated against porcine respiratory and reproductive syndrome or endotoxin showed a 15% decline in feed intake and 21% decline in daily weight gain during the challenge period (Spurlock et al. 1997). Similarly, lambs vaccinated with irradiated nematode larvae demonstrate a negative correlation between antibody titre and body weight gain

(Wagland et al. 1984). However, not all immune challenges of this type elicit hypermetabolic responses that lead to suppressed growth. Henken and Brandsma (1982) observed essentially no change in maintenance energy requirements or growth in extremely young poultry challenged with an injection of sheep red blood cells (SRBC). It is possible that more developed birds may respond more classically to such a challenge as indicated by Klasing et al. (1987) who observed about a 10% growth suppression in SRBC challenged chicks 3 weeks of age.

The degree to which immunological challenges elicits reductions in fitness among wild vertebrates has not been adequately explored, but the wealth of literature for livestock strongly suggests that such costs could be substantial. We would like to draw attention to the general observation that fitness returns as a function of a physical or physiological factor, such as growth rate or energy expenditure, are rarely linear. Therefore, reported changes in such factors as a result of variable investment in the immune system may over- or underestimate the fitness costs, depending on the shape of the fitness curve. The above observations are extremely relevant to life-history theory where species differ considerably in the pathogenicity of their environments. For example, species with more colonial than solitary social systems may be expected to have evolved mechanisms for devoting more nutrients to immune surveillance and response given the greater likelihood for disease transmission. Such species may benefit greatly from natural reductions in the pathogenicity of their environment or improvements in their nutritional status. Relative growth and reproductive performance would be predicted to be greater in populations residing in less pathogenic environments, as suggested from the livestock literature. Interestingly, rodents raised in germ-free environments outlive their conventionally raised counterparts in the laboratory (Wostmann et al. 1983).

## Conclusions

It is apparent from the above review of the literature that substantial nutritional and energetic costs are associated with immunological stress and maintenance of the system. Host protein metabolism during infection and the nitrogen-limited environments in which they reside may be the most important determinants driving life-history trade-offs among growth, reproduction, and host immunocompetence (Beisel 1977, Lochmiller and Dabbert 1993). Although no single quantitative estimate can be provided for these costs that would be relevant for all vertebrates, it is probably justified to hypothesise that these costs are comparable in magnitude to those for reproduction and growth. We would further predict that life history is as much a reflection of an organism's pathogenic environment as it is any other facet that may be driving complex evolutionary changes within a species, as suggested by Piersma (1997). Consequently, the physiological trade-offs that are necessary in a nutrient-limiting environment involve the seasonal balancing of reproductive, developmental, and immunological costs as dictated by seasonal environmental constraints. Such life-history trade-offs between fitness components are undoubtedly stochastic across seasons as suggested by Nelson and Demas (1996), who offered the "winter immunoenhancement hypothesis" to explain observed declines in reproductive activity and increased immune function in small mammals during stressful winter conditions (Lochmiller and Dabbert 1993, Lochmiller et al. 1994). Given the suggestions of Piersma (1997), one must wonder how much of the geographic variation in life-history attributes such as body size, rates of growth, and reproductive traits (Cameron and McClure 1988, Ims 1997) is attributable to trade-offs with immune function.

It is apparent that we advocate a viewpoint remarkably different from that of Klasing (1998) who suggested that costs of having and maintaining an immune

Table 5. Selected examples of studies examining the responses of avian and mammalian models to the growth-promoting influences of antibiotics that were supplemented in the diet of young growing animals. Percent change reflect comparisons of supplemented animals to a control group and studies reflect considerable variation in types of antibiotics, doses, and exposure duration.

Species	Measurement	Change	Reference
Chicken	Weight gain	+10–34%	Feighner and Dashkevycz (1987)
	Weight gain	+23%	Stutz et al. (1983)
	Efficiency of gain	+10–12%	Begin (1971)
Guinea fowl	Weight gain (12 weeks)	+11–14%	Oguntona (1988)
	Nitrogen retention	+26%	Oguntona (1988)
Swine	Weight gain	+5–9%	Foster et al. (1987)
	Weight gain	0%	Brumm et al. (1989)
	Weight gain	5%	Schrijver et al. (1990)
	Efficiency of gain	10%	Schrijver et al. (1990)



system are minimal to a host. While in terms of absolute mass the immune system appears to be a minor contributor to body mass, such a comparison fails to incorporate the plethora of costs associated with indirect effects of acute-phase responses, anorexia, cellular metabolic rates, and cellular-molecular turnover. To provide an example with another organ system, the gut tissues comprise about 3.7% of body mass in a non-lactating cow, yet because of its high rate of metabolic activity, the liver and gut collectively produce 40% of total heat production in a resting animal (Parker 1990). It may be more appropriate to look at the immune system using an analogy of the female reproductive system where the costs of maintenance are minor compared to the costs of actually using it. Given the antigenicity of environments, having a functional immune system may invariably mean using it upon challenge ("chronic immunologic stress"). If this is true, the benefits to the host of a reduced investment in maintenance of the immune system do not come from the actual economisation of nutrients required to maintain the system, but from avoiding the more costly up-regulation that results from immune challenges. Evolutionary theory predicts that the risks and costs of becoming infected are to be balanced against these benefits. Even if the risk of infection is low, the cost (for example death) may be such that it does not offset the benefits of avoiding the complex and nutritionally costly chain of events invoked by an immune challenge or infection. Similarly, the cost of up-regulation of the system during a response, whether to a benign or potentially deadly infection, may push the animal beyond the minimal levels of bodily reserves to survive. In the first example, the costs of failing to respond (death) is much higher than the nutritional costs of a functional immune response. This in contrast to the second example, where the immune response itself is deadly, independent of whether the pathogen evoking it is lethal or controllable. Thus, the optimal evolutionary strategy concerning the maintenance or down-regulation of the immune system will depend on the pathogenicity of the current environment of the animal, as well as on its body condition and the costs and success of mounting an immune response.

Unravelling the mysteries of life-history evolution can clearly benefit from further studies in the realm of ecological immunology (Sheldon and Verhulst 1996). The primary assumption that immunological defences are costly to the host appear well justified for providing the basis of myriad hypotheses that have been formulated to explain observed patterns in mate selection (Hamilton and Zuk 1982), reproductive strategies (Møller and Erritzøe 1996, Klein and Nelson 1998), sex-specific and seasonal survival rates (Nelson and Demas 1996), habitat use (Piersma 1997), and population regulation (Lochmiller 1996) among vertebrates. Thus, it is becoming increasingly clear that in addition

to growth and reproduction being inherently related life-history traits in vertebrates (Roff 1992), these traits are also inherently related to immunity (hence survival). Understanding of the significance of life history attributes will benefit from further research into ecological immunology.

**Acknowledgements** – Financial support for this research was provided through the National Science Foundation (IBN-9318066), Kerr Foundation of Oklahoma, and the Dept of Zoology, Oklahoma State Univ.

## References

- Bakker, R., Lasonder, E. and Bos, N. A. 1995. Measurement of affinity in serum samples of antigen-free, germ-free and conventional mice after hyperimmunization with 2,4-dinitrophenyl keyhole limpet hemocyanin, using surface plasmon resonance. – *Eur. J. Immunol.* 25: 1680–1686.
- Baracos, V. E., Whitmore, W. T. and Gale, R. 1987. The metabolic cost of fever. – *Can. J. Physiol. Pharmacol.* 65: 1248–1254.
- Begin, J. J. 1971. The effect of antibiotic supplementation on growth and energy utilization of chicks. – *Poult. Sci.* 50: 1496–1500.
- Beisel, W. R. 1977. Magnitude of the host nutritional response to infection. – *Am. J. Clin. Nutr.* 30: 1536–1544.
- Biolo, G., Toigo, G., Ciocchi, B. et al. 1997. Metabolic response to injury and sepsis: changes in protein metabolism. – *Nutrition* 13 (Suppl. 9): 52s–57s.
- Borel, M. J., Buchowski, M. S., Turner, E. A. et al. 1998. Alterations in basal nutrient metabolism increase resting energy expenditure in sickle cell disease. – *Am. J. Physiol., Endocrinol. Metab.* 274: E357–E364.
- Bos, N. A., Meeuwssen, C. G. and Westmann, B. S. 1988. The influence of exogenous antigenic stimulation on the specificity repertoire of background immunoglobulin-secreting cells of different isotypes. – *Cell. Immunol.* 122: 371–380.
- Brumm, M. C., Jesse, G. W., Mayes, H. F. and Clemens, E. T. 1989. Effects of feed or water restriction, antibiotic injection and receiving diet management on commingled feeder pig performance. – *J. Anim. Sci.* 67: 1183–1188.
- Cameron, G. N. and McClure, P. A. 1988. Geographic variation in life history traits of the hispid cotton rat (*Sigmodon hispidus*). – In: Boyce, M. S. (ed.), *Evolution of life histories in mammals: theory and pattern*. Yale Univ. Press, New Haven, CT, pp. 33–64.
- Campos, F., Flores, H. and Underwood, B. 1987. Effect of an infection on vitamin A status of children as measured by relative dose response (RDR). – *Am. J. Clin. Nutr.* 46: 91–94.
- Carlson, G. L., Gray, P., Arnold, J. et al. 1997. Thermogenic, hormonal and metabolic effects of intravenous glucose infusion in human sepsis. – *Brit. J. Surg.* 84: 1454–1459.
- Chiolero, R., Revelly, J. P. and Tappy, L. 1997. Energy metabolism in sepsis and injury. – *Nutrition* 13 (Suppl. 9): 45s–51s.
- Clark, M. A., Hentzen, B., Plank, L. D. et al. 1996. Sequential changes in insulin-like growth factor 1, plasma proteins, and total body protein in severe sepsis and multiple injury. – *J. Parent. Enter. Nutr.* 20: 363–370.
- Clayton, D.H. and Moore, J. 1997. *Host-parasite evolution*. – Oxford Univ. Press, Oxford.
- Coates, M. E., Dickinson, C. D., Harrison, G. F. et al. 1952. A mode of action of antibiotics in chick nutrition. – *J. Sci. Food Agric.* 1: 34–38.
- Cook, M. E., Miller, C. C., Park, Y. and Pariza, M. 1993. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. – *Poult. Sci.* 72: 1301–1309.

- Cooper, A. L., Horan, M. A., Little, R. A. and Rothwell, N. J. 1992. Metabolic and febrile responses to typhoid vaccine in humans: effect of B-adrenergic blockade. – *J. Appl. Physiol.* 72: 2322–2328.
- Cooper, A. L., Brouwer, S., Turnbull, A. V. et al. 1994. Tumor necrosis factor- $\alpha$  and fever after peripheral inflammation in the rat. – *Am. J. Physiol., Regul. Integr. Comp. Physiol.* 267: R1431–R1436.
- Cooper, E. S., Whyte-Alleng, C. A. M., Finizi-Smith, J. S. and MacDonald, T. 1992. Intestinal nematode infections in children: the pathophysiological price paid. – *Parasitology* 104: s91–s103.
- Crouser, E. D. and Dorinsky, P. M. 1996. Metabolic consequences of sepsis: correlation with altered intracellular calcium homeostasis. – *Clin. Chest Med.* 17: 249–261.
- Demas, G. E., Chefer, V., Talan, M. I. and Nelson, R. J. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. – *Am. J. Physiol., Reg. Integr. Comp. Physiol.* 273: R1631–R1637.
- Duke, J. H., Jorgensen, S., Broell, J. R. et al. 1970. Contribution of protein to caloric expenditure following injury. – *Surgery* 68: 168–175.
- Eggum, B. O. and Chwalibog, A. 1983. A study on energy requirement for maintenance and growth in rats with normal or reduced gut flora. – *J. Anim. Physiol. Anim. Nutr.* 49: 104–114.
- Elsasser, T. H., Rumsey, T. S., Hammond, A. C. and Fayer, R. 1988. Influence of parasitism on plasma concentrations of growth hormone, somatomedin-C and somatomedin-binding proteins in calves. – *J. Endocrinol.* 116: 191–200.
- Fearon, D. T. and Locksley, R. M. 1996. The instructive role of innate immunity in the acquired immune response. – *Science* 272: 50–55.
- Feighner, S. D. and Dashkevich, M. P. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytaurine hydrolase activity. – *Appl. Environ. Microbiol.* 53: 331–336.
- Festa-Bianchet, M. 1989. Individual differences, parasites, and the costs of reproduction for Bighorn Ewes (*Ovis canadensis*). – *J. Anim. Ecol.* 58: 785–795.
- Fewell, J. E., Ricciuti, F., Kondo, C. S. and Dascalu, V. 1991. Fever in young lambs: temperature, metabolic and cardiorespiratory responses to a small dose of bacterial pyrogen. – *J. Dev. Physiol.* 15: 229–235.
- Foster, A. G., Baker, D. H., Cline, T. et al. 1987. Effect of efratomylin on gain and feed efficiency for pigs from weaning until market weight. – *J. Anim. Sci.* 65: 877–880.
- Fox, M. T. 1997. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. – *Vet. Parasitol.* 72: 285–308.
- Frankenfield, D. C., Wiles, C. E., Bagley, S. and Siegel, J. H. 1994. Relationships between resting and total energy expenditure in injured and septic patients. – *Crit. Care Med.* 22: 1796–1804.
- Freeman, B. M., Manning, A. C. C., Harrison, G. F. and Coates, M. E. 1975. Dietary aureomycin and the response of the fowl to stressors. – *Br. Poult. Sci.* 16: 395–404.
- Fuller, R., Coates, M. E. and Harrison, G. F. 1979. The influence of specific bacteria and a filterable agent on the growth of gnotobiotic chicks. – *J. Appl. Bacteriol.* 46: 335–342.
- Furuse, M. and Yokota, H. 1984a. Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. – *Br. J. Nutr.* 51: 255–264.
- Furuse, M. and Yokota, H. 1984b. The effect of the gut microflora on growth of chicks fed diets high or marginally adequate in energy. – *Nutr. Rep. Intern.* 29: 1293–1300.
- Furuse, M. and Yokota, H. 1985. Effect of the gut microflora on chick growth and utilization of protein and energy at different concentrations of dietary protein. – *Br. Poult. Sci.* 26: 97–104.
- Furuse, M., Yokota, H. and Tasaki, I. 1985. Influence of energy intake on growth and utilization of dietary protein and energy in germ-free and conventional chick. – *Br. Poult. Sci.* 26: 389–397.
- Furuse, M., Yang, S. I., Choi, Y. H. and Okumura, J. 1991. Energy utilization in germ-free and conventional chicks fed diets containing sorbose. – *Br. Poult. Sci.* 32: 383–390.
- Gandra, Y. R. and Scrimshaw, N. S. 1961. Infection and nutritional status. II. Effect of mild virus infection induced by 17-D yellow fever vaccine on nitrogen metabolism in children. – *Am. J. Clin. Nutr.* 9: 159–163.
- Goossens, B., Osaer, S. and Kora, S. 1997. Long-term effects of an experimental infection with *Trypanosoma congolense* on reproductive performance of trypanotolerant Djallonke ewes and west African dwarf goats. – *Res. Vet. Sci.* 63: 169–173.
- Halliday, R. 1980. Interrelationships between immunity and growth. – In: Lawrence, T. L. (ed.), *Growth in animals*. Butterworths, London, pp. 65–82.
- Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites. – *Science* 218: 384–387.
- Hasselgren, P.-O. and Fischer, J. E. 1998. Sepsis: stimulation of energy-dependent protein breakdown resulting in protein loss in skeletal muscle. – *World J. Surg.* 22: 203–208.
- Hawkins, J. A. 1993. Economic benefits of parasite control in cattle. – *Vet. Parasitol.* 46: 159–173.
- Henken, A. M. and Brandsma, H. A. 1982. The effect of environmental temperature on immune response and metabolism of the young chicken. 2. Effect of the immune response to sheep red blood cells on energy metabolism. – *Poult. Sci.* 61: 1667–1673.
- Hentges, E. J., Marple, D. N., Roland, D. A. and Pritchett, J. F. 1984. Muscle protein synthesis and growth of two strains of chicks vaccinated for Newcastle disease and infectious bronchitis. – *Poult. Sci.* 63: 1738–1741.
- Hobler, S. C., Williams, A. B., Fischer, J. E. and Hasselgren, P.-O. 1998. IGF-I stimulates protein synthesis but does not inhibit protein breakdown in muscle from septic rats. – *Am. J. Physiol., Reg. Integr. Comp. Physiol.* 274: R571–R576.
- Hooijkaas, H., Benner, R., Pleasants, J. R. and Wostmann, B. S. 1984. Isotypes and specificities of immunoglobulins produced by germ-free mice fed chemically defined ultrafiltered “antigen-free” diet. – *Eur. J. Immunol.* 14: 1127–1130.
- Huhtanen, C. N. and Pensack, J. M. 1965. The role of *Streptococcus faecalis* in the antibiotic growth effect of chickens. – *Poult. Sci.* 44: 830–834.
- Ims, R. A. 1997. Determinants of geographic variation in growth and reproductive traits in the root vole. – *Ecology* 78: 461–470.
- Jin, M. B., Shimahara, Y., Yamaguchi, T. et al. 1995. The effect of a bolus injection of TNF- $\alpha$  and IL-1 $\beta$  on hepatic energy metabolism in rats. – *J. Surg. Res.* 58: 509–515.
- Klasing, K. C. 1988. Nutritional aspects of leukocytic cytokines. – *J. Nutr.* 118: 1434–1446.
- Klasing, K. C. 1998. Nutritional modulation of resistance to infectious diseases. – *Poult. Sci.* 77: 1119–1125.
- Klasing, K. C. and Barnes, D. M. 1988. Decreased amino acid requirements of growing chicks due to immunologic stress. – *J. Nutr.* 118: 1158–1164.
- Klasing, K. C. and Johnstone, B. 1991. Monokines in growth and development. – *Poult. Sci.* 70: 1781–1789.
- Klasing, K. C., Laurin, D. E., Peng, R. K. and Fry, D. 1987. Immunologically mediated growth depression in chicks: influence of feed intake, corticosterone and interleukin-1. – *J. Nutr.* 117: 1629–1637.
- Klein, S. L. and Nelson, R. J. 1998. Adaptive immune responses are linked to the mating system of arvicoline rodents. – *Am. Nat.* 151: 59–67.
- Klurfeld, D. M. 1993. *Nutrition and immunology*. – Plenum Press, New York.

- Kreymann, G., Grosser, S., Gottschall, P. et al. 1993. Oxygen consumption and resting metabolic rate in sepsis, sepsis syndrome, and septic shock. – *Crit. Care Med.* 21: 1012–1019.
- Kyriazakis, I., Tolkamp, B. J. and Hutchings, M. R. 1998. Towards a functional explanation for the occurrence of anorexia during parasitic infections. – *Anim. Behav.* 56: 265–274.
- Lee, K. and Reid, I. S. 1977. The effect of Marek's disease vaccination and day-old debeaking on the performance of growing pullets and laying hens. – *Poult. Sci.* 56: 736–740.
- Lev, M. and Forbes, M. 1959. Growth response to dietary penicillin of germ-free chicks and of chicks with a defined intestinal flora. – *Br. J. Nutr.* 13: 78–84.
- Levenson, S. M. 1978. The influence of the indigenous microflora on mammalian metabolism and nutrition. – *J. Parent. Enter. Nutr.* 2: 75–107.
- Levenson, S. M., Kan, D., Lev, M. and Dofst, F. 1966. Oxygen consumption, carbon dioxide production and body temperature in germ-free rats. – *Fed. Proc.* 25: 482.
- Lochmiller, R. L. 1996. Immunocompetence and animal population regulation. – *Oikos* 76: 594–602.
- Lochmiller, R. L. and Dabbert, C. B. 1993. Immunocompetence, environmental stress, and the regulation of animal populations. – *Trends Comp. Biochem. Physiol.* 1: 823–855.
- Lochmiller, R. L., Vestey, M. R. and McMurry, S. T. 1994. Temporal variation in humoral and cell-mediated immune function in a *Sigmodon hispidus* populations. – *Ecology* 75: 236–245.
- Long, C. L. 1977. Energy balance and carbohydrate metabolism in infection and sepsis. – *Am. J. Clin. Nutr.* 30: 1301–1310.
- Loye, J. E. and Zuk, M. 1991. Bird-parasite interactions. – Oxford Univ. Press, Oxford.
- McIntyre, J. and Hull, D. 1996. Metabolic rate in febrile infants. – *Arch. Dis. Child.* 74: 206–209.
- Michie, H. R. 1996. Metabolism of sepsis and multiple organ failure. – *World J. Surg.* 20: 460–464.
- Møller, A. P. and Erritzøe, J. 1996. Parasite virulence and host immune defense: host immune response is related to nest reuse in birds. – *Evolution* 50: 2066–2072.
- Muramatsu, T., Nakajima, S. and Okumura, J. 1994. Modification of energy metabolism by the presence of the gut microflora in the chicken. – *Br. J. Nutr.* 71: 709–717.
- Nelson, R. J. and Demas, G. E. 1996. Seasonal changes in immune function. – *Q. Rev. Biol.* 71: 511–548.
- Nesheim, M. C. 1993. Human nutrition needs and parasitic infections. – *Parasitology* 107: s7–s18.
- Newsholme, P. and Newsholme, E. A. 1989. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. – *Biochem. J.* 261: 211–218.
- Nordling, D., Andersson, M., Zohari, S. and Gustafsson, L. 1998. Reproductive effort reduces specific immune response and parasite resistance. – *Proc. R. Soc. Lond. B* 265: 1291–1298.
- Norris, K., Anwar, M. and Read, A.F. 1994. Reproductive effort influences prevalence of haematzoan parasites in great tits. – *J. Anim. Ecol.* 63: 601–610.
- Oguntona, T. 1988. Studies on the response of guinea fowls (*Numida meleagris*) to antibiotics. – *Br. Poult. Sci.* 29: 683–687.
- Oppliger, A., Christe, P. and Richner, H. 1996. Clutch size and malaria resistance. – *Nature* 381: 565.
- Parker, D. S. 1990. Manipulation of the functional activity of the gut by dietary and other means (antibiotics/probiotics) in ruminants. – *J. Nutr.* 120: 639–648.
- Pereira, P., Forni, L., Larsson, E. L. et al. 1986. Autonomous activation of B and T cells in antigen-free mice. – *Eur. J. Immunol.* 16: 685–688.
- Piersma, T. 1997. Do global patterns of habitat use and migration strategies coevolve with relative investments in immunocompetence due to spatial variation in parasite pressures. – *Oikos* 80: 623–631.
- Richner, H., Christe, P. and Oppliger, A. 1995. Paternal investment affects prevalence of malaria. – *Proc. Natl. Acad. Sci. USA* 92: 1192–1194.
- Rivera, D. L., Ollister, S. M., Liu, X. et al. 1998. Interleukin-10 attenuates experimental fetal growth restriction and demise. – *FASEB J.* 12: 189–197.
- Roe, C. and Kinney, J. 1965. The caloric equivalent of fever. II. Influence of major trauma. – *Ann. Surg.* 161: 140–148.
- Roff, D. 1992. Evolution of life histories. – Chapman and Hall, New York.
- Roura, E., Homedes, J. and Klasing, K. C. 1991. Potential mechanisms of action of antibiotics as growth promoters: modulation of immunological stress. – *Poult. Sci.* 70 (suppl. 1): 102.
- Schetter, T. A., Lochmiller, R. L., Leslie, D. M. et al. 1998. Examination of the nitrogen limitation hypothesis in non-cyclic populations of cotton rats (*Sigmodon hispidus*). – *J. Anim. Ecol.* 67: 705–721.
- Schrijver, R. De, Moreels, A. and Fremaut, D. 1990. Supplementing salinomycin to diets for growing-finishing pigs. – *Dtsch. Tierärztl. Wochen.* 97: 520–522.
- Scrimshaw, N. S. 1977. Effect of infection on nutrient requirements. – *Am. J. Clin. Nutr.* 30: 1536–1544.
- Scrimshaw, N. S. 1991. Effect of infection on nutrient requirements. – *J. Parent. Enter. Nutr.* 15: 589–600.
- Sewell, D. L., Wostman, B. S., Gairola, G. and Aleem, M. 1975. Oxidative energy metabolism in germ-free and conventional rat liver mitochondria. – *Am. J. Physiol.* 228: 526–529.
- Sheldon, B. C. and Verhulst, S. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. – *Trends Ecol. Evol.* 11: 317–321.
- Siikamäki, P., Ratti, O., Hovi, M. and Bennett, G. F. 1997. Association between haematzoan infections and reproduction in the Pied flycatcher. – *Funct. Ecol.* 11: 176–183.
- Snyder, D. L. and Wostmann, B. S. 1987. Growth rate of male germfree Wistar rats fed ad libitum or restricted natural ingredient diet. – *Lab. Anim. Sci.* 37: 320–325.
- Solomons, N. W. 1993. Pathways to the impairment of human nutritional status by gastrointestinal pathogens. – *Parasitology* 107: s519–s535.
- Spurlock, M. E., Frank, G. R., Willis, G. M. et al. 1997. Effect of dietary energy source and immunological challenge on growth performance and immunological variables in growing pigs. – *J. Anim. Sci.* 75: 720–726.
- Stephenson, C. B., Alvarez, J. O., Kohatsu, J. et al. 1994. Vitamin A is excreted in the urine during acute infection. – *Am. J. Clin. Nutr.* 60: 388–392.
- Stephenson, L. S. 1994. Helminth parasites, a major factor in malnutrition. – *World Health Forum* 15: 169–172.
- Stutz, M. W., Johnson, S. L., Judith, F. R. and Miller, B. 1983. In vitro and in vivo evaluations of the antibiotic efrotomycin. – *Poult. Sci.* 62: 1612–1618.
- Svensson, E., Raberg, L., Koch, C. and Hasselquist, D. 1998. Energetic stress, immunosuppression and the cost of an antibody response. – *Funct. Ecol.* 12: 912–919.
- Tanner, H. A. 1992. Energy transformations in the biosynthesis of the immune system: their relevance to the progression and treatment of AIDS. – *Med. Hypoth.* 38: 315–321.
- Taylor, D. E. and Piantadosi, C. A. 1995. Oxidative metabolism in sepsis and sepsis syndrome. – *J. Crit. Care* 10: 122–135.
- Tocco-Bradley, R., Georgieff, M., Jones, C. T. et al. 1987. Changes in energy expenditure and fat metabolism in rats infused with interleukin-1. – *Eur. J. Clin. Invest.* 17: 504–510.
- Tsigos, C., Papanicolaou, D. A., Defensor, R. et al. 1997. Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. – *Neuroendocrinology* 66: 54–62.
- Wagland, B. M., Steel, J. W., Windon, R. G. and Dineen, J. K. 1984. The response of lambs to vaccination and chal-

- lenge with *Trichostrongylus columbriformis*: effects of plane of nutrition on, and the inter-relationship between, immunological responsiveness and resistance. – Int. J. Parasitol. 14: 39–44.
- White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. – Springer-Verlag, New York.
- Wobeser, G. and Wobeser, A. G. 1992. Carcass disappearance and estimation of mortality in a simulated die-off of small birds. – J. Wildl. Dis. 28: 548–554.
- Wostmann, B. S., Larkin, C., Moriarty, A. and Bruckner-Kardoss, E. 1983. Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. – Lab. Anim. Sci. 33: 46–50.
- Yamanaka, M., Nomura, T. and Kametaka, M. 1977. Influence of intestinal microbes on heat production in germ-free, gnotobiotic and conventional mice. – J. Nutr. Sci. Vitaminol. 13: 221–226.
- Yen, J. T., Neinaber, J. A., Pond, W. G. and Varel, V. H. 1985. Effect of carbadox on growth, fasting metabolism, thyroid function and gastrointestinal tract in young pigs. – J. Nutr. 115: 970–979.
- Zuk, M. 1996. Disease, endocrine-immune interactions, and sexual selection. – Ecology 77: 1037–1042.