

**THERMOREGULATORY
CONSEQUENCES OF
STARVATION AND
DIGESTION IN BIRDS**

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LAURILA**

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AND DIGESTION IN BIRDS**

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Abstract

In homeothermic birds and mammals, several thermoregulatory adaptations have evolved for surviving in unstable, food-restricted conditions. This study focuses on two adaptive mechanisms in pigeons (*Columba livia*) and quails (*Coturnix coturnix japonica*): hypothermia and the adaptive use of obligatory heat production connected with feeding and digestion. The plasticity of the hypothermic response in fed and fasted birds and birds with restricted feeding was studied in laboratory and outdoor winter conditions. The other objective was to study adaptive timing of digestion, and substitution of facultative thermogenesis by obligatory heat production in cold and at thermoneutrality.

The results showed that fasting has a strong influence on the level of nocturnal hypothermia in laboratory conditions: hypothermia becomes progressively deeper when fasting continues. In outdoor conditions, ambient temperature and predation risk modulated the daily body temperature (T_b) pattern of fasting pigeons. In very cold conditions, diurnal T_b of fasted birds also dropped below the normal level of the active phase. Predation risk prevented diurnal hypothermia but also attenuated the depth of nocturnal hypothermia in fasting pigeons. This study provides the first empirical effects of predation risk on hypothermia in starving birds.

The study suggests that the presence of crop in pigeons allows adaptive timing of digestion. At thermoneutrality, peak digestion appeared late in the dark phase in birds with fed in the morning. Because the T_b of the birds increases to diurnal levels late in the dark phase, this obligatory heat from digestion can be used to aid re-warming by such timing. On the other hand, the results of this study were partly opposite to the classical model of thermoregulatory substitution. In line with the classical model, a postprandial increase in metabolic rate (heat increment of feeding, HIF) was seen at thermoneutrality but not in cold. However, electromyographic measurements showed that there was no postprandial decrease in the intensity of shivering in the fed birds in cold. This indicates that true thermoregulatory substitution may be less common than assumed and suggests a role for facultative thermogenesis in HIF.

Keywords: birds, digestion, facultative thermogenesis, fasting, heat increment of feeding (HIF), hypothermia, obligatory thermogenesis, predation, shivering, substitution

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Oulu, February 2005

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Abbreviations

BAT	brown adipose tissue
BMR	basal metabolic rate
DIT	diet-induced thermogenesis
DRT	digestion-related thermogenesis
EMG	electromyogram
HIF	heat increment of feeding
NST	non-shivering thermogenesis
RQ	respiratory quotient
SDA	specific dynamic action of feeding
SDE	specific dynamic effect of feeding
ST	shivering thermogenesis
T_a	ambient temperature
T_b	body temperature
$T_{b(\min)}$	T_b minimum
UCP	uncoupling protein
VO_2	oxygen consumption

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Laurila M, Pilto T & Hohtola E (2005) Testing the flexibility of fasting-induced hypometabolism in birds: effect of photoperiod and repeated food deprivations. *J Therm Biol* 30:131-138.
- II Laurila M & Hohtola E (2005) The effect of ambient temperature and simulated predation risk on fasting-induced nocturnal hypothermia of pigeons in outdoor conditions. *J Therm Biol* (*accepted*).
- III Laurila M, Hohtola E, Saarela S & Rashotte ME (2003) Adaptive timing of digestion and digestion-related thermogenesis in the pigeon. *Physiol Behav* 78:441-448.
- IV Laurila M, Impiö K & Hohtola E (2005) Heat increment of feeding in the pigeon: the role of crop and facultative thermogenesis. *J Comp Physiol* (*submitted*).

In addition, this thesis also includes some original unpublished data.

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1 Introduction

Homeothermic birds and mammals maintain their body temperature (T_b) within a narrow range. The stable T_b of endothermic homeotherms is based on metabolic heat production: they have a high basal metabolic rate and active intrinsic thermogenesis, unlike poikilotherms, whose ability to regulate T_b depends on ambient temperature (T_a). In addition, the strictly regulated T_b of homeothermic animals requires controlled heat dissipation (see Scholander *et al.* 1950c, Stevens 1973). The balance between heat production and heat dissipation is possible by numerous autonomic and behavioural thermal responses that are controlled by the central nervous system (Simon *et al.* 1986, Marsh & Dawson 1989). In the cold, metabolic heat production increases and thermal conductance diminishes. The heat production ensues from all vital obligatory processes in the body (e.g., activity of enzymes in cells, or digestion and absorption of nutrients), but also from facultative mechanisms, muscular thermogenesis (shivering and gross activity of the muscles; Scholander *et al.* 1950b) and high metabolism of brown fat tissues (non-shivering thermogenesis in mammals). For lowering the heat loss, the endothermic animal increases insulation by pilo- or ptiloerection, balling up and decreasing the circulation in the skin (Scholander *et al.* 1950c, Steen 1958).

An endothermic lifestyle is very costly. Endotherms have a high metabolic rate and thus also high food demand and consumption. There is a continuous trade-off between feeding and starvation and the costs related to them, in addition to which choices are made in terms of energy reserves: whether energy is used for facultative thermogenesis or for other reactions of the body in cold conditions. Several thermoregulatory adaptations have evolved for surviving in food-restricted conditions. In birds, these include nocturnal hypometabolism and hypothermia that enable metabolic savings (see Steen 1958). Another mechanism of adaptation is to utilise the extra heat produced by the obligatory processes of the body (e.g., the processes of digestion). This thesis views shallow nocturnal hypothermia and the adaptive use of digestion as mechanisms for saving energy and thus increasing survival.

2 Review of literature

2.1 Body temperature, hypothermia and hypometabolism in birds

The energetically expensive endothermic lifestyle requires continuous balancing between accumulation of energy reserves and energy consumption. Maintaining a stable deep body temperature in unstable environmental conditions involving variable foraging success consumes a large amount of energy reserves. Endothermic animals have developed several mechanisms for saving energy: thermoregulatory mechanisms, other than behaviour and insulation, that can save energy in starved endotherms are hibernation, torpor and shallow regulated rest-phase hypothermia and hypometabolism.

Regulated hypothermia is a phenomenon in which deep body temperature (T_b) is below its normal range during the inactive phase (see Calder & King 1974, IUPS 2001). Rest-phase hypothermia, hypothermia during the sleeping phase, is not a collapse of thermoregulation but a controlled decrease of T_b (see Chaplin 1976). True torpor, by contrast, is a state of inactivity and depressed responsiveness to external stimuli (IUPS 2001). It may also be referred to as profound hypothermia where heart and respiratory rates and metabolism are greatly reduced and where coordination and alertness are absent (see Calder & King 1974). According to Calder and King (1974), hibernation is a state of torpor throughout a long period of food deficiency in the winter (see also Nedergaard & Cannon 1990, Buck & Barnes 2000). It can be defined as a winter lethargy in some homeothermic animals in which T_b and metabolism diminish (IUPS 2001). The difference between controlled nocturnal hypothermia and torpor is not unambiguous. McKechnie and Lovegrove (2002) discuss the most prevalent controversial issues concerning the differences between these two phenomena. Some studies support the view that nocturnal hypothermia and torpor represent a physiological response continuum (see Reinertsen 1996, McKechnie & Lovegrove 2002), while others describe these phenomena as discrete physiological states (see Prinzinger *et al.* 1991, Merola-Zwartjes & Ligon 2000, McKechnie & Lovegrove 2002). This question is difficult to answer. Some attempts to define these states have, however, been made. Prinzinger and co-authors (1991) have suggested that a nocturnal T_b minimum ($T_{b(\min)}$) of 25°C is the limit between hypothermia and torpor. Reinertsen (1996) has argued that $T_{b(\min)} < 30^\circ\text{C}$

indicates torpor. In some species, however, $T_{b(\min)}$ of non-torpid birds can diminish below 25°C, and T_b of torpid birds may range above 25°C (see Merola-Zwartjes & Ligon 2000, McKechnie & Lovegrove 2002). McKechnie and Lovegrove (2002) have also shown that the metabolic rate during hypothermic states is useless in distinguishing nocturnal hypothermia from torpor, although T_b during hypothermia depends on the level of metabolism (see Scholander *et al.* 1950b). Metabolic rates in hypothermia have not been investigated in many bird species and thus metabolic rate as a criterion for distinguishing between hypothermia and torpor cannot be definitely interpreted. The difference between rest-phase hypothermia and torpor is best described by the relationship between metabolic rate, T_b and T_a and also by the relationship between physiological state and the ability of a bird to respond to external stimuli (McKechnie & Lovegrove 2002).

Three distinct phases can be seen in the physiological state during rest-phase hypothermia in which alertness of birds is slightly diminished even though responsiveness to stimuli is retained (see McKechnie & Lovegrove 2002). 1) During the entry phase, T_b decreases due to metabolic downregulation. 2) T_b remains low but constant during the maintenance phase. 3) During the arousal phase, T_b increases by metabolic heat production. However, the pattern of hypothermia varies within bird species and between individuals: a single phase may be lacking, another may be remarkably long or short. The variation of rest-phase hypothermia may be due e.g., to communal roosting behaviour and clustering (McKechnie & Lovegrove 2001, 2002).

Nocturnal hypothermia is a mechanism for decreasing energy expenditure and an adaptation to unstable environmental conditions (Scholander 1950a, Steen 1958, Chaplin 1976, Reinertsen 1983, McKechnie & Lovegrove 2002). It has an important role in avian energy balance: birds use rest-phase hypothermia together with other mechanisms (increasing of insulation, huddling, being motionless, seeking shelter) in order to save energy to survive during the night (Scholander 1950a, b, c, Steen 1958, Reinertsen 1983). However, energetic stress is not always a prerequisite for the occurrence of hypothermia: it may also be used to speed up fat accumulation prior to migration, e.g., in the hummingbird (*Selasphorus rufus*; Carpenter & Hixon 1988) or barnacle goose (*Branta leucopsis*; Butler & Woakes 2001). The amount of energy spared depends on the depth of hypothermia, its duration and costs of re-warming (Reinertsen 1983). T_b during the night cannot diminish indefinitely; in small arctic passerine birds nocturnal T_b does not decrease below 30°C (Reinertsen 1983). Non-energetic costs (e.g., predation risk) or physiological restrictions may prevent further decrease in T_b (Hohtola *et al.* 1991). The depth of hypothermia is set as a result of a compromise: energy is saved only to the extent where a sufficient state of alertness can be maintained. However, during the long nights the increased duration of nocturnal hypothermia permits saving of energy without a very strong decrease in nocturnal T_b . The third factor that has an influence on the amount of energy saving in rest-phase hypothermia is re-warming. The increase in T_b in the arousal phase occurs by energy-requiring shivering thermogenesis in birds. If nocturnal T_b is extremely deep, birds have to shiver more intensely for re-warming. On the other hand, as it takes more time to increase T_b to the level of the active phase, thus the plateau phase (2), the energy saving phase, of nocturnal hypothermia becomes shorter. In some species (e.g., Alaskan chickadee, Grossman & West 1977, see Reinertsen 1983), hypothermia is not useful for energy economics because of high re-warming costs.

Rest-phase hypothermia occurs throughout the avian phylogeny and especially across the entire range of avian body mass (Prinzinger *et al.* 1991, McKechnie & Lovegrove 2002). The largest bird species in which deep hypothermia has been found are cloven-feathered dove (*Drepanoptila holosericea*, 200 g; Schleucher 2001) and tawny frogmouth (*Podargus strigoides*, 500 g; Körtner *et al.* 2000). In smaller bird species, the drop of nocturnal T_b is deeper than in larger bird species during hypothermia (McKechnie & Lovegrove 2002). Interestingly, T_b of the active phase decreases with increasing body mass in birds, but not in mammals (Prinzinger *et al.* 1991). The reason may be the high cost of flight in small birds: a small bird needs much more energy for flight than a mammal of the same-size needs for walking or running. Some studies suggest that in birds, body mass may correlate with nocturnal T_b (e.g., willow tit, *Parus montanus*; Reinertsen 1982), or that body weight has no influence on the depth of nocturnal hypothermia (e.g., black-capped chickadee, *Parus atricapillus*; Chaplin 1976). In large birds, both the decrease in T_b to the nocturnal level and re-warming take a lot of time, and thus deep hypothermia is restricted to a maximum of few hours (Schleucher 2001). There are also other factors than body mass that affect the level of nocturnal hypothermia. Photoperiod, ambient temperature, feeding success (see Reinertsen 1983, McKechnie & Lovegrove 2002), breeding cycle (see Prinzinger *et al.* 1991, McKechnie & Lovegrove 2002), migration (Carpenter & Hixon 1988, Butler & Woakes 2001) and predation risk (Pravosudov & Lucas 2000, 2001, Welton *et al.* 2002) have an effect on rest-phase T_b . In the following, factors that modulate hypothermia are discussed.

2.1.1 Effect of photoperiod on T_b

Many physiological functions have a connection with photoperiod: it is an environmental cue and evokes changes in reproduction and secretion of sex hormones, body mass, coloration of fur as well as nesting and burrowing activity (see the review Heldmaier *et al.* 1989a). The amplitude of T_b circadian rhythm also varies according to photoperiod. For example, in black-capped chickadee (Chaplin 1976) and willow tit (Reinertsen & Haftorn 1983) seasonal changes influence the level of nocturnal T_b . When the light phase becomes shorter, the circadian fluctuation of T_b is enhanced (Heldmaier *et al.* 1989a, Basco *et al.* 1996), and stable nocturnal T_b is also replaced by an increased number of ultradian variations in the short photoperiod (Heldmaier *et al.* 1989a). On the other hand, in continuous bright light, a non-cyclic decrease in T_b has been observed in birds (Berger & Phillips 1988), whereas in constant darkness, the circadian rhythm of T_b is preserved with diurnal hypothermia (in subjective daytime) during fasting (Underwood *et al.* 1999).

In short-day conditions, diurnal T_b of pigeons is higher and nocturnal T_b lower than in long-day conditions (Basco *et al.* 1996). An increase of diurnal O_2 consumption and a higher RQ (respiratory quotient) have also been observed in birds in short-day conditions. This is probably because birds have less time available for feeding, and therefore foraging intensity increases and the entire daily food load is ingested during a few hours. The long night may result in energy depletion, and birds save energy by decreasing their nocturnal metabolism and T_b in the long nights. However, the high RQ shows that some

birds can retain extra food in the crop and thus use nocturnal digestion to regulate their T_b (see e.g., Basco *et al.* 1996, Boon *et al.* 2000; this will be discussed in chapter 2.2.2). It has also been shown that daily body weight and energy gain, which interact with T_b , depend on the length of photoperiod via food availability (Boon *et al.* 2000).

Melatonin, a hormone synthesised largely in the pineal gland, drives circadian and seasonal rhythms via suprachiasmatic nuclei and hypothalamus (Saarela & Reiter 1994). Both photoperiod and the amount of light have an effect on melatonin excretion. Thus melatonin functions as a signal of seasonal environmental changes for organs to adapt to photoperiod changes. Besides having an influence on circadian rhythms, melatonin participates in cold acclimation and improves cold tolerance in mammals (Heldmaier *et al.* 1985) and in birds (Saarela & Heldmaier 1987). In mammals, melatonin as well as short photoperiod augments thermogenesis in brown adipose tissues (BAT): its metabolism, the amount of mitochondria and enzyme activity increase in BAT (Heldmaier *et al.* 1985). In adrenal glands and BAT, both melatonin and short photoperiod enhance synthesis of catecholamines, mediators of thermogenesis (see Heldmaier *et al.* 1985). Melatonin also has an influence of the gastrointestinal system: e.g., melatonin enhances contraction in muscles of a crop and caeca (Poon *et al.* 1997).

2.1.2 Effect of ambient temperature on T_b

When ambient temperature (T_a) is lower than thermoneutral, the difference between T_b and T_a and heat loss (radiation, convection, conduction) increases by insulative adjustments. Thermoneutrality is a zone of T_a at which T_b is regulated without active metabolic heat production or evaporative heat loss (IUPS 2001). Below thermoneutrality, heat production starts by shivering thermogenesis, non-shivering thermogenesis or, in some instances, by other heat production mechanisms (see thermogenesis later in chapter 2.2). Animals have to maintain the diurnal/active phase T_b for foraging, reacting to environmental stimuli and escaping predators. In the cold, the level of energy consumption becomes higher and thus a high diurnal T_b is an energetic cost. Minimisation of energy consumption and saving of energy by a low T_b is usually only possible in the scotophase (dark phase). Normally, diurnal T_b remains at a constant level despite large decreases in T_a (e.g., in siskins and greenfinches, Saarela *et al.* 1995). Also, when T_a ranges from cold to warm (above thermoneutrality), no significant changes in diurnal T_b in quails are seen (Spiers *et al.* 1983), but in acute cold exposure, the core T_b of pigeons seems to increase distinctly (Østnes & Bech 1998). The same has been observed in Pekin ducklings (Østnes & Bech 1997).

In some bird species, the depth of nocturnal hypothermia is correlated with prevailing T_a (Reinertsen 1983, Reinertsen & Haftorn 1983). When T_b decreases in very cold conditions, the difference between T_b and T_a diminishes and thus heat loss is reduced. This allows a decrease in metabolic rate and results in energy savings. The level of nocturnal T_b depends on T_a in Siberian tit (Haftorn 1972), willow tit (Reinertsen & Haftorn 1986) and black-capped chickadee (Chaplin 1976), but not in great tit, common redpoll (Reinertsen & Haftorn 1986) and greenfinch (Saarela *et al.* 1995). In non-

passerines, Hohtola and co-workers (1991) have shown that in quails, nocturnal hypothermia is not augmented by low T_a . Obviously, some physiological constraints prevent further decrease in T_b .

Variation of T_a is a less reliable environmental cue for seasonal acclimatisation than photoperiod (see Heldmaier *et al.* 1985). In winter- or cold-acclimatised birds, thermal insulation is significantly better and thermogenic capacity improves. Thus, cold limit, the lowest T_a at which a bird can maintain its T_b , is remarkable lower (Saarela *et al.* 1995). During acclimatisation, blood circulation changes in extremities, and the mass of feathers, subcutaneous fat and the amount of available free fatty acids and carbohydrates increase (Saarela & Heldmaier 1987). Also in cold-acclimatised small mammals, metabolic capacity in brown adipose tissue (non-shivering thermogenesis) increases and thus cold tolerance improves (Heldmaier *et al.* 1985).

2.1.3 Effect of nutrition and foraging success on T_b

Feeding success is very variable in precarious environmental conditions. Animals may face periods of food deprivation and even starvation. Most homeothermic species become starved even after a few days of fasting. There are, however, species that have adapted to long-term fasting: e.g., penguins fast several months during breeding, and bears fast during the winter sleep (Le Maho 1977, 1983, Cherel *et al.* 1987, 1988, Castellini & Rea 1992). Castellini and Rea (1992) discussed the difference between fasting and starvation: in fasting, metabolic homeostasis is maintained but during starvation, “homeostatic control is lost and critical organ function becomes compromised”. However, fasting endurance depends on body mass and the amount of endogenous fat reserves in all species (Millar & Hickling 1990). Consequently, starvation (or fasting), compromises and survival expedient connecting with T_b are covered in non-fasting adapted birds.

Feeding success and nutritional state have an influence on the depth of hypothermia in birds (Chaplin 1976, Reinertsen & Haftorn 1983, 1986, Graf *et al.* 1989, Rashotte *et al.* 1989, Prinzing *et al.* 1991, Ostheim 1992) but also in mammals (e.g., Yoda *et al.* 2000). The level of hypothermia depends on the amount of food eaten: the more a bird eats during the light phase, the less is the probability of hypothermia in scotophase (Rashotte *et al.* 1989). The level of nocturnal hypothermia is re-set with the state of energy reserves (see e.g., Hohtola *et al.* 1991) and via humoral or neural signals from the gastrointestinal system (Reinertsen & Bech 1994), but hypothermia in the dark phase is also due to re-setting of thermoregulatory system in the central nervous system (Graf *et al.* 1989). As fasting continues, nocturnal T_b decreases in a stepwise fashion but diurnal T_b is maintained at a constant level in birds (e.g., Graf *et al.* 1989, Hohtola *et al.* 1991, Ostheim 1992, Underwood *et al.* 1999) and in mammals (e.g., Yoda *et al.* 2000). During shortage of food, metabolism decreases and thus T_b declines, and this is an evident strategy for saving energy in both bird and mammals. According to general view, food deprivation has no effect on diurnal T_b because diurnal metabolism and T_b need to be maintained at normal level for foraging, reacting to environmental stimuli and escaping predators. In some studies, a very long fasting period (21 days) in non-fasting-adapted

birds causes a notable decline of diurnal T_b (Sartori *et al.* 1995) and on the other hand, in constant darkness T_b decreases in all phases of the circadian rhythm resulting from the lack of the masking effect of light (Underwood *et al.* 1999).

2.1.4 Predation risk and nocturnal hypothermia

Birds that face unstable environmental conditions or variable foraging success use nocturnal hypothermia as a mechanism of energy sparing. However, nocturnal hypothermia has its costs. These comprise, among other things, the energy and time required for re-warming (e.g., Reinertsen 1983) and the nocturnal predation risk resulting from a lower state of alertness (Reinertsen & Haftorn 1986, Reinertsen 1996, Welton *et al.* 2002). If the birds cannot use nocturnal hypothermia, they may resort to accumulating more fat reserves for the night (Pravosudov & Lucas 2000). On the other hand, fat reserves are not cost-free: maintenance and carrying of fat is expensive, manoeuvrability declines or diurnal predation risk increases (Gosler *et al.* 1995, Pravosudov & Lucas 2000). Empirical test effects have not been reported on the predation risk on T_b , but Pravosudov and Lucas (2000) and Welton and co-authors (2002) have created a dynamic model of nocturnal hypothermia that predicts advantages and disadvantages of hypothermia in relation to e.g., predation and feeding success. According to the dynamic models, birds have to balance between the costs of starvation, diurnal/nocturnal predation risk, maintenance of massive fat reserves and nocturnal hypothermia. Changes of predation risk during the day influence the pattern of energy/fat accumulation (Pravosudov & Lucas 2001), and the decision of using nocturnal hypothermia depends on the amount of energy reserves (Pravosudov & Lucas 2000). If foraging time is too short to accumulate energy, food supply is scarce or nocturnal predation risk is low, the birds use nocturnal hypothermia for sparing energy (Pravosudov & Lucas 2000, Welton *et al.* 2002). If diurnal predation risk is high, the birds reduce foraging and energy accumulation and use nocturnal hypothermia. If the birds are not able to use hypothermia in the scotophase, they gather more fat reserves, thus facing mass-dependent predation risk (Welton *et al.* 2002). Bird species may have different tactics of using hypothermia in different environmental conditions.

2.2 Thermogenic function of digestion

When T_a falls below thermoneutrality the basal metabolic rate (BMR) cannot maintain T_b and thermoregulatory heat production mechanisms are required to compensate for the increased heat loss. Thermogenesis can be divided into two categories, facultative thermogenesis and obligatory thermogenesis (see e.g., Hohtola 2002). Facultative thermogenesis contains shivering thermogenesis (ST) and non-shivering thermogenesis (NST), and obligatory thermogenesis comprises BMR, activity and heat production from feeding, digestion and faecal excretion. Obligatory processes in the body produce heat

that either has to be dissipated into the environment or may be used to substitute for facultative thermogenesis. Heat production is usually measured by indirect calorimetry, i.e., registering oxygen consumption (VO_2). In the following, facultative thermogenesis is reviewed briefly and then obligatory thermogenesis connected to feeding and digestion is discussed in detail.

2.2.1 *Facultative thermogenesis*

Shivering thermogenesis (ST) is the major thermogenic mechanism in adult birds (e.g., Hohtola 2004) and nestlings (Marjoniemi & Hohtola 1999). ST is non-voluntary electrical activity in the skeletal muscles, especially in the pectoralis muscles (e.g., Steen & Enger 1957, Hohtola 1982, IUPS 2001). Motor units, comprising muscle cells and an α -motoneuron, contract non-voluntarily and asynchronously without external work. Heat is released concomitantly from e.g. two reactions in muscle cells: extramitochondrial ATP (adenosinetriphosphate) breaks up in connection with interactions between actin and myosin, and intramitochondrial ADP (adenosinediphosphate) is phosphorylated to ATP using energy from cellular fuels (e.g., Hohtola 1982, Block 1994). ST is the main heat production mechanism in birds and in warm-acclimated mammals. In cold-acclimated mammals, ST may be activated if the capacity of non-shivering thermogenesis (NST) is not sufficient at very low $T_{a,s}$ (Heldmaier *et al.* 1989b). On the other hand, the significance of ST increases in aging mammals as metabolism diminishes in brown adipose tissue (Florez-Duquet & McDonald 1998). Heldmaier and co-workers (1989b) have noted that ST also has costs: “it interferes with the use muscles for locomotion and other behaviour” and “shivering at high intensities may disturb the insulative layers of air in the fur and plumage and thus cause additional heat loss” (Heldmaier *et al.* 1989b). However, Hohtola and co-authors (1998) have shown that various types of behaviour (walking, preening, feeding, drinking, pecking, dropping) do not enhance or inhibit shivering in birds, but that motor activity can indirectly decrease the need of shivering because obligatory heat production increases and substitutes for ST.

In cold-acclimated small mammals, brown adipose tissue is the main component for thermogenesis (non-shivering thermogenesis, NST; Cannon & Nedergaard 2004). Brown adipose tissue (BAT) around the kidneys, aorta, in the axilla, interscapular regions and between cervical muscles (see Rothwell & Stock 1985) is found in small-sized mammals and newborn young of large mammals (e.g., in calves of reindeers; Soppela *et al.* 1991). Large adult mammals lack BAT, but insulation of fur and subcutaneous fat together with ST is sufficient. In BAT, oxidative phosphorylation of mitochondria and respiratory chain have been uncoupled by uncoupling protein 1 (UCP1); UCP1 breaks up proton gradient in the inner membrane of the mitochondrion and heat is released (e.g., Rothwell & Stock 1985, Cannon & Nedergaard 2004). Birds do not have BAT or UCP1 (Saarela *et al.* 1989, 1991). However, many UCP homologues expressed in BAT, muscles, white adipose tissue, brain tissue and other tissues have been found in mammals, fish, birds, insects and plants (see Raimbault *et al.* 2001, Vianna *et al.* 2001, Argyropoulos & Harper 2002), and they play important roles in mitochondrial metabolism connected or un-connected with

thermogenesis (Argyropoulos & Harper 2002). However, the uncoupling activity has not been shown in other UCP homologues *in vivo* (see e.g., Nedergaard *et al.* 1999). Despite the lack of BAT and UCP1, the existence of NST in birds has been suggested repeatedly. For instance, Saarela and Heldmaier (1987) showed in quails that when T_a decreases to a sufficiently low level, ST remains constant but metabolic rate increases. This suggests heat production without shivering. NST in newly hatched birds has been suggested to occur in skeletal muscles (Barré *et al.* 1987, Duchamp & Barré 1993, Duchamp *et al.* 1999). According to Duchamp and co-workers (1999), there are two candidate mechanisms for skeletal muscle NST: 1) uncoupling of oxidative phosphorylation in mitochondria of muscle cells and 2) increasing Ca^{++} cycling, dependent on ATP. Both mechanisms are connected with the metabolism of free fatty acids.

2.2.2 Obligatory digestion-related thermogenesis

Obligatory thermogenesis contains basal metabolism, feeding and digestion as well as locomotor activity (Blaxter 1989, Hohtola 2002). As none of these can proceed at 100% economy, heat is released as a consequence of these processes in cells. The post-prandial heat associated with mechanical and chemical processing of food in the gastrointestinal system is referred to as heat increment of feeding (HIF). The heat results from released energy of endogenous energy reserves, used for the uptake and processing of newly ingested nutrients in the gut (Schlitz & Murphy 1997). The avian digestive system is composed of oesophagus, crop, proventriculus (a glandular stomach), gizzard (a muscular stomach), small intestine, caeca, large intestine and cloaca (see Denbow 2000). In different avian species, gastrointestinal anatomy varies, e.g., in Columbiformes the caeca are absent (McLelland 1989, Denbow 2000), and thus digestion of food and the rate of processes and absorption may differ between species.

Postprandial heat has also been termed specific dynamic action (SDA), specific dynamic effect (SDE), diet-induced thermogenesis (DIT) as well as digestion-related thermogenesis (DRT). Although these terms are used as synonyms in the literature, they do however have their own specific meanings (see IUPS 2001). HIF covers all processes of feeding, digestion, absorption and faecal excretion, and accordingly has two components: ingestion- and digestion-related thermogenesis. DRT includes only the processes of digestion, absorption and excretion. On the other hand, there are also two components in DIT: DRT and heat production associated with types and texture of the diet. In a strict sense, hereafter the occurrence mentioned is referred to as DIT. In mammals, metabolism in BAT increases as a response to hyperphagia or high fat and carbohydrate concentrations of the meal, and this is regarded as a component of DIT (see e.g., Rothwell & Stock 1985, Lowell *et al.* 1993). This review concentrates on DRT, i.e., digestion and absorption of food, peristalsis and defecation.

The extra heat produced by DRT either has to be dissipated into the environment or may be used to substitute for facultative thermogenesis. For instance, Rashotte and co-workers (1999) have shown that fed birds shiver less during the dark phase. Thus, nocturnal DRT decreases the need of ST. Many other studies also support substitution of

facultative thermogenesis. Postprandial heat production decreases the need of facultative thermogenesis e.g., in starlings (*Sturnus vulgaris*; Biebach 1984), in kestrels (*Falco tinnunculus*; Masman *et al.* 1989), in blue grouse (*Dentragapus obscurus*; Pekins *et al.* 1992) in house wren chicks (*Troglodytes aedon*) at low T_a (Chappell *et al.* 1997), in pigeons during the nocturnal phase (Rashotte *et al.* 1997, Rashotte *et al.* 1999) and in bronze mannikins (*Lonchura cucullata*; Seagram *et al.* 2001). However, in large birds, e.g., in mallard ducks (*Anas platyrhynchos*) fed food with low protein concentration, substitution is less (Kaseloo & Lovvorn 2003). Klaassen and co-authors (1989) showed that substitution does not occur in some bird species, e.g., in Arctic tern chicks (*Sterna paradisica*). It is noteworthy that DRT does not necessarily substitute for ST directly but birds can benefit from it indirectly: the extra heat from digestion increases T_a in the microclimate around birds, and this benefit is significant in huddling birds during the inactive phase (Campbell *et al.* 2000, Seagram *et al.* 2001).

It has been shown in many studies that diurnal feeding success has an effect on the T_b daily pattern and that fasted birds show deeper nocturnal drop in T_b (see e.g., Chaplin 1976, Reinertsen & Haftorn 1983, 1986, Graf *et al.* 1989, Rashotte *et al.* 1989, Prinzinger *et al.* 1991, Ostheim 1992, Rashotte *et al.* 1999). By studying hypothermia induced by fasting and food-restriction, the significance of DRT or SDA for regulative thermogenesis can be studied. According to the experiments of Rashotte and co-authors (1995, 1999) and Geran and Rashotte (1997), the intensity of ST diminishes in pigeons as the amount of food eaten increases. Nocturnal DRT decreases the need of shivering. Interestingly, the nutritional value of food has an influence on SDA, but also on DRT. As mentioned above, the heat production is related to the chemical composition and texture of the diet (DIT): different types of nutrients are digested and absorbed with different efficiency and thus the amount of heat released varies. Low-protein foods produce a very low level of DRT and substitution of facultative thermogenesis does not occur (see Blaxter 1989, Kaseloo & Lovvorn 2003). Reinertsen and Bech (1994) and Geran and Rashotte (1997) have found that even non-nutritive food (cellulose) has a thermogenic effect in birds and influences the level of nocturnal T_b . This indicates that actual chemical processing of nutrients is not the only source of heat. Cellulose pellets in the moist gastrointestinal tract expand more than the same volume of normal food. Thus, cellulose pellets evoke more signals in distension-sensitive receptors, and these neural signals with the endocrine pathways affect the setting level of nocturnal T_b (Reinertsen & Bech 1994, Geran & Rashotte 1997). However, non-nutritive food produces less DRT than normal food (lack of digestible nutrients in cellulose pellets). Therefore facultative thermogenesis is not substituted (Geran & Rashotte 1997).

Small meals and sub-maintenance food intake produces very low levels of SDA or DRT (see Blaxter 1989, Kaseloo & Lovvorn 2003). In cold-acclimatised birds, meals are large, feeding is fast and gut size also increases during long-term exposure (Savory 1986). Savory (1986) has shown that the efficiency of digestion improves in low T_a s. Henderson and co-workers (1992) have also found that the amount of food eaten increases in cold-acclimatisation (see also Steen 1957). Food intake increases especially at the end of the light phase. Thus, the large amount of ingested food at late photophase compensates the nocturnal fasting period (Henderson *et al.* 1992, Buyse *et al.* 1993; see also Kendeigh *et al.* 1969). Not only the amount but also the timing of feeding has a strong influence on T_b pattern (see Rashotte *et al.* 1995). Birds that have a crop or another

part of the oesophageal tract for retaining food can eat large meals at the end of the light phase and ensure a prolonged energy supply during the night (Jackson & Duke 1995). Buyse and co-workers (1993) have demonstrated that the content of the crop in broiler chickens passes progressively to the stomach and the lower gut in the dark phase, and transit time of food (in the gastrointestinal system) is longer in scotophase than in photophase. The experiments of Rashotte and co-authors (1997) also show that a large amount of the crop content is emptied during the inactive phase in pigeons. In birds that are able to retain food in their oesophageal system digestion does not necessarily follow food intake in a ballistic (=fixed) fashion, and therefore the two components of SDA, ingestion- and digestion-related thermogenesis, are separated in time (see also Rashotte *et al.* 1999).

3 Aims of the study

The main goal of the present work was to study the thermoregulatory consequences of fasting/starvation and digestion. In papers I and II, the flexibility of hypothermia and hypometabolism was studied during variable photoperiods, feeding success and ambient temperature. It is noteworthy that in the avian species of the present study, only shallow hypothermia occurred, not torpor. The obligatory heat production related to digestion and its utilisation in substituting for facultative thermogenesis were studied in papers III and IV. The specific objectives were:

1. To study adaptive shallow hypothermia during variable photoperiods and metabolic responses to repeated food deprivations in birds;
2. To study the body temperature responses of birds to fasting and predation risk in semi-natural winter conditions;
3. To investigate whether adaptive timing of obligatory heat production (digestion-related thermogenesis) is possible in birds with a crop;
4. To study timing of digestion and digestion-related thermogenesis during constant and fluctuating ambient temperatures; and
5. To study heat increment of feeding and substitution for facultative thermogenesis in a bird with a crop.

4 Materials and methods

The following descriptions of materials and methods are summaries of the main experimental procedures that have been presented in detail in the original papers.

4.1 Animals

Male and female adult pigeons (*Columba livia*; I-IV and the experiments related to unpublished results that are presented in this thesis) and male adult quails (*Coturnix coturnix japonica*; I) were used in the experiments. The birds were obtained from the Zoological Gardens of the Department of Biology, University of Oulu, Finland. Several weeks before the experiments the pigeons were brought into indoor aviaries (except II). Photoperiod was maintained at a 12:12 LD cycle and ambient temperature (T_a) ranged from 20 to 22°C before the experiment. The primary measurements were done in indoor conditions (I, III and the experiments related to unpublished results), outdoor aviaries (II) and metabolic chambers (I, IV). Each experimental protocol was approved by the Animal Care and Use Committee of the University of Oulu.

4.2 Food

Commercial pelleted food (Huippu-Punahelhta, Suomen Rehu, Finland; 16.5% protein, 5.5% fat, 4.0% fibre) with oat grains was given to the pigeons, and pure commercial pellets (Kana-Herkku Aktiivi, Rehuraisio, Finland; 15.5% protein, 4.5% fat, 3.8% fibre) to the quails. Thus, the commercial food might be richer in nutrients than the natural food of these birds. During the actual experiments, food was given *ad libitum* (I, II and the experiments related to unpublished results), a 1-h food-pulse each day (III), using a restricted feeding protocol (IV) or not at all during the fasts (I, II, IV). The amount of

food eaten was measured by weighing a pyramidal dish before and after filling (I, IV and the experiments related to unpublished results). Water was available *ad libitum*.

4.3 Body temperature

For measuring of body temperature (T_b), radiotelemetric transmitters (I, III) or temperature loggers (II, IV and the experiments related to unpublished results presented in this thesis) were implanted into the abdominal cavity of the birds under anaesthesia. The transmitters (Model VM-FH, Mini-Mitter, USA) encode temperature by wideband RF bursts. The temperature loggers (iButton ThermoChron DS 1921H, Dallas Semiconductors, USA) register and record temperature values directly.

4.4 Body mass

The birds were weighed manually (I, III and the experiments related to unpublished results) or automatically by perch scales (I). In experiment I, the body mass of the quails was measured manually just before the dark phase. The body mass of the pigeons was registered for the whole dark phase with perch scales on which the birds sat and slept. In III, the post-feeding body mass was measured during an initial experiment. The pigeons were weighed manually two hours after lights on in the experiments related to unpublished results.

4.5 Dropping mass

Fresh dropping mass was measured in experiment III and dry mass in experiments I and III, and in the experiments related to unpublished results. The daily rhythm of digestion was measured indirectly by recording fresh dropping mass by load cells (III). Fresh dropping mass accumulated continuously in the recording device placed under each cage. Faecal excretion was measured in two groups, both of which received the same experimental treatments and were measured in turns: morning-pulse birds (food was given for 1 h per day in the morning) and evening-pulse birds (food was given for 1 h per day in the evening). In addition, all excreta accumulated during the day were collected manually for dry mass determination.

In experiment I, total dry mass of excreta during the day was determined in the quails. In the experiments related to unpublished results, diurnal and nocturnal digestion index (amount of food eaten (g)/excreta (g)/day) were determined. Dropping mass was collected separately in two 12-h periods and dried for 48 hours. The first period (for diurnal digestion determination) began two hours after lights on, the second period (for

nocturnal digestion determination) two hours after lights off. Because of the gut transit time, the period began two hours later than the light phase or the dark phase.

4.6 Oxygen consumption and carbon-dioxide production

Oxygen consumption (VO_2) was recorded by open-circuit respirometry (I, IV). For measuring oxygen concentration, a Servomex 1440C oxygen analyser was used. In addition, carbon dioxide production (VCO_2) was measured with a Servomex 1440C carbon dioxide analyser. The birds were in metabolic chambers from which air was directed to the analysers. The airflow to the metabolic chamber was regulated with mass-flow controllers [FMA-A2407, Omega Engineering (I); Bronkhost HI-Tec (IV)]. Respiratory quotient (RQ) was calculated as a proportion of CO_2 production to O_2 consumption.

4.7 EMG, electromyography

For determining of shivering (IV) in the cold, EMG was recorded from a pectoral muscle (*m. pectoralis major*) with three monopolar electrodes. The EMG signal picked up by the electrodes was amplified, filtered and then displayed on an oscilloscope. Shivering was quantified as 10-s r.m.s. EMG values.

4.8 Statistical analyses

To compare the data of experiments I, III and IV, a *t* test for paired samples was used. A one-way ANOVA was also used to test data in I. Differences between treatments were analysed by a two-way repeated-measures ANOVA in II, III, IV and in the experiments related to unpublished results presented in this thesis. In experiment II, the data were also compared by Pearson's correlation.

5 Results

5.1 Nocturnal hypothermia

5.1.1 Flexibility of fasting-induced hypothermia (I)

A rather fixed nocturnal hypothermia developed during fasting in variable photoperiods (12L:12D, 16L:8D, 20L:4D; Fig. 1). In the fasting quails, nocturnal T_b was not lower during the shortest dark phases, but T_b was actually kept on a higher level. Instead, a diurnal hypothermia occurred during the fast in 16L:8D and 20L:4D. Photophase T_b decreased immediately after food removal in 20L:4D.

Food deprivations induced a progressive nocturnal hypothermia at each dark phase of a 3-d fast in the pigeons and in the quails. With repeated fasts, nocturnal hypothermia became deeper in the quails but in contrast to the quails, progressively deeper hypothermic reactions were not produced in the pigeons. Also, re-warming during the end of the scotophase occurred later and more slowly in the quails as the birds met fasting again. A slight diurnal hypothermia developed with repeated food deprivations in the quails but not in the pigeons. VO_2 decreased markedly in the fasting quails, and the decrease became slightly more pronounced during repeated fasts. The decrease of VO_2 was most obvious on the second day of fasting. In the pigeons, body mass increased during the interval days between fasts: mean body mass before the first fast 361.0 ± 31.5 g (mean \pm S.E.M.) and after the last fast 370.1 ± 20.4 g. In contrast to the pigeons, the quails lost body mass in the intervals: mean body mass before the first fast was 159.8 ± 5.9 g and after the last fast 152.0 ± 6.0 g.

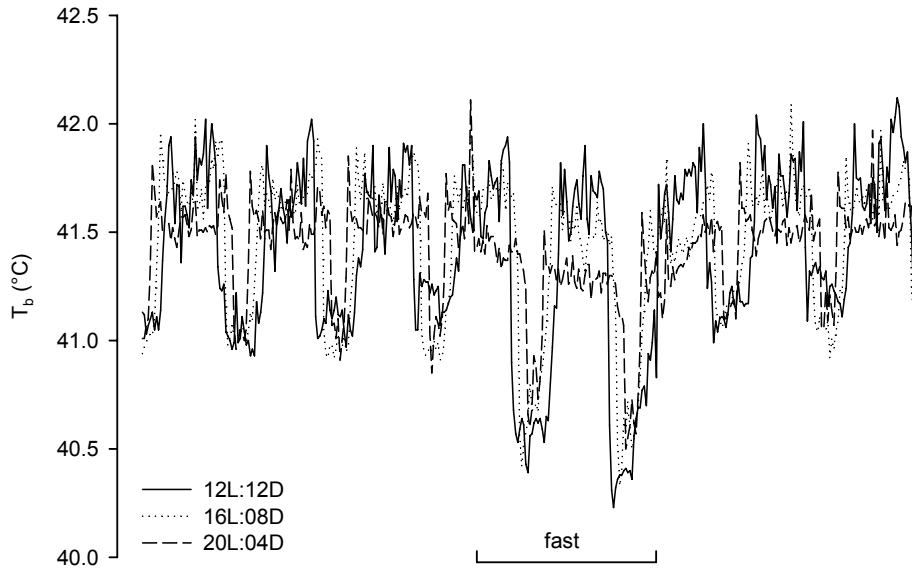


Fig. 1. Mean body temperature (T_b) of six quails in different photoperiods [12L:12D (solid), 16L:08D (dotted), 20L:04D (long dash)] during seven days. The 2-d fast has been marked by the horizontal line. Error bars are omitted for clarity. Re-drawn from paper I.

5.1.2 Effects of environmental factors on hypothermia in semi-natural conditions (II)

In semi-natural conditions, the normal amplitude of pigeons' T_b cycle was ca. 2.5°C, but nocturnal T_b dropped during starvation and the amplitude increased to 6°C. There was a negative correlation between ambient temperature (T_a) and diurnal T_b . However, nocturnal T_b did not correlate with prevailing T_a in the fed birds.

During fasting, nocturnal hypothermia was progressively augmented. In addition, diurnal T_b decreased during the fasts in cold conditions. The prevailing T_a had an influence on diurnal and nocturnal T_b of the fasting pigeons in semi-natural conditions: a deeper hypothermia developed when T_a was low (Fig 2A). The difference in nocturnal T_b was as much as two degrees between mild (T_a ca. 0°C) and very cold T_a s (T_a ca. -25°C).

The effect of simulated predation on nocturnal hypothermia during the fasts was studied using stuffed goshawks. A perched predator had no effect on nocturnal T_b but a “flying” goshawk suppressed nocturnal hypothermia during fasting (Fig. 2B). The duration of hypothermia also diminished. Remarkably, diurnal T_b of the fasting pigeons did not decrease in the case of the flying predator.

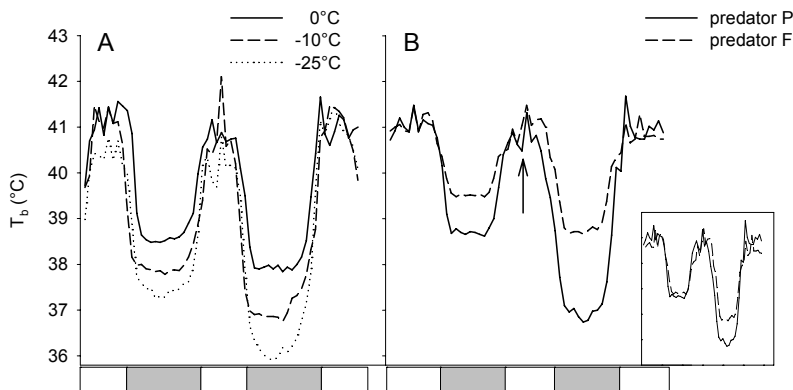


Fig. 2. Mean body temperature (T_b) of six pigeons during the 2-d fast A) in different ambient temperatures [$T_a = 0^\circ\text{C}$ (solid), $T_a = -10^\circ\text{C}$ (long dash), $T_a = -25^\circ\text{C}$ (dotted)] and B) exposed to a perched predator P (solid line) or to a “flying” predator F (long dash line). The arrow indicates the time when the simulated predator was brought into the aviary. White and grey bars indicate approximated light and dark phases, respectively. Error bars are omitted for clarity. Inset: Because there was an initial difference in nocturnal T_b , the data were normalised for the drop in T_b during the first night. Modified from paper II.

5.2 Thermogenic functions of digestion

5.2.1 Adaptive timing of digestion (III)

For measuring indirectly the daily rhythm of digestion in pigeons, dropping mass accumulation was recorded continuously throughout the day (III). A temperature-dependent peak in dropping mass appeared in the pigeons on the morning feeding regime (morning-pulse; Fig. 3). The peak was absent in the evening-pulse birds both at thermoneutrality and in cold. In the morning-pulse birds, a peak of the excreta occurred in the first hour of the photophase at thermoneutrality, but in the cold T_a , faecal excretion remained relatively constant throughout the day. In the evening-pulse pigeons, nocturnal excretion was a little higher than diurnal in both T_a s and the morning faecal excretion peak was thus not so pronounced. At both T_a s, nocturnal T_b of the morning-pulse pigeons was ca. 0.4°C lower than that of the birds with the evening feeding regime.

In the cyclic T_a (photophase $T_a = 22^\circ\text{C}$, scotophase $T_a = 2^\circ\text{C}$), the total amount of droppings was determined for light and dark phases separately (unpublished data). The mass of excreta, which was used as an indirect marker for digestion, was higher in the

light than in the dark phase both at cyclic T_a and at constant T_a (12°C). Diurnal processing of food was thus more intense in both temperature conditions. However, diurnal assimilation of food became weak in the thermoneutral light phase (of the cyclic T_a), compared with assimilation in the cold light phase (of the constant cold T_a).

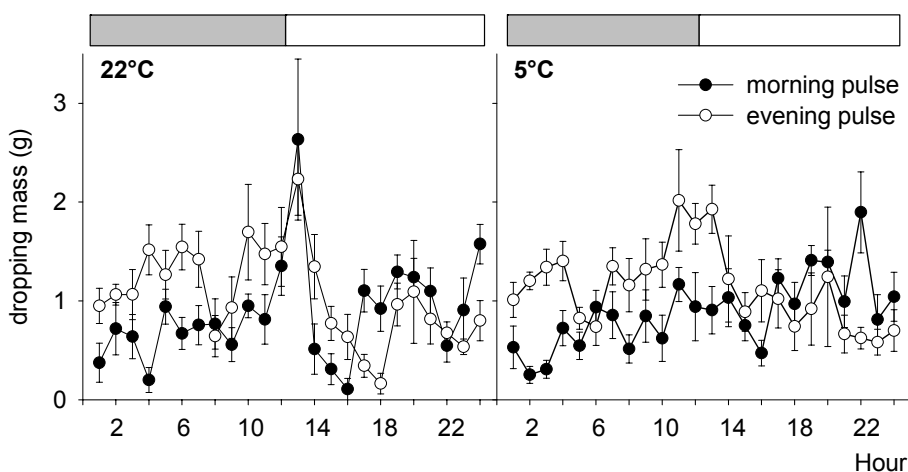


Fig. 3. Mean daily dropping mass of four pigeons in different ambient temperatures (22°C , 5°C). The grey bar refers to the dark phase and the white bar to the light phase (Hour 0 = starting time of the dark phase). Closed and open circles indicate hourly dropping mass of the pigeons on the morning and evening feeding regime, respectively. Dropping mass of Hour 15 in the morning pulse birds and Hour 22 in the evening pulse birds has been estimated by interpolation because dropping mass was not measured during the 1-h feeding period. Redrawn from paper III.

5.2.2 Heat increment of feeding and its use in the substitution for shivering thermogenesis (IV)

At thermoneutrality ($T_a = 23^\circ\text{C}$), the fed birds consumed more oxygen than the controls (birds without food) and VO_2 remained high for several hours after feeding (Fig. 4). In cold ($T_a = 0.5^\circ\text{C}$), VO_2 of the fed pigeons and the controls was on a higher level than at thermoneutrality, but VO_2 of the fed birds was on the same level or even lower than in the controls. Ambient temperature had no influence on RQ: at both T_a s, RQ increased during feeding and remained at a high level, whereas RQ of the controls did not change. Surprisingly, shivering intensity in the cold was identical both in the fed birds and the controls. The amount of food eaten ranged between 2.5-16.0 g at thermoneutrality and 0-8.6 g in the cold. T_b of the fed birds was higher after feeding than T_b of the controls at

thermoneutrality. In the cold, there was no difference between T_b of the fed pigeons and the controls.

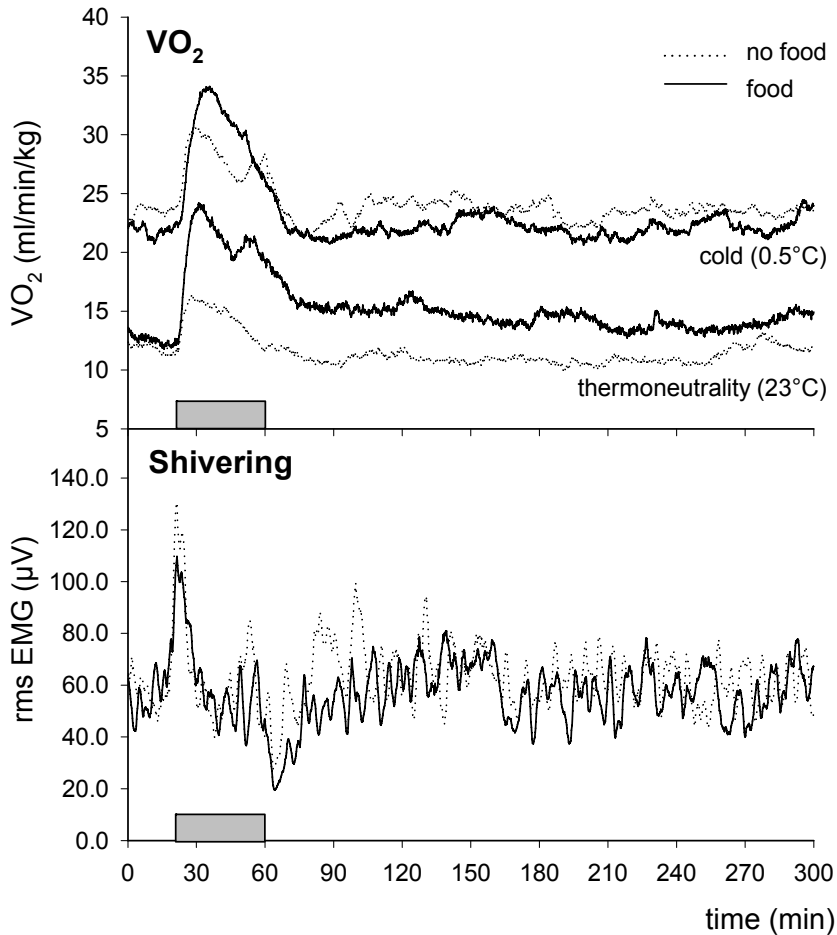


Fig. 4. Mean oxygen consumption (VO_2) and shivering intensity (EMG) of six pigeons. VO_2 was measured in the cold (0.5°C ; upper curves) and at thermoneutrality (23°C ; lower curves), shivering only in the cold. Solid and dotted lines indicate the data of the fed birds and the controls (the birds without food), respectively. The grey bar refers to a 40-min light-on phase when food and water were available for the fed birds and only water for the controls. Error bars are omitted for clarity. Maximum S.E.M. of VO_2 in controls is ± 1.2 ml/min/kg at thermoneutrality and ± 2.6 ml/min/kg in the cold conditions, and in fed birds ± 2.8 and ± 3.9 ml/min/kg, respectively. Modified from paper IV.

6 Discussion

6.1 Hypothermia and hypometabolism

6.1.1 Flexibility of hypothermia and hypometabolism

Energy savings are equal to the decrease of metabolic rate (VO_2). In theory, the relative decrease of oxygen consumption is equal to the decrease of the gradient between T_b and T_a . Hohtola and co-workers (1991) have calculated that the daily saving from a 5-degree hypothermia of quails corresponds to 35% of metabolic rate at thermoneutrality. However, in many studies VO_2 decreases more than predicted by the changes in temperature gradients (I). The present study shows that fasting quails did not compensate the decreased energy savings by a deeper nocturnal hypothermia during the very short scotophase (I). Previous studies suggest that physiological constraints or non-energetic costs and environmental factors may prevent further decrease of nocturnal T_b (see e.g., Hohtola *et al.* 1991). However, the amount of savings depends on both the depth and duration of hypothermia. During a short night less energy is saved, and during the day phase more energy is consumed. When this is combined with fasting, birds have to decrease their energy consumption. The fasting quails of the present study reacted to the short dark phase by diurnal hypothermia for saving energy, thus compensating the higher energy consumption during the longer day. A common hypothesis connected with thermoregulation of birds is that hypothermia in fasting birds occurs only in the scotophase because light suppresses the hypothermic effect of fasting (Underwood *et al.* 1999). However, the present study shows that the thermoregulatory system is such a flexible mechanism that this suppression can be overridden. Similarly, Berger and Phillips (1988) showed that in constant light the daily rhythm of T_b disappears and T_b decreases below the normal diurnal level. In addition, hypothermia can also recur without fasting: nocturnal and diurnal hypothermia can help migrating birds to decrease energy consumption (Butler & Woakes 2001) or to accumulate fat (Carpenter & Hixon 1988).

In the present study (I), the fasting quails saved energy by diurnal hypothermia during the long photophase. However, diurnal hypothermia is not harmless. The lower T_b decreases a bird's vigilance and alertness, making it thus an easier prey (e.g., Reinertsen & Haftorn 1986, Reinertsen 1996, Welton *et al.* 2002). In addition, foraging and accumulation of energy and fat reserves are less effective in hypothermic birds. But birds have to balance between surviving and risks, between energy saving and disadvantages (see Pravosudov & Lucas 2000, Welton *et al.* 2002), and the present study demonstrates that diurnal hypothermia may also be an adaptive mechanism. In the present study, diurnal hypothermia in the fasting quails occurred immediately as food was removed, although they were not yet starved. This suggests that the hypothermic reaction was conditioned to food removal. By contrast, Petherick and Waddington (1991) have shown in domestic fowls (*Gallus gallus domesticus*) that the birds cannot learn a cue that predicts a period without food: food intake and crop filling do not increase in spite of the cue. The fowls have no capability for true cognitive anticipation (Petherick & Waddington 1991).

Nocturnal hypothermia of fasting pigeons and quails becomes deeper when fasting continues (I). Many previous studies have shown that nocturnal metabolism of birds decreases gradually during a depletion of energy reserves (e.g., Chaplin 1976, Reinertsen & Haftorn 1983, 1986, Graf *et al.* 1989, Rashotte *et al.* 1989, Prinzinger *et al.* 1991, Ostheim 1992). There may also be species-specific and phylogenetic differences in the thermoregulatory system: the pigeons reacted to fasting by a deeper and more well-defined nocturnal hypothermia than the quails. In addition, randomly repeated fasts did not modulate the daily pattern of T_b in the pigeons, whereas in the quails nocturnal hypothermia became deeper, re-warming at the end of the night occurred later and more slowly and a slight diurnal hypothermia occurred as well. Interestingly, the quails, whose food was removed at the L:D transition, dropped their nocturnal T_b immediately in repeated fasts although they were not yet starved. It cannot, however, be decisively concluded that the quails would have developed conditioned hypothermic response to food removal because there was only a slight change in VO_2 connected with repeated fasts.

Hypothermic responses are connected with energy reserves (e.g., Rashotte *et al.* 1989, Hohtola *et al.* 1991). In the pigeons, the daily pattern of T_b was not modulated by repeated fasts. A key to this might be the slightly positive energy balance during the interval days between repeated food deprivations. Pigeons have a crop, which is an important, flexible part of the gastrointestinal system. Birds can store large amounts of food in their crop, and timing of digestion is possible in birds with a crop (Rashotte *et al.* 1999, III). By timing of digestion, birds can utilise the heat produced by digestion and use it to substitute for facultative thermogenesis. Thus, food intake and digestion can be separated in time. Ekman and Hake (1990) have shown that fat reserves of greenfinches (*Carduelis chloris*) increase as the environmental conditions become unfavourable (colder and/or unpredictable foraging success; see also Lehtikoinen 1986). Increased fat reserves assure lower starvation risk but also increase mass-dependent predation risk. In contrast to the pigeons, the quails had a negative energy balance in the present study. The differential response may be explained by the fact that quails have a much smaller crop for storing extra food, or that they are otherwise incapable of regaining the body mass lost during a fast.

According to the present study (I), diurnal hypothermia of the fasting quails in the long photophase was not due to conditioning. The birds compensated smaller energy savings during the short dark phase by diurnal hypothermia. Fasting itself has a strong influence on the daily T_b pattern and hypothermic responses in birds. Conversely, other environmental factors, e.g., photoperiod and fasting history, modulate metabolic and thermogenic responses only slightly, especially in laboratory conditions.

6.1.2 Effects of environmental factors on hypothermia in semi-natural conditions

Earlier studies have shown that fasting and restricted feeding may induce different responses in captive and free-living birds (Lovegrove & Smith 2003). Therefore, the depth and pattern of hypothermia and the daily T_b cycle may be affected by experimental conditions. In addition, even different stocks of the same species may have different thermoregulatory responses to starvation (I, Hohtola *et al.* 1991, Rikke *et al.* 2003). According to Schleucher (2001), columbiform birds have a very pronounced hypothermic reaction to starvation, but variations do occur within the species as well.

In the present study (II), effects of environmental factors on the daily T_b cycle of birds were investigated. In the semi-natural outdoor conditions, diurnal T_b of the well-fed pigeons increased as prevailing T_a became colder. This is in contrast to several previous studies, in which it has been demonstrated that T_b , especially nocturnal T_b , is parallel with T_a (e.g., Reinertsen 1983, Heldmaier *et al.* 1989a, Pravosudov & Lucas 2000, Welton *et al.* 2002). According to Reinertsen (1983), birds have less time to forage in the short day, and therefore accumulation of energy reserves is restricted and T_b is lower for saving energy. It is possible that diurnal T_b increases because birds enhance locomotor activity (behavioural thermogenesis) including foraging and feeding in the cold. In general, however, pigeons are typically inactive at very low T_a s. Increased activity incurs a diurnal predation risk (see Pravosudov & Lucas 2000, Welton *et al.* 2002). In this study (II), however, diurnal T_b increased as a response to colder T_a in the predation-free conditions of the outdoor aviary. Østnes and Bech (1998) have also shown that acute cold exposure induces an increase of the metabolic rate and deep T_b in the pigeon. Nocturnal T_b of the fed pigeons was not influenced by T_a . Non-energetic costs and physiological limitation may prevent further decrease of nocturnal T_b (see Reinertsen 1983, Hohtola *et al.* 1991). Also, during the long nights, the increased duration of nocturnal hypothermia augments the savings even with a small drop of nocturnal T_b (Hohtola *et al.* 1991). Interestingly, it was shown in the previous study (I) that shortened duration of hypothermia (during the short dark phases) is not compensated by a deeper nocturnal hypothermia. Instead, a diurnal drop of T_b occurs in fasting quails (I).

Nocturnal hypothermia of the fasting birds was progressively enhanced in the present study (II). This is a typical phenomenon in birds if scarcity of food continues (e.g., Hohtola *et al.* 1991). In the outdoor pigeons, T_a also had a distinct effect on the daily T_b cycle of the starved birds: hypothermia was deeper at cold T_a s. Diurnal T_b was also dependent on T_a during fasting: diurnal T_b was lower in the colder T_a . If birds are not

exposed to a diurnal predation risk, decreasing of diurnal T_b may be used for energy saving (see Pravosudov & Lucas 2000, Welton *et al.* 2002). A similar drop of diurnal T_b appeared in very long photoperiods in the previous study (I) at thermoneutrality. Butler and Woakes (2001) have also reported diurnal hypothermia in migrating geese in natural conditions. According to Underwood and co-authors (1999), food deprivation has an effect on T_b at all phases of the circadian rhythm, but light masks it. They demonstrated that in continuous darkness, the drop of T_b occurs in the subjective day as a response to food deprivation, but in the 'normal-light' day, fasting has only a slight effect on diurnal T_b (Underwood *et al.* 1999).

The dynamic models of hypothermia show that at night, hypothermic birds face nocturnal predation risk (Pravosudov & Lucas 2000, Welton *et al.* 2002). The hypothesis was tested empirically in the present study (II), and the results agreed with the dynamic models. The "flying" goshawk attenuated nocturnal hypothermia as fasting continued. In addition, the "flying" predator had a slight influence on diurnal T_b : the decrease of "afternoon T_b " seen previously during starvation did not occur. When the "flying" predator was present, the vigilance of the fasting birds remained high longer at the end of the light phase and re-warming occurred earlier in the late dark phase. Therefore, the duration of nocturnal hypothermia was shortened. Nocturnal hypothermia incurs an increased predation risk: the state of alertness declines and thus the ability to escape a predator decreases (Reinertsen 1996, Welton *et al.* 2002). It may therefore be possible that the birds balanced between risks and benefits: nocturnal hypothermia occurred but it was less deep and shorter, which might relate to vigilance. According to Pravosudov and Lucas (2000), birds use nocturnal hypothermia only at very low T_{as} or at higher levels of variability in foraging success when nocturnal predation risk increases. During starvation, hypothermia and low activity are the most effective mechanisms for energy sparing. On the other hand, if predation risk is diurnal, birds should exclusively use nocturnal hypothermia for diminishing energy consumption. This would decrease the time needed for feeding in daytime. There is also a question whether the type of sleep changes as a response to predation risk (see Rattenborg *et al.* 1999). Rashotte and co-workers (1998) demonstrated that the proportion of slow-wave sleep and paradoxical sleep changes during the nocturnal hypothermia in pigeons. In addition, some birds are even able to observe the environment for approaching predators by unihemispheric slow-wave sleep: birds may have ability to facultative control of sleep and wakefulness simultaneously in different regions of the brain (Rattenborg *et al.* 1999).

It is noteworthy that a perched predator had no influence on the daily cycle of T_b in the starving birds. It is probable that the perched and motionless goshawk did not represent a threat. No comparison of flying and perched predators exists, but van der Veen (1999) has demonstrated that a perched sparrow hawk had an influence on the body mass and food intake pattern of yellowhammers (*Emberiza citrinella*).

If the nocturnal predation risk is high or if the birds are not able to use hypothermia, fat reserves are accumulated more effectively for surviving in unstable feeding conditions (Pravosudov & Lucas 2000, Welton *et al.* 2002). However, maintenance of the fat reserves is costly as large amounts of fat reduce manoeuvrability, thus increasing mass-dependent predation risk (Ekman & Hake 1990, Gosler *et al.* 1995, Pravosudov & Lucas 2000). It has been shown that the body mass of small birds (Gosler *et al.* 1995) and daily feeding and foraging patterns (Pravosudov & Lucas 2001) depend on the diurnal

predation risk. For example, yellowhammers reduce feeding time if there is the threat of predation (van der Veen 1999). Birds also have other strategies for saving energy that do not incur predation risk: they can ruffle their feathers to increase insulation and huddle together to reduce heat loss and find a sheltered place for roosting (see Steen 1958).

This work (II) together with several other studies shows that the depth of nocturnal hypothermia depends on T_a and foraging success. This work presents empirical evidence that predation risk suppresses fasting-induced hypothermia. However, without predation risk birds may enhance foraging and behavioural thermogenesis for surviving in the cold T_a s.

6.2 Thermogenic function of digestion

6.2.1 Adaptive timing of digestion

Feeding is strongly biphasic in birds, and feeding bouts have an effect on the daily T_b rhythm (Rashotte & Stephan 1996). In the present study (III), the effect of feeding time on timing of digestion was investigated and faecal excretion activity was used as an indirect marker of digestion. At thermoneutrality, a clear peak of defecation appeared during the first hour of the photophase in the pigeons with morning feeding regime. As the birds had fed in the morning, this excretion peak on the following morning indicates regulated retention of food in the crop and regulated timing of digestion. The exact gut transit time is not known in pigeons, but e.g., in broiler chickens (ca. ten times heavier than a pigeon) it is about three hours (Buyse *et al.* 1993). Therefore, it can be assumed that a digestion peak in the pigeons occurred at the end of the dark phase. Earlier studies have demonstrated that 40% of daily food intake is retained in the crop for the starvation phase during the night (Rashotte *et al.* 1997). Digestion, absorption of nutrients and peristalsis are processes that produce obligatory heat in the body and this heat production, digestion-related thermogenesis (DRT), depends on the amount of food (Rashotte *et al.* 1999). In thermoneutral conditions, heat from digestion is extra heat and has to be dissipated into the environment. By timing of digestion, DRT can be utilised for re-warming, the only phase where this extra heat can be used. It has been shown that unfed and partially fed birds shiver more at the late dark-phase re-warming (Rashotte *et al.* 1999). It is therefore economical that DRT of well-fed birds substitutes for shivering in the re-warming phase at thermoneutrality.

In the cold T_a , energy requirements are higher than at thermoneutrality. Birds have to digest food at a constant rate throughout the day because of the large energy demand. A digestion peak of the birds with the morning feeding regime was therefore absent in the cold. On the other hand, because heat from DRT is not extra heat in the cold, DRT can be used to substitute for shivering throughout the day. Thus energetic benefits can be achieved even when food is not stored in the crop for the re-warming phase.

The birds with the evening feeding regime excreted more during the night than during the photophase at both T_a s. A clear faecal excretion peak did not appear in the evening-

pulse birds, but nocturnal excretion increased gradually and masked a regulated digestion peak. Although part of the food was digested ballistically following food intake, especially in the evening-pulse birds, the birds with the morning feeding regime were able to retain part of the food in the crop throughout the day. The morning-pulse feeding represents a restricted feeding regime, and nocturnal T_b dropped to a deeper level in the morning-pulse birds. Many earlier studies have shown that birds with slightly restricted feeding react by shallow nocturnal hypothermia in unstable energy conditions (Henderson *et al.* 1992, Hohtola *et al.* 1991, Rashotte *et al.* 1995, Saarela *et al.* 1995). Some studies have also demonstrated that the level of nocturnal metabolism has been pre-set according to the amount of energy reserves in the evening (Reinertsen & Haftorn 1983, 1984, Hohtola *et al.* 1991, Reinertsen & Bech 1994). Restricted feeding might force birds with morning-pulse regime to economise their energy reserves more strictly, and this may explain why timing of DRT was seen so obviously only in the morning-pulse birds. It has also been shown that food intake has an influence on gut size, gut transit time and thus also on the efficiency of digestion (Savory 1986). However, the modulation of gut size cannot explain the digestion peak, because the delay from feeding to the faecal excretion peak was very long in the morning-pulse birds.

What are the benefits of adaptive timing of DRT? If birds are able to time digestion, they have more options for feeding times. In addition, the amount of food can be reduced, thus decreasing foraging time that can be used for other functions. Daily foraging also carries diurnal predation risk (Gosler *et al.* 1995, Pravosudov & Lucas 2001, Welton *et al.* 2002) and therefore decreasing and optimising of feeding times generate better possibilities of predator avoidance. In natural conditions, T_a usually drops during the scotophase and this is another benefit of nocturnal digestion: heat from nocturnal digestion can be used to substitute for facultative thermogenesis, especially in the re-warming phase.

However, in the cyclic T_a (22°C in photophase, 2°C in scotophase), which imitates natural conditions, adaptive timing of digestion (total diurnal and total nocturnal digestion were only measured, not by 1-h definition) did not occur in *ad lib*-fed pigeons (unpublished data). Diurnal faecal excretion was higher than nocturnal, but some amount of food was retained in the crop for the nocturnal 'starvation' phase. This has also been shown in earlier studies (e.g., Jackson & Duke 1995). One reason may be that birds do not have mechanisms to adapt their food intake to short-term fluctuations in T_a and therefore their metabolic and even gastrointestinal systems are not capable of adapting to daily shifts of T_a . Prinzinger (1982) demonstrated that the influences of quick sinusoidal temperature fluctuations on metabolic rate result from inertia of the entire metabolic system. However, diurnal digestive efficiency (the proportion of total food eaten to dry mass of excreta) was lower at thermoneutrality than in the cold T_a (12°C), and digestion efficiency was lower in the photophase than at night at both T_a s. The efficiency of digestion depends on ingestion rate, activity of enzymes, nutrient absorption and gut transit time (see Karasov 1996, Brown & Downs 2003). These processes also result in DRT, which increases with digestive/assimilation efficiency. It has been shown, however, that small birds especially have a limited ability to assimilate food in different T_a s (McWorther & Martínez del Río 2000) or with different diets (Brown & Downs 2003).

By adaptive timing of digestion and storing food in the crop at thermoneutrality, birds can utilise obligatory heat production to substitute for energy-demanding regulated

thermogenesis in the late dark phase, during re-warming. Birds can thus avoid dissipation of the extra heat. In the cold T_{as} , obligatory heat from digestion can be used for substitution throughout the day, and timing is thus not needed. In the cyclic T_a , timing of digestion does not occur, but modulation of assimilation efficiency results in changes of DRT.

6.2.2 Heat increment of feeding and substitution for shivering

Postprandial heat production, termed HIF can be studied indirectly by measuring the increase of VO_2 consumption after a meal. In the present study (IV), postprandial VO_2 of the fed birds was markedly higher than in the controls at thermoneutrality (controls = the birds without food). Thus, increasing VO_2 suggests that the feeding bout and subsequent digestion incurred HIF. The usual pattern of HIF is that metabolic rate increases sharply after feeding, especially in mammals. This study showed a more fixed and constant increase without any clear peaks in metabolic rate after feeding. It suggests steady release of food to the gastrointestinal system. Birds with a crop or another storage compartment of their gastrointestinal system can store large amounts of food and digestion can occur later and gradually (Rashotte *et al.* 1997, III). Such a model of HIF has also been seen in white-crowned sparrows (*Zonotrichia leucophrys*; Schieltz & Murphy 1997) and in bronze mannikins (Seagram *et al.* 2001).

In the cold T_{as} , a smaller increase of postprandial metabolic rate in fed birds is usually taken as an indication of a substitution of facultative thermogenesis by HIF. Many studies have demonstrated that the need for shivering thermogenesis reduces in well-fed birds (e.g., Rashotte *et al.* 1999, Marjoniemi 2000, Bech & Præsteng 2004). The present study also showed smaller metabolic increase in VO_2 after a meal in cold. This would indicate “classical” substitution. It has been suggested in many avian species, e.g., in kestrels (*Falco tinnunculus*; Masman *et al.* 1989), in house wren chicks (*Troglodytes aedon*; Chappell *et al.* 1997), in guillemots (*Uria lomvia*; Hawkins *et al.* 1997), in pigeons (*Columba livia*; Rashotte *et al.* 1997, 1999), in chickens (Koh and Macleod 1999) and in tawny owls (*Strix aluco*; Bech and Præsteng 2004). Although substitution for facultative thermogenesis would have energetic advantages, substitution may be less in large birds and birds with protein-poor food, e.g., in mallard duck (*Anas platyrhynchos*; Kaseloo & Lovvorn 2003) or be totally absent (Campbell *et al.* 2000, Rosen and Trites 2003).

Although the pattern of VO_2 indicates to substitution in the present study, there were no electromyographic indications of a decrease in shivering of the fed birds. This indicates that thermoregulatory, facultative thermogenesis did not decrease and was thus not substituted. There are a number of explanations for this contradiction. First, the amount of food eaten might be too small for substitution. Kaseloo & Lovvorn (2003) have shown such threshold effects: only large amounts of food and sufficient protein content of a meal produce HIF in mallards. However, increase of RQ indicated that digestion occurred in the present study and the identical RQs at both T_{as} suggest a similar digestion rate. Therefore, the RQ results allow rejecting the threshold effects in the pigeons with the crop. It has been shown that non-nutritive food has no effect of RQ in

pigeons but incurs modifications in metabolic rate and T_b (Geran & Rashotte 1997). On the other hand, it is well known that HIF depends on the protein content of a meal (Blaxter 1989, Kaseloo & Lovvorn 2003). In the present study, the birds were fed on food with oat grains and protein-rich pellets (1:2), and the protein content of food was quite high. Therefore this does not explain the paradox.

Second, it has been shown that non-nutritive food modifies thermoregulatory system in pigeons (Reinertsen & Bech 1994, Geran & Rashotte 1997). Modification is transmitted by nervous signals: there are mechanoreceptors in the crop and other parts of the gastrointestinal system and they react to stretching (see Tashani & Leek 1994). The presence of food may alone have an influence on the thermoregulatory system. If a well-fed animal relies less on insulation, this might necessitate facultative thermogenesis (shivering) despite the decrease in energetic benefits. The increase of oxygen consumption might be due to a thermoregulatory effect in the present study. Higher T_b of the fed birds supports this explanation. However, none of the previous studies have found facultative thermogenesis in fed birds at thermoneutrality. Therefore, the postprandial increase of VO_2 and higher T_b were probably not due to facultative thermogenesis but rather to an increase and a shift in basal metabolic rate. At thermoneutrality, the fed birds expend energy but in the cold, this energetic shift was prevented.

These results raise different and partially contradicting explanations of HIF and the energetic model of feeding and thermoregulation, in contrast to previous viewpoints. The present study raises the question whether postprandial HIF truly exists in birds with a crop or whether the immediate increase in VO_2 after feeding results from other metabolic and thermoregulatory shifts. However, in birds, in which digestion follows ingestion directly, true postprandial HIF and its role in substitution for facultative thermogenesis are still prominent. As shown in this study, in birds with a crop substitution can occur uncoupled from the food intake time.

7 Conclusions

This study focused on two adaptive mechanisms in birds: hypothermia and obligatory heat production connected with feeding and digestion. Nocturnal hypothermia has been studied extensively; the energetic and hypothermic responses to ambient temperature, photoperiod and variable feeding success, specifically starvation, have been clarified in several previous studies. This study shows that flexibility of hypothermia in converting experimental factors to adaptive modifications of hypothermia is restricted, especially in laboratory conditions, in galliform and columbiform birds. Fasting birds do not compensate the decreased duration of nocturnal hypothermia by a deeper nocturnal drop of T_b in the short scotophase, but energy saving may be achieved by diurnal hypothermia. Thus, fasting itself (not e.g., fasting history) is the strongest modulator of the daily T_b cycle of these species.

However, in semi-natural, outdoor conditions, flexibility of hypothermia is obvious. In fasting birds, ambient temperature has a clear effect and photophase duration also a slight effect on the level of nocturnal hypothermia. Birds also resort to diurnal decline of T_b for minimising energy consumption in very cold, fasting conditions. On the other hand, in the cold well-fed birds can exploit behavioural thermogenesis.

This study also clarified experimentally the effects of predation on hypothermia in fasting birds. This is the first time that the theoretical predictions concerning predation risk and hypothermia have been tested empirically. The study showed that a simulated, flying predator prevents the occurrence of deep nocturnal hypothermia. In addition, decreased duration of nocturnal hypothermia increases the vigilance state of birds. Birds have to maintain sufficient alertness to avoid falling prey. Nocturnal hypothermia is an adaptive mechanism for diminishing energy consumption in food-restricted conditions. It is also a flexible mechanism in the natural conditions: the level of hypothermia may be changed especially by threat of predation and coldness.

Another focus of this study was obligatory heat production associated with digestion. Several previous investigations have demonstrated substitution of facultative thermogenesis by this type of obligatory thermogenesis. A new aspect is that birds with a crop can time digestion adaptively: at thermoneutrality, obligatory heat from digestion can be utilised during re-warming in the late dark phase. In the cold, substitution can occur throughout the day.

Heat production after feeding, HIF, also differs between birds with a crop and animals in which digestion follows ingestion in a ballistic fashion. In HIF of birds with a crop, a clear peak does not occur after feeding, but heat production remains at the increased and fixed level for a longer time. This study also showed that HIF does not substitute for shivering after feeding in the cold. This suggests a new aspect of HIF: does HIF immediately after feeding exist at all? Is the putative HIF only a change of basal metabolic rate after feeding? True postprandial HIF and its role in substituting are obvious in birds without a crop or where HIF is uncoupled from the feeding time. This latter focus of the study evokes several new questions of HIF and obligatory heat production and its energetic importance.

In summary, this study raised four new points regarding the thermoregulatory consequences of fasting and feeding:

1. In laboratory conditions, hypothermic responses to environmental factors are ballistic; only fasting itself has a clear effect on the daily T_b pattern in birds and the daily T_b cycle is not obviously modulated by ambient factors.
2. Ambient temperature and predation risk affect the daily T_b pattern of fasting birds in outdoor conditions. This study showed the first empirical effects of predation risk on hypothermia in unfed birds.
3. Adaptive timing of digestion occurs in birds with a crop. At thermoneutrality, obligatory heat from digestion can be utilised during re-warming by adaptive timing.
4. This study evokes questions as to the existence of postprandial heat production (HIF) and substitution of facultative thermogenesis immediately after feeding. More studies where true facultative thermogenesis is measured are needed.

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