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Abstract Turkey (*Meleagris gallopavo*) and guinea fowl (*Numida meleagris*) chicks (0–27 days posthatch) were exposed to decreasing or increasing ambient temperatures. Root mean square electromyographic activity of musculus pectoralis (m. pect.) and musculus iliotibialis (m. iliot.) was recorded simultaneously with O_2 consumption and CO_2 production. From both muscles, relative mass, water fraction and fibre type were determined. M. iliot. participated in shivering from hatching onwards. The relationship between its root mean square electromyographic activity and ambient temperature resembled that of metabolic rate and ambient temperature, and the shivering threshold temperature was indistinguishable from the lower critical temperature. This suggests that the leg muscles are major contributors to shivering thermogenesis. M. pect. participated in shivering only at days 6–20 in turkeys and at days 6–10 in guinea fowl. Both water fraction and histological analysis indicated that m. pect. was less developed than m. iliot. at hatching. We hypothesize that a minimal level of maturity is required before a muscle can participate in shivering, which is probably represented by a water fraction of about 0.85. Both species recruited the aerobic leg muscles first; the anaerobic breast muscle was recruited only when the rate of mass-specific heat loss was high.

Key words Galliform chicks · Electromyography · Level of functional maturity · Muscle fibre type · Thermoregulation

Abbreviations *EMG* electromyogram · *FG* fast-glycolytic fibres · *FOG* fast-oxidative-glycolytic fibres · *M* metabolic rate · M_{MS} mass-specific metabolic rate · *m. pect.* musculus pectoralis major · *m. iliot.* musculus iliotibialis · *rms EMG* root mean square of the electromyogram signal · *RMR* M_{MS} in the thermoneutral zone · *SO* slow-oxidative fibres · T_A ambient temperature · T_{LC} lower critical temperature · T_{ST} shivering threshold temperature · $\dot{V}_{O_2}$ volume oxygen consumed · $\dot{V}_{CO_2}$ volume carbon dioxide produced · *WF* water fraction

Introduction

It is well accepted that shivering is the principal means of regulatory thermogenesis in birds (Hohtola 1982; Stevens et al. 1986). In most adult birds the breast muscles form the largest muscle mass, and are often assumed to be the major heat-producing tissue during shivering (Aulie 1976a; Dawson et al. 1992). However, the leg muscles may contribute substantially to heat production as well (Stevens et al. 1986). This is especially the case in adult galliforms, which activate the oxidative leg muscles first for shivering, while the glycolytic, anaerobic breast muscles are only activated when the heat loss becomes severe (Aulie and Tøien 1988) or the core temperature decreases further (Tøien 1993a). In galliform hatchlings, the leg muscles are larger and more developed than the breast muscles (Ricklefs 1983), and are assumed to contribute substantially more to shivering thermogenesis (Aulie and Grav 1979), which is in accordance with the case of adult galliforms. However, owing to their unfavourable surface-to-volume ratio, the mass-specific heat

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loss of hatchlings is considerable. This may necessitate the participation of both the breast muscles and the leg muscles in shivering thermogenesis in very young chicks.

Three factors may affect the participation of a muscle in shivering: 1. *Relative muscle mass*, as the contribution of a muscle to shivering thermogenesis will increase with muscle mass (Aulie 1976a; Aulie and Grav 1988; Dawson et al. 1992); 2. *Level of functional maturity*, as the mode of functional development has a major impact on the shivering capacities of a muscle (Aulie and Grav 1979; Grav et al. 1988); and 3. *Fibre type*, as oxidative muscle fibres are able to sustain prolonged shivering, whereas glycolytic, anaerobic muscle fibres have only limited capacity for energy production (Choi et al. 1993). In adult galliforms, the breast muscle is glycolytic, and it may be expected that in galliform hatchlings, the breast muscle is glycolytic as well. However, some muscles that are glycolytic in adults may be oxidative in hatchlings. During development, these originally oxidative muscles differentiate gradually into glycolytic, anaerobic muscles (Bass et al. 1970; Gauthier et al. 1982; McLennan 1983). It is thus possible that the contribution of breast and leg muscles to regulatory thermogenesis changes during development with a change in fibre type.

The aim of this study was to investigate the participation of the breast and leg muscles in shivering thermogenesis in galliform chicks, and to determine whether this participation changes during development, and if so, which of the three factors mentioned above are involved. Chicks of domestic turkey, *Meleagris gallopavo*, and guinea fowl, *Numida meleagris*, were used from 0 to 27 days after hatching. Muscle mass and fibre type, and water and fat content of the breast and leg muscles were determined. The latter two were used to calculate the water fraction of the muscles, which is considered to be an index of functional maturity (Ricklefs 1983; Ricklefs and Webb 1985).

Materials and methods

Animals and housing

Eggs of turkeys (strain: B.U.T. Big 6) and guinea fowl (strain: Galor) were incubated at our laboratory. Until 8–12 h post-hatch the chicks stayed in the incubator (37.5 °C). Thereafter, they were housed on a litter floor inside a temperature-controlled room (24 °C) with an L:D cycle of 23:1 (in accordance with the standard raising conditions). A 100-W infrared bulb supplied additional heat. Water and commercial food were available ad libitum. The ambient temperature underneath the infrared bulb was gradually decreased to room temperature with increasing age.

Experimental design

Subcutaneous surface electromyographic electrodes were placed on the cranial part of the right (m. pectoralis major) and (m. iliotibialis) of the chick without damaging the muscle, and fixed with adhesive tape. A safety-pin electrode was placed as a ground wire in the neck skin. Thereafter, the chick was placed in a darkened metabolic chamber, in which the ambient temperature (T_A, °C) was either within the thermoneutral zone (Dietz 1995) or well below lower critical temperature (T_{LC}). The T_A range used varied with age and species. Dry outdoor air was pulled through the chamber. Flow rates of the outlet air, measured with a wet-precision gas meter, were adjusted to maintain the O_2 concentration above 20%. The outlet air was dried (molecular sieve 3 Å; Merck) before measuring O_2 and CO_2 concentrations. After an equilibration period of 30 min, O_2 and CO_2 concentrations of the outlet air were measured continuously with a paramagnetic O_2 analyzer (Taylor Servomex OA184) and an infrared CO_2 analyzer (Heraeus Leybold Binos), respectively, until a stable recording of at least 15 min was obtained. During this period, T_A inside the chamber and EMGs of both muscles were measured as well. Before calculating metabolic rate (M), the O_2 concentrations of the outlet air were corrected for the differences in volume of the inlet and outlet air at respiratory exchange ratios below 1, according to Eq. (6) in Hill (1972). Average M (W) was computed by:

$$M = 4.49\dot{V}_{O_2} + 1.39\dot{V}_{CO_2}$$

with $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in l(STPD)·h^{-1} (Romijn and Lokhorst 1961). After the trial the chick was weighed (±0.1 g).

After the first measuring period, the T_A was either lowered or increased repeatedly with steps of about 2 °C. After an adjustment period of at least 15 min, stable O_2 and CO_2 concentrations, T_A and EMG were measured for at least 15 min, whereafter T_A was changed again. Measurements were made at 0 (i.e. 10–24 h post-hatch), 3, 6, 10/11 (guinea fowl/turkeys), 15 (guinea fowl only), 20 and 27 days of age. Six chicks were used at each age, of which three were exposed to increasing and three to decreasing temperatures. With the exception of hatchlings, all chicks had free access to food and water prior to measurement.

EMG measurement

The subcutaneous surface EMG electrodes used were slightly modified from the model described by Aulie and Tøien (1988) and Tøien (1992). Two insulated multicord stainless steel wires (Capable; 1 wire consisted of 12 stainless steel wires of Ø 0.044 mm; total Ø 0.43 mm including the insulation of low density polyethylene) were glued into position in a Teflon catheter while the tip of one was curved backwards inside the catheter. Two 1-mm holes were cut through the catheter and the insulation of the EMG wire, one at the tip of the electrode and one 5 mm behind the tip.

The EMG signals were led to a differential amplifier (Schwarzer, model UME47) with a high-pass filter with a cut-off frequency of 50 Hz. The amplified EMG signal was converted to its root mean square (rms EMG) with a 10-s sliding time average. The direct and rms EMG were recorded (Schwarzer, model U8008); in addition, the rms EMG was sampled by a data logger (Grant Squirrel, model 1200) with a time interval of 2 s. The recorder registrations were used to identify and eliminate artefacts resulting from electrical noise and movements of the chicks from the data logger recordings before averaging rms EMG. The rms EMG signals were corrected for individual differences in base level owing to differences in location of the electrodes. For each experiment the lowest average rms EMG value was taken as base level, and subtracted from all average rms EMG values during that experiment.

Muscle composition

After the trial the chick was weighed to the nearest 0.1 g and killed by decapitation. Samples were taken within 10 min from the cranial part of the right m. pect. and m. iliot. for histological analysis of fibre type. Fresh masses of m. pect., m. iliot. and all leg muscles (upper and lower leg) were obtained from the left side of the body. To calculate total fresh mass of a muscle (group), the fresh mass was multiplied by two. In the following text and figures, 'muscle mass' refers to the total fresh mass. Dry contents of m. pect. and m. iliot. were determined by freeze-drying to constant mass. Water content was calculated by subtracting dry mass from wet mass. After drying, fat content was determined by extraction

with petroleum ether (40–60 °C, at least 24 h). The water fraction (WF) was calculated as WF = (water content)/(fat-free wet mass) (Ricklefs and Webb 1985). Contents of the gastrointestinal tract were weighed and subtracted from body mass to calculate the empty body mass. Empty body mass was used to calculate relative muscle mass (%) and mass specific metabolic rate (M_{MS}, $W \cdot kg^{-1}$).

Histological analysis

Samples of the muscles were immediately quick-frozen in isopentane cooled in liquid N_2, and kept at −80 °C until analysis. Transverse serial sections (10 μm) were cut in a cryostat at −20 °C and mounted on microscope slides for histochemical staining. Fibre types were distinguished on the basis of ATPase activity (Bancroft and Stevens 1982). The pH during the preincubation period was 4.2 in turkeys and 4.4 in guinea fowl, and the incubation temperature was set at 39 °C. This resulted in a dark staining of the aerobic slow-oxidative (SO) fibres. It was not possible to distinguish between fast glycolytic (FG) and fast-oxidative glycolytic (FOG) fibres. In the following text, all non-SO fibres in a muscle cross section are referred to as 'FG/FOG' fibres.

In m. iliot. cross sections, segments could often be distinguished based on the content of SO fibres. The percentage SO fibres of a section was determined by counting fibres in five randomly chosen areas of a constant surface area of either the whole section or each distinguishable segment. The magnification used depended on species (×400 in turkeys and ×200 in guinea fowl). The total number of fibres counted per area depended on species, muscle and age; range 100–350 fibres.

Statistics

Comparisons between groups were made by Student's *t*-test or analysis of variance. Linear regression equations and correlation coefficients were calculated by the least-squares method. Analysis of covariance was used to test if the slopes or intercepts of two regression lines differed significantly. The method described by Nickerson et al. (1989) was used to estimate continuous two-phase linear regression parameters.

Results

Metabolic rate

The relationship between M_{MS} and T_A showed the well-established pattern with the lowest levels in the thermoneutral zone and a negative correlation between M_{MS} and T_A below T_{LC} at all ages (Fig. 1). T_{LC}, estimated by a continuous two-phase linear regression, decreased with age and was higher in guinea fowl than in turkeys, except at day 0. M_{MS} in the thermoneutral zone was significantly higher in guinea fowl ($P < 0.025$, $n = 24$–33; Table 1), except at day 0 ($P > 0.5$, $n = 21$). At T_As below T_{LC}, M_{MS} was higher in guinea fowl than in turkeys at each age and T_A, again with the exception of day 0 where M_{MS} did not differ between the species ($P > 0.5$, $n = 46$).

Body temperature was not measured; therefore, it was not possible to calculate thermal conductances (McNab 1980). However, the thermal coefficient, i.e. absolute slope of the regression lines of M_{MS} with T_A below T_{LC}, may be used as an estimate of the thermal conductance. Thermal coefficients did not differ significantly between the species within age, except at days 10/11 and 20 where the thermal coefficients were higher in guinea fowl ($P < 0.001$, $n = 82$, and $P < 0.001$, $n = 76$, respectively).

EMG measurements

In Fig. 2, representative examples are given of the direct and rms EMG registrations of the m. pect. and m. iliot.

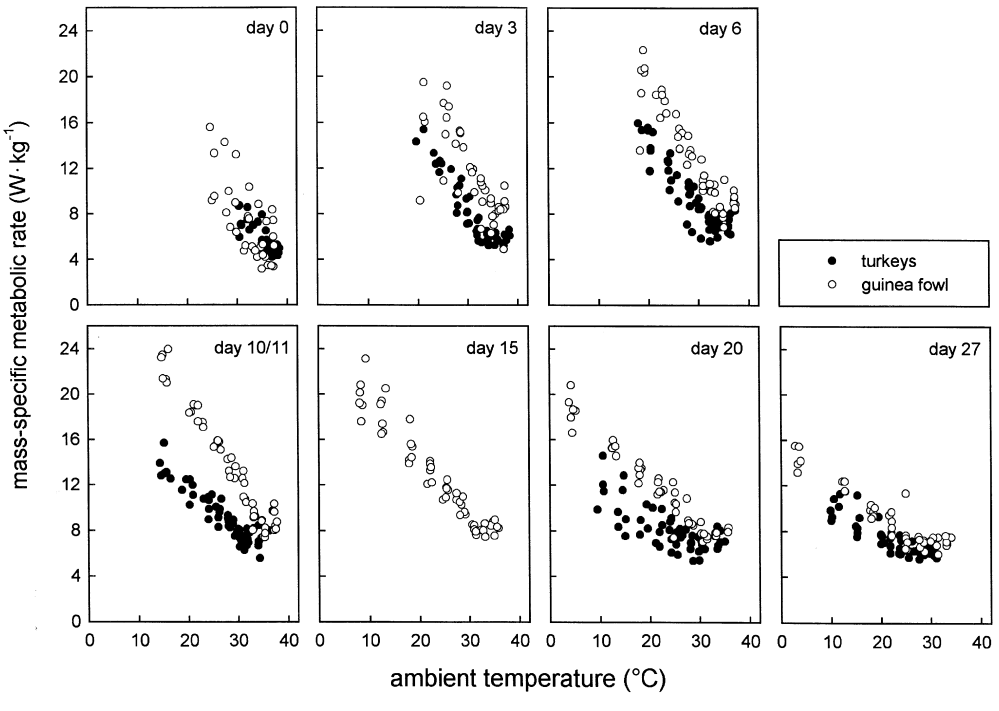

Fig. 1 Mass-specific metabolic rate as a function of ambient temperature in turkey and guinea fowl chicks from 0 to 27 days post-hatch

Table 1 Parameters of the continuous two-phase linear regression model for M_{MS} with T_A in turkeys and guinea fowl from 0 to 27 days posthatch. The absolute value of the slope represents the thermal coefficient. In addition, T_{ST} of the m. iliotibialis is given. N_1 = number of measurements below T_{LC}; N_2 = number of measurements within the thermoneutral zone; and RMR is given as mean ± SEM

Age (days)	Slope ($W \cdot kg^{-1} \cdot °C^{-1}$)	N_1	RMR ($W \cdot kg^{-1}$)	N_2	T_{LC} (°C)	T_{ST} (°C)
Turkeys						
0	−0.45	24	4.86 ± 0.14	9	37.0	36.5
3	−0.71	27	5.90 ± 0.12	16	33.3	33.9
6	−0.60	37	7.06 ± 0.20	15	32.3	33.1
11	−0.39	41	7.42 ± 0.26	15	31.2	33.7
20	−0.25	34	6.84 ± 0.19	17	28.1	29.1
27	−0.26	30	6.21 ± 0.10	17	25.5	26.6
Guinea fowl						
0	−0.73	22	5.14 ± 0.52	12	35.0	37.1
3	−0.61	32	8.35 ± 0.55	8	35.8	37.2
6	−0.72	36	8.84 ± 0.32	12	34.3	35.1
10	−0.72	41	8.40 ± 0.30	10	35.0	34.8
15	−0.52	44	8.21 ± 0.15	12	31.7	34.6
20	−0.41	42	7.72 ± 0.11	8	31.4	31.4
27	−0.29	33	7.04 ± 0.12	16	27.8	28.7

of 0- and 6-day-old turkey chicks. Figure 3 shows the average rms EMG of the m. iliot. versus T_A of each species. M. iliot. was involved in shivering in both species from hatching onwards. The relationship between the rms EMG of m. iliot. and T_A resembled that of M_{MS} with T_A. At all ages a shivering threshold temperature could be estimated with the continuous two-phase linear regression model (Table 1). In both species, shivering threshold temperature (T_{ST}) and T_{LC} were positively linearly correlated, and the linear regressions did not differ significantly between the species. Thus, one linear regression was calculated for the species combined: T_{ST} = 3.04 (±2.81 SEM) + 0.94 (±0.09 SEM) T_{LC}; (r^2 = 0.914, $P < 0.001$, n = 13). The slope of this regression line did not differ from 1 ($P > 0.05$), and the intercept was indistinguishable from 0 ($P > 0.05$). Therefore, the T_{ST} of m. iliot. coincided with T_{LC} at each age.

Unlike m. iliot., m. pect. did not participate in shivering immediately after hatching (Fig. 4). In both species, the rms EMG of m. pect. was correlated with T_A at day 6 (turkey: $P < 0.001$, n = 42; guinea fowl: $P < 0.001$, n = 37) and 10/11 (turkey: $P < 0.05$, n = 45; guinea fowl: $P < 0.001$, n = 49); in turkeys a significant correlation was also found at day 20 ($P < 0.05$, n = 43). At none of these ages could a T_{ST} be detected with the continuous two-phase linear regression model.

Muscle composition

At hatching, relative leg muscle mass was twice as large as that of m. pect. ($P < 0.001$, n = 12, and $P < 0.001$,

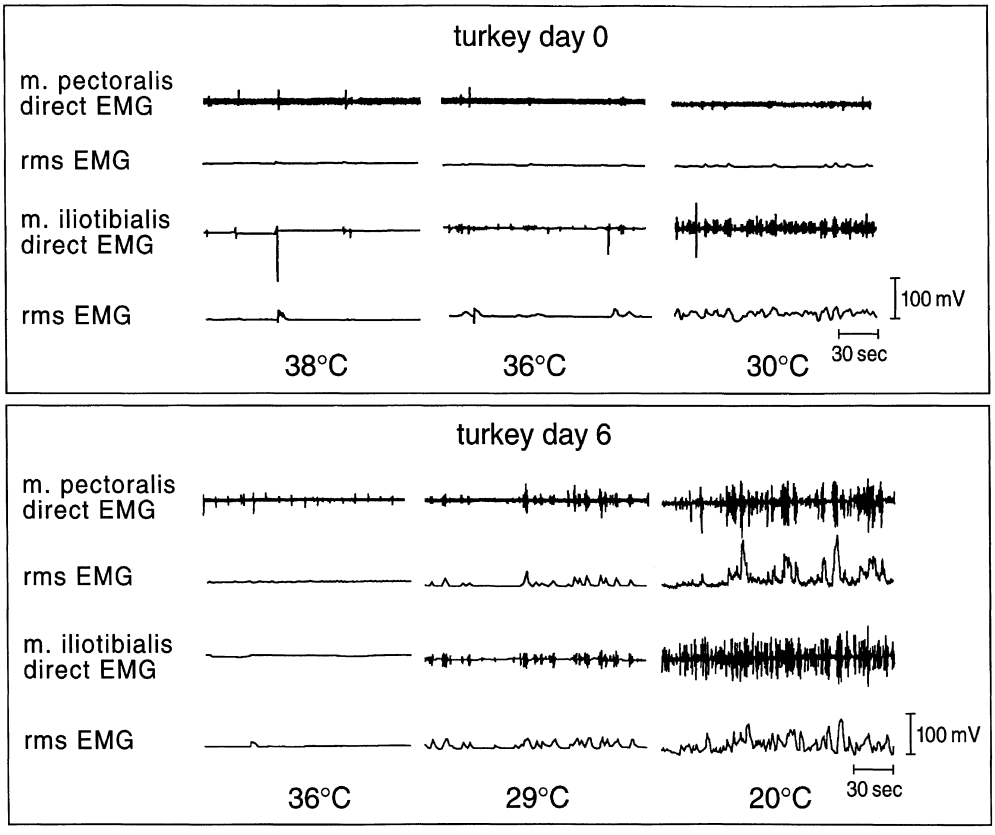

Fig. 2 Representative examples of direct and rms EMG recordings of m. pectoralis and m. iliotibialis in 0 and 6 day-old turkeys. Ambient temperatures (from left to right) are representative for the thermoneutral zone, just below T_{LC} and a temperature well below T_{LC}

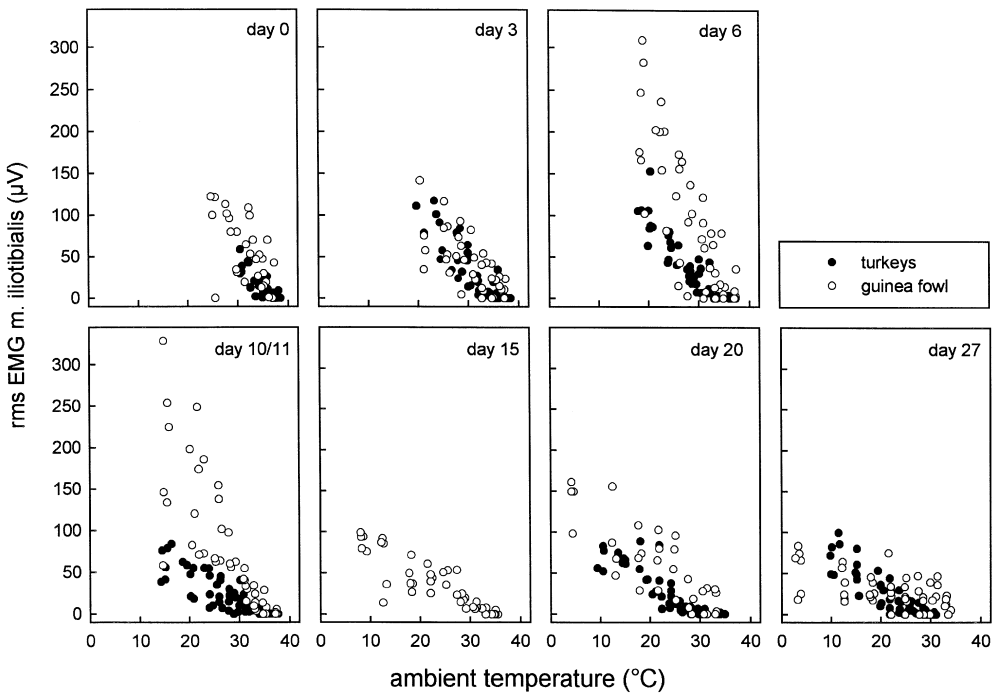

Fig. 3 Root mean square EMG of m. iliotibialis as a function of ambient temperature in turkey and guinea fowl chicks from 0 to 27 days post-hatch

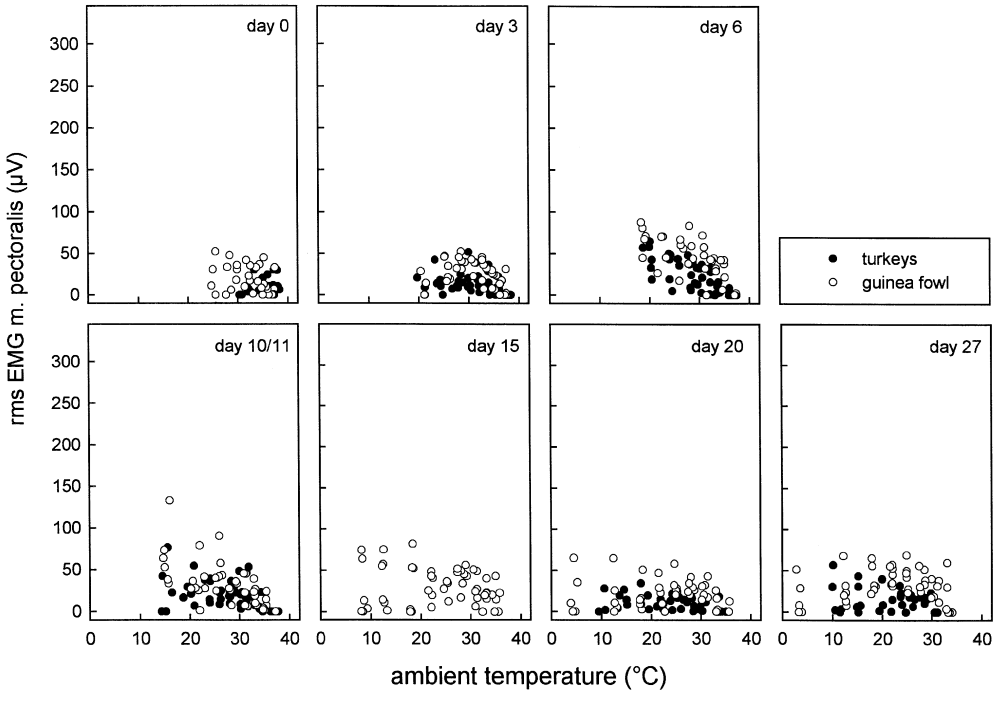

Fig. 4 Root mean square EMG of m. pectoralis as a function of ambient temperature in turkey and guinea fowl chicks from 0 to 27 days post-hatch

$n = 12$, for turkeys and guinea fowl, respectively, Fig. 5B). Relative m. pect. mass increased considerably more with age than relative leg muscle mass. Relative m. iliot. mass decreased slightly with age. In turkeys, relative m. pect. mass equalled that of the leg muscles at day 11 ($P > 0.1$, $n = 12$); thereafter, m. pect. was significantly larger. In guinea fowl, relative m. pect. mass remained significantly smaller than that of the leg muscles. With the exception of day 3, relative m. pect. mass was significantly larger in turkeys than in guinea fowl. Relative leg muscle mass, however, was significantly larger in guinea fowl than in turkeys from day 10/11 onwards.

At hatching, WF of m. pect. was higher than that of m. iliot. ($P < 0.001$, $n = 10$, and $P < 0.01$, $n = 12$, for turkeys and guinea fowl, respectively, Fig. 5C), indicating that the level of maturity was lower in m. pect. During the first week after hatching, WF of m. pect.

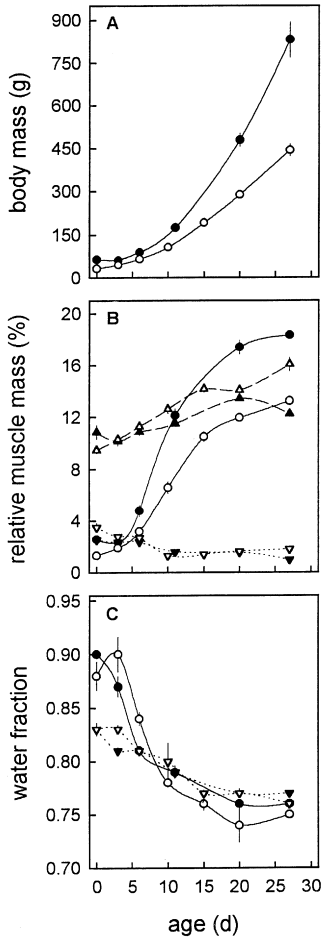

Fig. 5 A Body mass (mean ± SEM) as a function of age in turkeys (●) and guinea fowl (○). **B** Relative mass (mean ± SEM) of m. pectoralis (●, ○), m. iliotibialis (▼, ▽), and all leg muscles (▲, △) as a function of age in turkeys (*closed symbols*) and guinea fowl (*open symbols*). **C** Water fraction (mean ± SE) of m. pectoralis and m. iliotibialis as a function of age in turkeys and guinea fowl (symbols as in B)

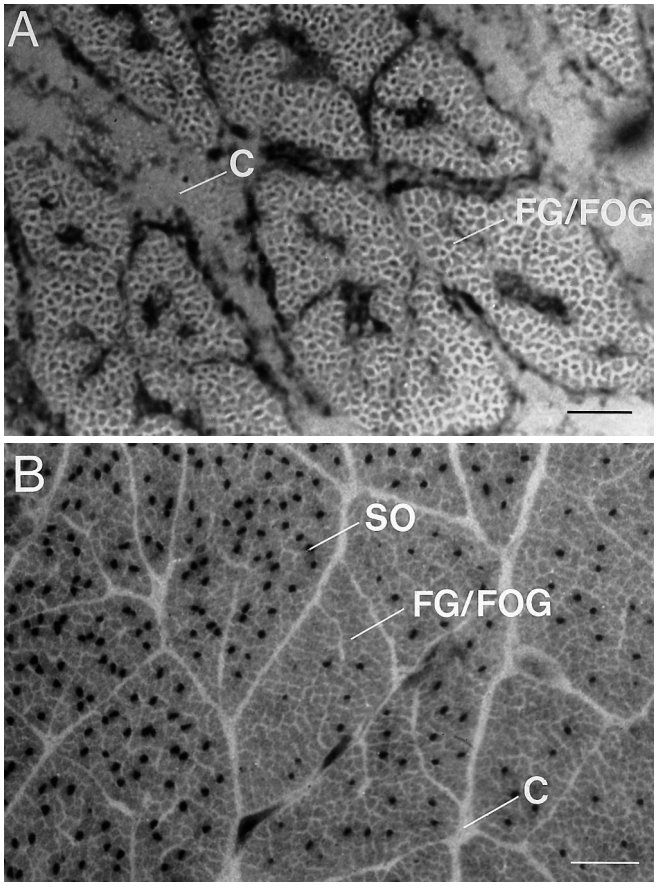

Fig. 6A, B Representative examples of m. pectoralis (**A** ×200; length scale: 20 μm) and m. iliotibialis (**B** ×100; length scale: 10 μm) cross sections of turkey hatchlings stained for ATPase activity. Red, slow-oxidative fibres were stained darkly (*SO*), while both white, fast-glycolytic, and intermediate, fast-oxidative-glycolytic fibres were not stained (*FG/FOG*). Note the high amount of connective tissue (*C*) in the m. pectoralis

decreased considerably. Thereafter, rates of decrease in WF with age were about equal in m. pect. and m. iliot. In turkeys, WF of m. pect. and m. iliot. did not differ significantly from day 6 onwards, and in guinea fowl they did not differ from day 10 onwards.

Histological analysis

Histological analysis revealed that in both species m. pect. was less developed than m. iliot at hatching. Hatchling m. pect. consisted of loosely packed fibres with a considerable amount of connective tissue between them, while m. iliot. had larger fibres in a more closely packed structure with a smaller amount of connective tissue (Fig. 6). During development the fibre diameter increased, while the amount of connective tissue decreased.

In turkeys, some m. pect. cross sections contained a few SO fibres at all ages. The SO fibres were concentrated in a very small segment of the section, located at the outer (skin) side of the muscle. At day 20 and 27, some sections contained giant SO fibres, randomly distributed over the section. Main fibre type was FG/FOG at all ages. In guinea fowl, some m. pect. cross sections contained a few SO fibres, also concentrated in a very small segment at the outer (skin) side of the muscle. At day 10, 15 and 20 some sections contained giant SO fibres, again randomly distributed. As in turkeys, main fibre type was FG/FOG at all ages.

All sections of the m. iliot. contained SO fibres. In turkeys, three different segments could be distinguished in the muscle cross sections, based on presence of SO fibres. A large segment was located at the inner side of the muscle, forming about half of the section. The rest of the section, the outer half, i.e. skin side, could be divided in two, about equal-sized segments. The larger inner segment contained no SO fibres. The two skin-side segments contained different numbers of SO fibres. The percentage of SO fibres in these segments did not exceed 10% (Table 2). In guinea fowl, only two segments could

Table 2 Percentage of SO fibres (mean ± SEM) in m. iliotibialis samples in turkeys and guinea fowl from 0 to 27 days posthatch. In turkeys, values are given for the three segments that could be distinguished: *A* segment in the outer (skin) side of the muscle cross section with high number of SO fibres; *B* segment in outer (skin) side of the muscle cross section with low number of SO fibres; *C* inner half of the muscle cross section. In guinea fowl, the percentage of SO fibres is given for that part of the section that contained SO fibres. *n* = the number of samples

Turkeys					Guinea fowl		
Age (days)	*n*	Segment A (%)	Segment B (%)	Segment C (%)	Age (days)	*n*	Segment with SO fibres (%)
0	5	8.2 ± 0.9	1.6 ± 1.0	1.2 ± 1.1	0	6	9.9 ± 3.2
3	4	2.8 ± 1.4	1.7 ± 1.3	0.0 ± 0.0	3	5	20.4 ± 5.4
6	6	3.7 ± 0.8	2.5 ± 1.0	0.0 ± 0.0	6	6	13.1 ± 3.6
11	6	4.2 ± 1.2	0.3 ± 0.3	0.0 ± 0.0	10	5	36.5 ± 12.5
					15	5	36.1 ± 8.8
20	6	2.0 ± 1.0	1.1 ± 0.7	0.0 ± 0.0	20	6	26.9 ± 10.9
27	5	7.8 ± 2.0	4.3 ± 1.9	0.0 ± 0.0	27	6	26.5 ± 11.3

be distinguished in the pectoralis sections, roughly the outer, skin-side half and the inner half. SO fibres were initially only present in the outer half of the muscle cross section. Size of this segment increased gradually with increasing age. From day 20 onwards, some SO fibres were always present in the inner half. The percentage SO fibres in the segment with SO fibres was much higher in guinea fowl than in turkeys (Table 2).

Discussion

This study is the first to record the distribution of shivering by simultaneous EMG recordings from several muscles in developing young birds. The major finding was that leg muscles (as represented by m. iliot.) were the major contributors to shivering and participated in shivering from hatching onwards, while the breast muscles only contributed to shivering for a period from day 6 onwards. Absolute quantitative information about the contribution of muscles to heat production may not be obtained from EMG recordings in a growing animal because level and range of the rms EMG depend not only on location of the electrode, but also on relative size of the electrode as compared to the muscle. In growing chicks, muscle masses vary with age and differ between species at equal age. However, EMG contains useful information on whether a muscle is involved in shivering, and possibly at which T_A it starts to shiver (T_{ST}). Also several other factors have to be considered when evaluating the participation of muscles in shivering in developing birds.

One of those factors is muscle fibre type, because aerobic muscles are major contributors to shivering thermogenesis in adult birds (Aulie and Tøien 1988; Carey et al. 1989a,b; Tøien 1993a). In both species in the present study, there were no major changes in fibre type in either m. pect. or m. iliot. during the first month of development. Changes in the participation of a muscle in shivering thermogenesis were thus not correlated with a change in muscle fibre type. M. pect. cross sections contained only very few SO fibres, just like in most adult galliforms (Kaiser and George 1973; Smith and Fletcher 1988). Some m. pect. sections contained randomly distributed giant SO fibres. Giant SO fibres represent a degenerative characteristic that is thought to be correlated with the selection for relatively large, rapid growing breast muscles (Sosnicki et al. 1989; Wilson et al. 1990). Possibly, the growth of the connective tissue cannot keep up with muscle fibres growth thereby depriving the muscle fibres of extracellular support (Wilson et al. 1990). In the m. iliot. cross sections, SO fibres were mainly found in the outer (skin) half side of the muscle. In turkeys, percentages SO fibres in that half were on average lower than that found in the m. iliot. of 9- to 12-week-old domestic chickens (*Gallus domesticus*) by MacNaughtan (1974) who reported approximately 7% SO fibres. Ono et al. (1993), however, were unable to detect SO fibres in the m. iliot. of 1- to 35-week-old domestic chickens. In guinea fowl, the percentages of SO fibres were considerably higher than in turkeys and chickens, while the segment containing SO fibres increased gradually with age.

Although m. pect. is an anaerobic muscle, it might be expected that very young chicks could compensate their very high mass-specific heat loss through shivering of both the breast and leg muscles. Nevertheless, unlike the leg muscles, the breast muscles were not involved in shivering in hatchlings. This is consistent with the findings in capercaillie (*Tetrao urogallus*) hatchlings (Hissa et al. 1983), but in contrast to the findings in willow ptarmigans (*Lagopus lagopus*) hatchlings (Aulie 1976b). However, in willow ptarmigan hatchlings shivering activity of the breast muscle was low, and Aulie (1976b) suggests that it did not contribute much to the heat production. Perhaps m. pect. did not contribute to shivering because its relative mass was considerably smaller than that of the leg muscles (Fig. 5); however, the lower level of development of the m. pect. was probably a more important factor. Our histological samples showed that upon hatching, muscle tissue of m. pect was much less developed than that of m. iliot.

(Fig. 6). In accordance with this, WF was high in m. pect., as was also the case in capercaillie hatchlings [Milonoff and Lindén (1989b) cited in Saarela et al. (1990)]. This indicates that at hatching the level of functional maturity of m. pect. was low (Ricklefs 1983; Ricklefs and Webb 1985).

The WF of m. pect. decreased rapidly with subsequent development, reflecting a rapid increase in functional maturity. In both species, the breast muscles contributed to shivering thermogenesis at day 6. At this age, relative m. pect. mass was still much smaller than that of the leg muscles (Fig. 5B), suggesting that the change in the participation in shivering of m. pect. was not related to a change in relative muscle mass. At day 6, the WF of m. pect. had decreased to levels about equal to (guinea fowl) or lower (turkeys) than the WF of m. iliot. at hatching, when m. iliot. already participated in shivering. We therefore hypothesize that a minimum level of functional maturity is required to enable a muscle to participate in shivering. This minimum level could be represented by a WF of approximately 0.85, because in both species WF of m. iliot. was at hatching slightly below 0.85, while WF of m. pect. had decreased to below this level at day 6 (Fig. 5C).

After day 20 in turkeys and day 10 in guinea fowl, m. pect. participated no more in shivering (within the T_A range used), despite a sufficient level of functional development. In turkeys, relative breast muscle mass was larger than that of the leg muscles from 20 day onwards; but in guinea fowl, relative breast muscle mass remained smaller than that of the leg muscles. Apparently, relative muscle mass had again little effect on the change in participation of the breast muscle in shivering. Since neither functional maturity, relative muscle mass nor a fibre-type change were involved in this second change in participation in shivering of the breast muscle, a fourth factor must be involved. This factor may well be the rate of mass-specific heat loss in combination with muscle fibre type as found in adult bantams, where the anaerobic glycolytic breast muscle participated only in shivering when the heat loss was severe or core temperature decreased considerably (Aulie and Tøien 1988; Tøien 1993a). After day 6 in turkeys and day 10 in guinea fowl, empty body mass increased to about 100 g and the thermal coefficient decreased considerably (Table 1). In other words, there was a large decrease in rate of mass-specific heat loss and the chicks should be able to maintain a constant body temperature with a much a smaller increase in M_{MS}. Consequently, the required level of mass-specific shivering thermogenesis would have been lower. In guinea fowl, the large decrease in rate of mass-specific heat loss after day 10 coincided with the cessation of the participation of the breast muscle in shivering. This suggests that indeed the participation of the anaerobic, glycolytic breast muscles in shivering was correlated with the rate of mass-specific heat loss.

Turkeys, however, did not respond in the same manner. The breast muscle continued to participate in shivering until day 20 although the rate of mass-specific heat loss had decreased after day 6. This could be due to several reasons: firstly, the percentage of SO fibres in the m. iliot. was much lower in turkeys than in guinea fowl. This may have resulted in a relatively lower shivering thermogenesis of m. iliot., which could be compensated by shivering of the breast muscle. However, it seems likely that a relatively low shivering thermogenesis of m. iliot. will initially be compensated by a higher shivering thermogenesis of the other leg muscles, because those are generally oxidative muscles. Overall shivering thermogenesis of the leg muscles may thus not have differed between turkeys and guinea fowl, and participation of the breast muscle in turkeys after day 6 is then not expected. A second explanation could be that not only the percentage of SO fibres is important, but that also the percentage of FOG fibres influences the participation in shivering. In adult turkeys, the percentage of FOG fibres in the breast muscle is low, about 5% (Wiskus et al. 1976). If the percentage of FOG fibres in turkey breast muscles exceeded this adult level at day 20, a correlation between a change in FOG fibres and shivering participation could be present. However, no data are available on percentages FOG fibres in young turkeys, nor for guinea fowl breast muscles, and this assumption may be invalid. Finally, it cannot be excluded that the relative contribution to shivering of the leg muscles was initially much lower in turkeys than in guinea fowl, and that of the breast muscles higher. If the contribution of the breast muscles to shivering thermogenesis was relatively important in turkeys, this could account for the continuation of the participation in shivering of m. pect. after their decrease in mass-specific heat loss.

Unlike m. pect., m. iliot. was involved in shivering during the whole study period, from hatching to 1 month post-hatch. We assumed that shivering of the m. iliot. was representative of shivering in other leg muscles. This is supported by the finding that in adult bantams, EMG activity of m. iliot. and musculus gastrocnemius were of about the same magnitude and occurred synchronously (Tøien 1993a,b). At all ages the relationship between the rms EMG of m. iliot. and T_A resembled that of M_{MS} and T_A. This indicates that the leg muscles contributed considerably to shivering thermogenesis during development. Moreover, T_{ST} of m. iliot. was indistinguishable from T_{LC}. Also in non-acclimated 4-week-old muscovy ducklings (*Anas barbariae*) and 6- to 9-months-old king penguins (*Apenodytes patagonicus*) the T_{ST} of the leg muscles and T_{LC} were similar (Barré et al. 1985; Duchamp et al. 1989). In these non-acclimated chicks, regulatory non-shivering thermogenesis was thus most likely not present, as opposed to cold-acclimated muscovy duck and king penguin chicks in which the T_{ST} of the leg muscles was about 14 and 9 °C below T_{LC}, respectively (Barré et al. 1985; Duchamp et al. 1989).

Unexpectedly, the percentage SO fibres in m. iliot. was relatively low, especially because half of the muscle did not contain any SO fibres. The SO fibres were lo-

cated at the outer (skin) part of the muscle, which seems not the most efficient location to generate heat to warm the body. Possibly, FOG fibres were involved in shivering as well. In domestic chickens, the percentage FOG fibres of m. iliot. increased from about 22% at week 1 to about 54% at week 35 (Ono et al. 1993). Probably shivering in other leg muscles with a high content of SO/FOG fibres make important contributions to the heat production, and it may well be that part of the rms EMG picked up by the electrode on m. iliot. originates from these underlying muscles.

In conclusion, it appears that two of the three factors mentioned in the Introduction influence the participation of breast and leg muscles in shivering in young galliforms. The first factor, relative muscle mass, had apparently no effect on the participation in shivering of the breast and leg muscle. The second factor, level of maturity, was an important constraint for participation in shivering. Apparently a minimal level of maturity is required before a muscle can participate in shivering, which may be represented by a WF of approximately 0.85. Further research is necessary to investigate whether this level of WF represents a general threshold to the participation in shivering of muscles in young birds. The third factor, muscle fibre type, may in combination with mass-specific heat loss influence the role of a muscle in shivering. In both species there was a preference to first recruit the more aerobic muscles followed by the less oxidative muscles when the rate of mass-specific heat loss was high. This phenomenon has not only been shown in young and adult galliforms (Aulie and Tøien 1988; Tøien 1993a; this study), but also in adult house finches (*Carpodacus mexicanus*) where the breast muscle is more oxidative than the leg muscles (Carey et al. 1989a,b). Thus, the present study supports the hypothesis that aerobic muscles are recruited first in birds.

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