

# Comparison of physical body growth and metabolic and reproductive endocrine functions between north and south climates of Japan in trained Thoroughbred yearling horses

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*This study aimed to compare body growth, metabolic, and reproductive hormonal changes in trained Thoroughbred yearling horses under different climate conditions with and without light supplementation (LS). Thoroughbred yearlings raised at research centers of the Japan Racing Association in Hokkaido (north) or Miyazaki (south) were divided into control and LS groups. In the LS groups, 44 colts and 47 fillies from Hokkaido and 11 colts and 11 fillies from Miyazaki were exposed to LS with an extended photoperiod of 14.5 hr of daylight and 9.5 hr of darkness. One week before and once a month after LS, circulating total thyroxine (T4), insulin-like growth factor-1 (IGF-1), prolactin (PRL), cortisol, and progesterone (P4) concentrations were measured by radioimmunoassay and fluoroimmunoassay, respectively. Growth parameters, including body weight, height, girth, and cannon bone circumferences, were measured monthly. Hair coat (HC) condition was scored. Under natural conditions, the T4 concentrations of Hokkaido yearlings tended to be higher, whereas the IGF-1 (colt) and PRL levels were significantly lower than those of yearlings in Miyazaki. Growth parameters and HC scores were lower in Hokkaido yearlings. With LS, the PRL and P4 concentrations in Hokkaido and Miyazaki were higher, and the first ovarian activity tended to be earlier than in the controls. Only LS Hokkaido yearlings showed significantly higher HC scores than the controls. Comparing the different climates among the LS yearlings, the levels of PRL and P4 and the HC scores in Hokkaido yearlings increased and reached levels similar to those in Miyazaki yearlings. The body weight and girth increment percentages of Hokkaido yearlings in January dramatically decreased and then eventually increased to levels similar to those of Miyazaki yearlings. This suggested that yearlings in naturally colder Hokkaido exhibit higher basal metabolism to maintain homeostasis. However, providing LS may help to improve growth and early development of reproductive function in Hokkaido yearlings to levels equal to those of Miyazaki horses.*

**Key words:** artificial light, climate, growth, reproductive function, Thoroughbred yearlings

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Horses are expected to show not only good performances but also sound body physical conditions for achievement in the racing industry. They are long-day seasonal breeders, with their highest reproductive activity in spring to summer. Artificial light supplementation is widely used to extend the daylight hours in clinically healthy and anovulatory broodmares and stallions to enhance reproductive functions during short-day periods, i.e., the nonbreeding season. In Japan, most of Thoroughbred racehorses have been produced in Hokkaido, which is located in the northern temperate climate zone and has cold weather in winter. For decades, Hokkaido has been known as the main area for Thoroughbred horse breeding in Japan. It has been observed that Thoroughbred horses raised in Miyazaki, a southern subtropical climate with warm weather, grow well and seem to be bigger than horses in the north. The length of the day in autumn through winter in Hokkaido is shorter than in Miyazaki. Recently, there have been reports showing that an extended photoperiod affects the gonadal functions of yearling horses reared in Hokkaido and Miyazaki; however, the knowledge concerning the mechanism of the effect on growth in yearlings is still quite limited.

Thyroid hormones play important roles in thermogenesis, metabolism, and growth in humans and animals [3, 4, 10, 27]. Moreover, thyroid hormones can be an indicator of metabolic rate in the resting stage under various environmental and climatic conditions [16, 29, 31, 43]. Increased thyroxine (T4) and triiodothyronine (T3) concentrations in horses have been observed as a result of cold exposure [26]. Previous researchers have reported that T4 and T3 increased metabolism of most cells and stimulated growth in young animals [9, 25, 39].

The present study aimed to compare the changes in circulating thyroxine, body physical growth, and reproductive hormones in the presence and absence of artificial light supplementation between trained Thoroughbred yearling horses in Hokkaido and Miyazaki.

## Materials and Methods

All procedures in this study were performed according to the guidelines of the Institutional Animal Welfare and Experiment Management Committee of the Japan Racing Association (JRA) Hidaka Training and Research Center.

### *Animals and diets*

During two experimental periods (from autumn of 2012 to spring of 2013 and from autumn of 2013 to spring of 2014), a total of 160 trained Thoroughbred yearling horses were subjected to the research from September (autumn) at 1 year of age to April (spring) at less than 2 years of age at 2 facilities of the JRA: the Hidaka Training and Research

Center in Hokkaido (temperate north, latitude 42.2° and longitude 142.8°) and the Miyazaki Training Yearling Farm in Miyazaki (subtropical south, latitude 31.9° and longitude 131.4°). The animals were randomly divided into 2 groups at each facility: the control and light supplementation (LS) groups. There were 25 control horses (12 colts, 13 fillies) and 91 LS horses (44 colts, 47 fillies) in Hokkaido and 22 control horses (10 colts, 12 fillies) and 22 LS horses (11 colts, 11 fillies) in Miyazaki. All horses were fed hay (*Poa pratensis*), oats, and pellet feed (JRA original 10, NOSAN Corporation, Kanagawa, Japan) containing vitamins and trace mineral supplementation in their individual stalls 4 times per day. Water was provided *ad libitum*. Through the end of December, Miyazaki horses were led to pasturing and fresh grass grazing daily, while Hokkaido horses were pastured in small paddocks and ate dried Timothy grass. For winter pasturing beginning in January, the Hokkaido and Miyazaki horses had free access to hay and water provided by a heated automatic waterer.

### *Exercise program*

The horses were trained with training system conducted in accordance with JRA original training programs for growing horses. Both Hidaka and Miyazaki horses warmed up on a walking machine for 30 min/day through the end of January, whereas they warmed up by trotting 800 m during February and March. Low- and high-intensity training were performed by having the horses canter and gallop on 800 m flat-track and 1,000 m slope courses in Hokkaido and a 1,600 m flat-track course in Miyazaki; the horses then cooled down by walking.

### *Light supplementation*

Artificial light supplementation was conducted by using a timer-linked 100 watt white light bulb that was set in the ceiling of the horse stall (3.6 × 3.6 m). An extended photoperiod was created with 14.5 hr of daylight and 9.5 hr of darkness, equal to the summer conditions, from December 25th to April 16th in each year.

### *Growth parameters and hair coat conditions*

Body weight, height, girth, and cannon bone circumferences were measured monthly. Increment percentages of all parameters were calculated as follows: (value of current month-value of previous month)/ value of previous month × 100. Means of raw values and increment percentages were used for comparison between groups. In addition, hair coat condition, i.e., type of hair, thickness, shininess, and shedding were evaluated and scored by three veterinarians in November, January, and April in each year for two experimental periods. The scoring system was categorized as follows: 1=poor, 2=normal, and 3=excellent. Mean

scores were compared between groups.

### *Endocrine parameters*

Blood samples were collected from jugular veins into Vacutainers with no anticoagulant or heparinized Vacutainers between 9:00 and 12:00 hr, one week before LS (mid-December) and then once a month from January to April (late January, late February, mid-March, and early April) in each year for two experimental periods. After centrifugation, sera (for Miyazaki) and plasma (for Hokkaido) were harvested and stored at  $-20^{\circ}\text{C}$  until assayed. For parameters of endocrine function, circulating total T4, insulin-like growth factor-I (IGF-1), prolactin (PRL), cortisol, and progesterone levels were measured.

### *Hormonal assay*

Determination of the circulating total T4 concentration was carried out by double-antibody radioimmunoassay (RIA) system using  $^{125}\text{I}$ -labeled radioligands. Rabbit polyclonal anti-thyroxine BSA serum (65850), thyroxine-BSA conjugate (Cat No. 8960) for radioiodination, and L-Thyroxine (T2376), as the reference standard, were used in the assay. The intra- and inter-assay coefficients of variation were 9.7 and 5.9%, respectively.

Circulating IGF-1 concentrations were measured by RIA as described previously [30] using rabbit anti-serum against human IGF-1 (AFP4892898) and recombinant human IGF-1 (Lot. No. 090701) for radioiodination and as the reference standard. The intra- and inter-assay coefficients of variation were 8.6 and 5.0%, respectively.

Prolactin concentrations in blood circulation were determined by RIA as described previously [30] using rat anti-serum against equine PRL (AFP-261987), purified equine PRL (AFP-8794B) for radioiodination, and equine PRL (AFP-7730B) as the reference standard. The intra- and inter-assay coefficients of variation were 10.0 and 5.3%, respectively.

Circulating cortisol concentrations were determined by RIA system as previously described [2] using rabbit anti-cortisol (HAC-AA71-02RBP85), Cortisol-3-CMO-BSA (80-IC20) for radioiodination, and Hydrocortisone (H-4001) as the reference standard. The intra- and inter-assay coefficients of variation were 9.6 and 1.3% for cortisol, respectively.

Measurement of progesterone concentrations in fillies was performed by time-resolved fluoroimmunoassay (DELFIA Eu-Labeling kit, PerkinElmer, Waltham, MA, U.S.A.) according to the kit's protocol. Fluorescence was measured using a time-resolved fluorimeter (Wallac 1420 Multilabel Counter, PerkinElmer). The intra- and inter-assay coefficients of variation were 4.6 and 4.9%, respectively.

### *Determination of ovarian function*

The first expected ovarian activity in fillies was arbitrarily defined as occurring when the concentration of progesterone reached 1 ng/ml or higher.

### *Statistical analysis*

All statistical analyses were performed using R software. The results are presented as the mean  $\pm$  standard error of the mean (SEM). The repeated measures data in longitudinal sampling were analyzed using generalized least-squares (GLS). Differences in means of all parameters between groups and among times during the study were adjusted by Bonferroni's multiple comparison tests. The significances level was set at  $\alpha=0.05$ .

## **Results**

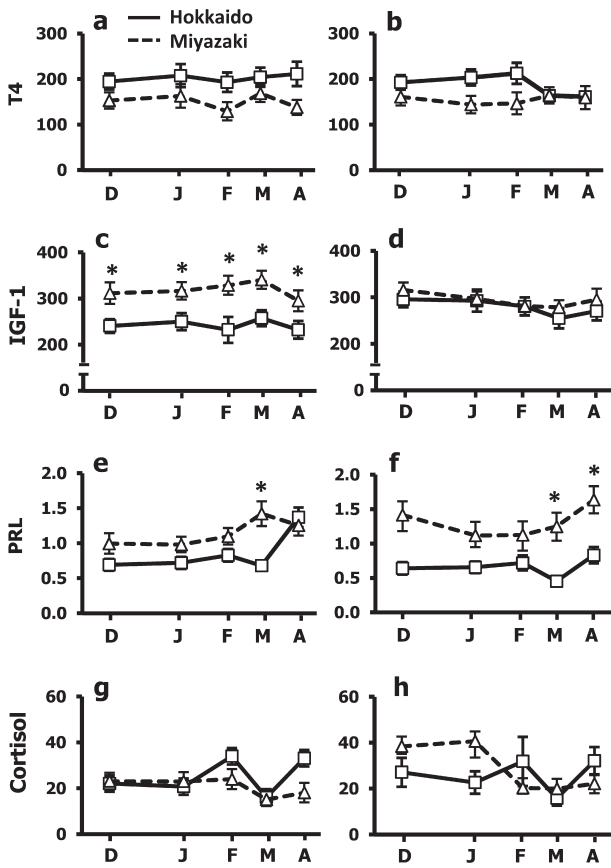
### *Comparison between Hokkaido and Miyazaki horses under natural light conditions*

Comparison of circulating total T4, IGF-1, PRL, and cortisol between Hokkaido and Miyazaki horses under natural conditions are shown in Fig. 1. Circulating T4 concentrations in Hokkaido colts and fillies tended to be higher than those in Miyazaki colts and fillies (Fig. 1a and 1b). No significant differences in T4 concentrations were found among periods. In contrast, the IGF-1 concentrations in Hokkaido colts were significantly lower than those in Miyazaki colts, while the IGF-1 concentrations were not significantly different in fillies (Fig. 1c and 1d). Also, Miyazaki colts and fillies had significantly higher prolactin levels when compared with those of Hokkaido horses (Fig. 1e and 1f). The concentrations of cortisol were not significantly different between the Hokkaido and Miyazaki groups, but the levels in both Hokkaido colts and fillies tended to be higher than those of Miyazaki colts and fillies in late February and early April (Fig. 1g and 1h).

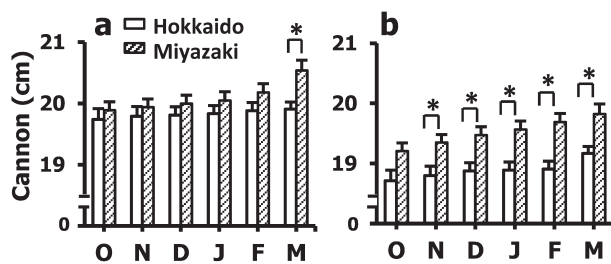
Comparison of the monthly means of cannon bone circumferences between the control Hokkaido and Miyazaki horses are shown in Fig. 2. Cannon bone circumferences were significantly lower in Hokkaido colts and fillies when compared with those of Miyazaki colts and fillies (Fig. 2a and 2b). No significant differences were noted in the means of body weight, height, and girth circumference (data not shown); however, Hokkaido colts and fillies tended to have lower values for these three parameters than Miyazaki horses.

### *The effects of LS under the differing climates of Hokkaido and Miyazaki*

Changes in endocrine functions in the LS and control groups from Hokkaido are shown in Fig. 3 (data not shown for the Miyazaki groups). Circulating T4 concentrations

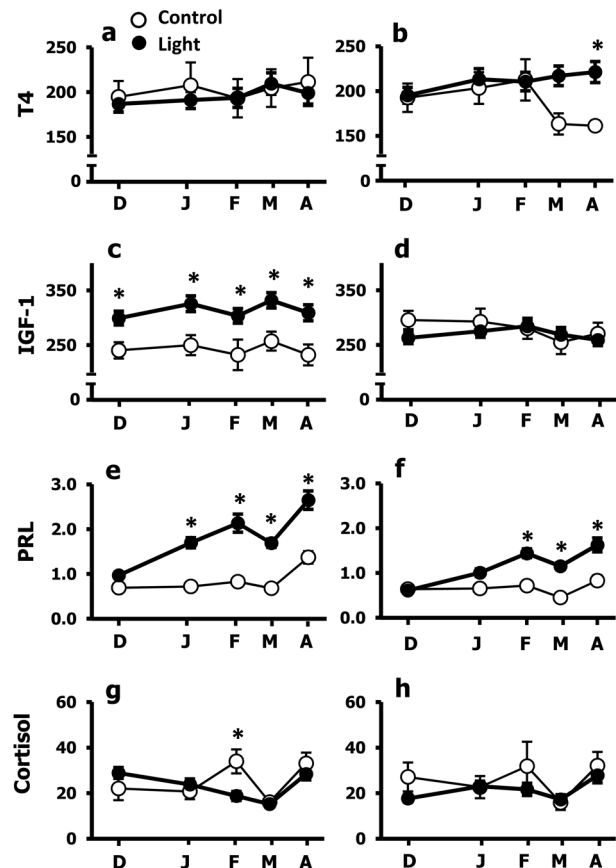


**Fig. 1.** Changes of circulating total T4 (a, b), IGF-1 (c, d), PRL (e, f), and cortisol (g, h) concentrations (ng/ml) in colts (a, c, e, g) and fillies (b, d, f, h) from Hokkaido (—) and Miyazaki (---) under natural conditions from December to April. Values are expressed as the mean ± SEM. \*Significant differences ( $P < 0.05$ ) between different climates in the same period in each sex.



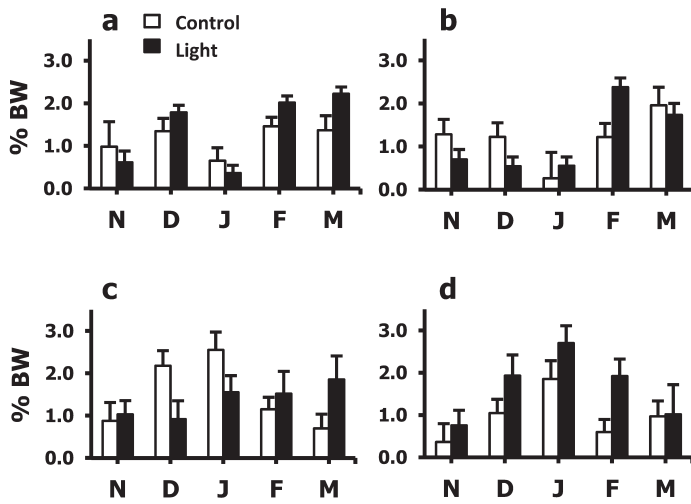
**Fig. 2.** Comparison of cannon bone circumferences between Hokkaido (□) and Miyazaki (▨) colts (a) and fillies (b) under natural conditions from October to March. Values are expressed as the mean ± SEM. \*Significant differences ( $P < 0.05$ ) between different climates in the same period in each sex.

showed no significant differences between the LS and control groups in Hokkaido colts (Fig. 3a) and in Miyazaki

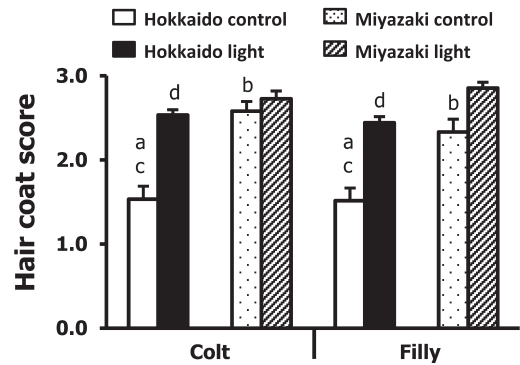


**Fig. 3.** Changes of circulating total T4 (a, b), IGF-1 (c, d), PRL (e, f), and cortisol (g, h) concentrations (ng/ml) in colts (a, c, e, g) and fillies (b, d, f, h) from Hokkaido in control (○) and light supplementation groups (●) from December to April. Values are expressed as the mean ± SEM. \*Significant differences ( $P < 0.05$ ) between different treatment groups in the same period in each sex.

colts and fillies. In Hokkaido fillies, the T4 concentrations were significantly higher in the LS group than the controls in early April (Fig. 3b). Only Hokkaido LS colts showed significantly higher levels of IGF-1 than controls throughout the periods (Fig. 3c). No significant differences in IGF-1 levels were noted between the control and LS groups in Hokkaido fillies (Fig. 3d) and Miyazaki yearlings. The LS groups of Hokkaido (Fig. 3e and 3f) and Miyazaki horses showed significantly higher prolactin concentrations than the controls. In Hokkaido, the prolactin level rose in early April in control groups, but it increased in late January in LS groups (Fig. 3e and 3f). In Miyazaki, the prolactin concentration rose around mid-March in control groups, whereas it increased in late January in LS groups. In addition, there were no significant differences in circulating cortisol concentrations between LS and control groups in Hokkaido



**Fig. 4.** Comparison of increment percentages of body weight (BW) between the control (□) and light supplementation (■) groups of colts and fillies from Hokkaido (a, b), and Miyazaki (c, d) from November to March. Values are expressed as the mean ± SEM.



**Fig. 5.** Hair coat condition scores of colts and fillies from Hokkaido and Miyazaki under natural (□, ▨) and light supplementation (■, ▩) conditions, respectively, in April. Values are expressed as the mean ± SEM. <sup>a, b</sup>Significant differences ( $P < 0.05$ ) between different climates in the same treatment group and period in each sex. <sup>c, d</sup>Significant differences ( $P < 0.05$ ) between different treatment groups in the same period in each place and sex.

fillies (Fig. 3h) and Miyazaki colts and fillies. However, the cortisol levels in Hokkaido LS colts were significantly lower than those of the controls in late February (Fig. 3g).

The monthly means of the body weight increment percentages of the Hokkaido and Miyazaki horses are shown in Fig. 4. The body weight increment percentages of the Hokkaido colts and fillies in both the control and LS groups apparently decreased in January and eventually increased during February and March (Fig. 4a and 4b), whereas a notable decline in body weight increment was not observed in Miyazaki horses during January (Fig. 4c and 4d). Moreover, these trends were similarly found in the increment percentages for height, girth, and cannon bone circumferences (data not shown). In comparisons between LS and control groups, the increment percentages for body weight (Fig. 4) and other growth parameters, i.e., height, girth, and cannon bone circumferences (data not shown), were not significantly different in both colts and fillies from Hokkaido and Miyazaki. However, the increment percentages for body weight in the LS groups from both Hokkaido and Miyazaki tended to be higher than those of the controls during February and March (Fig. 4).

The changes in hair coat condition scores in April are shown in Fig. 5. Both Hokkaido colts and fillies in control groups had hair coat condition scores that were significantly lower than those of the Miyazaki horses. With LS, the scores were increased and significantly higher in Hokkaido LS colts and fillies than the controls, whereas Miyazaki horses did not show differences in these scores.

Changes in progesterone concentrations in Hokkaido

and Miyazaki fillies under control and LS conditions are shown in Fig. 6. In the control, circulating progesterone was maintained at basal levels from December to March in Hokkaido (Fig. 6a) and Miyazaki (Fig. 6c) horses, and the levels then increased and reached 1 ng/ml in early April. With LS, the progesterone concentrations were markedly higher in LS groups than the controls in both Hokkaido and Miyazaki. Also, the first ovarian activity in LS groups from Hokkaido (Fig. 6b) and Miyazaki (Fig. 6d) was earlier (late February) than in the controls (early April).

*Comparison between Hokkaido and Miyazaki horses under light supplementation*

Comparison of the changes in circulating total T4 concentrations between Hokkaido and Miyazaki LS horses are shown in Fig. 7. With LS, the total T4 concentrations in Hokkaido colts and fillies tended to be higher than in the Miyazaki colts and fillies throughout the experimental period, with significant differences in late February (colts and fillies) and early April (fillies) (Fig. 7a and 7b). There were no significant differences in the levels of IGF-1 between Hokkaido and Miyazaki LS horses (data not shown). Circulating prolactin levels in Hokkaido LS colts increased and reached levels similar to those of the Miyazaki LS colts, whereas those of the fillies did not (data not shown). The Hokkaido LS fillies did show a trend toward increased progesterone levels, which was similar to results observed for the Miyazaki LS fillies. The circulating cortisol concentrations in Hokkaido LS horses tended to be lower than those of Miyazaki LS horses during February, whereas

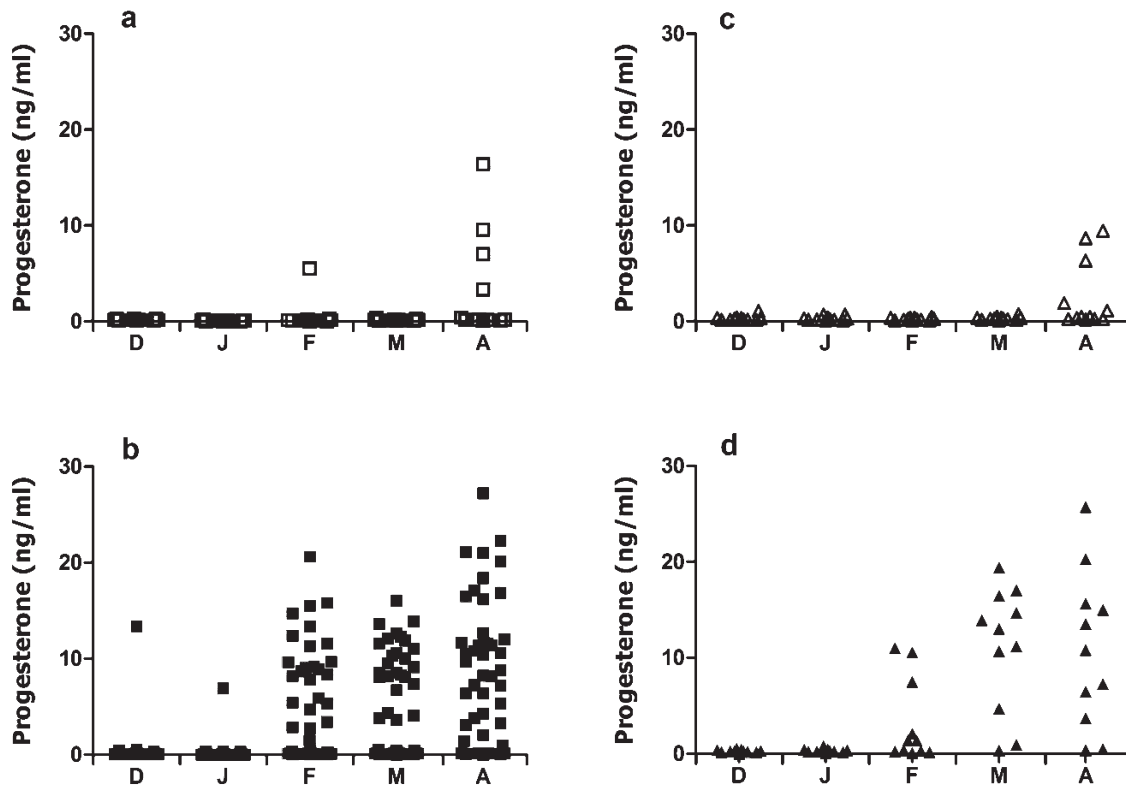


Fig. 6. Changes of circulating progesterone concentrations (ng/ml) from December to April in individual Hokkaido (a, b) and Miyazaki (c, d) fillies under natural (a, c) and light supplementation (b, d) conditions, respectively.

they tended to be higher in April (data not shown).

Comparison of the increment percentages of body weight and girth circumference between the Hokkaido and Miyazaki LS groups are shown in Fig. 8. In January, the results showed that the body weight and girth increments of Hokkaido colts and fillies decreased dramatically and became lower than those of the Miyazaki horses. Then, the increments increased to levels similar to those of the Miyazaki horses, without significant differences, during February and March (Fig. 8).

Finally, comparisons of hair coat conditions in April between Hokkaido and Miyazaki LS horses are shown in Fig. 5. The scores of the Hokkaido colts and fillies were not significantly different from those of the Miyazaki horses.

## Discussion

The present study was clarified that under natural conditions, Hokkaido horses raised in the north tended to be inferior to Miyazaki horses (south). However, we confirmed that providing of artificial light supplementation may help to improve body physical growth and early development of reproductive function in Hokkaido yearlings to levels

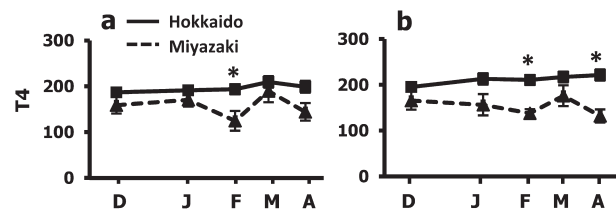
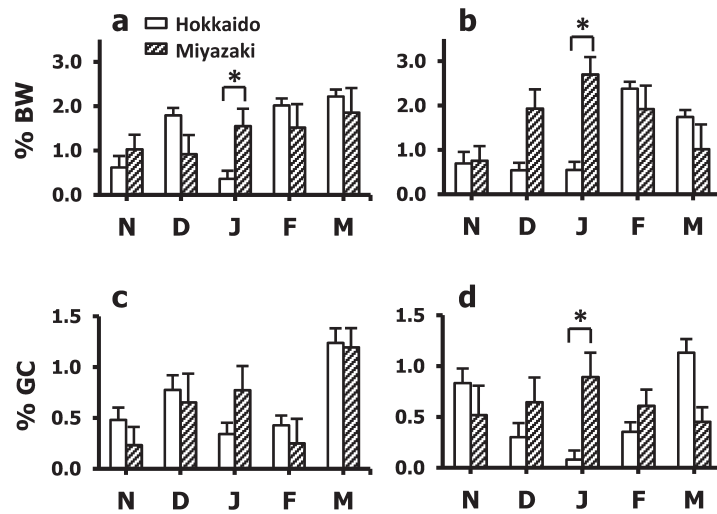


Fig. 7. Changes of circulating total T4 concentrations (ng/ml) in colts (a) and fillies (b) from Hokkaido (—) and Miyazaki (---) under light supplementation from December to April. Values are expressed as the mean  $\pm$  SEM. \*Significant differences ( $P < 0.05$ ) between different climates in the same period in each sex.

equal to those of Miyazaki horses. With light supplementation, our study found that the increment percentages of all 4 growth parameters tended to be higher in LS groups from both Hokkaido and Miyazaki compared with the controls. Moreover, the present study clearly demonstrated that the LS groups in Hokkaido, both colts and fillies, had better hair coat scores in April than control groups, whereas no significant differences were observed in Miyazaki. These findings were consistent with the increase in prolactin



**Fig. 8.** Comparison of increment percentages of body weight (BW; a, b) and girth circumference (GC; c, d) between Hokkaido (□) and Miyazaki (▨) colts (a, c) and fillies (b, d) under light supplementation from November to March. Values are expressed as the mean  $\pm$  SEM. \*Significant differences ( $P < 0.05$ ) between different climates in the same period in each sex.

concentrations in Hokkaido LS colts and fillies from late January to early April. Regarding reproduction, the first ovarian activity designated by the levels of progesterone was obviously earlier in LS fillies from both Hokkaido and Miyazaki. The present results in conjunction with similar findings of previous studies [24, 38] suggested that artificial light supplementation was able to stimulate prolactin secretion and the hypothalamic-pituitary-gonadal axis, resulting in enhancement of gonadal function and acceleration of growth in winter Hokkaido yearlings to levels similar to those of Miyazaki yearlings.

Under natural conditions, our results showed that body growth and development of gonadal function were slower in Hokkaido yearlings compared with the Miyazaki yearlings. Interestingly in winter (January), we found a dramatic decrease in all growth increments in Hokkaido horses followed by an eventual increase in February and March, but not in Miyazaki horses. This remarkable point indicated that in Hokkaido, January, the coldest month, was a difficult time and resulted in a slow growth rate in Hokkaido horses. Miyazaki horses were able to keep growing throughout the winter and reach as close to the maximum limit as possible. However, the body and reproductive developments of Hokkaido horses began to increase again in early spring. According to the weather reports from the Japan Meteorological Agency from 2012 to 2014, the years in which this experiment was performed, the mean daily minimal temperature in the Hidaka area of Hokkaido was lower than that in Miyazaki throughout the experiment years

(9–10°C approximately). In the winter months, January showed especially low temperatures in Hokkaido, around –9°C, whereas the temperature was 2–3°C in Miyazaki. In addition, the number of daylight hours during the winter period was about one hour shorter in Hokkaido than in Miyazaki. These climate distinctions were in accordance with our results suggested that the differences in climate between the north and south of Japan may affect growth and early reproductive development in young horses, which is consistent with a previous report [30].

Thyroxine secreted by the thyroid gland plays the key roles in regulation of body temperature, metabolism, and growth. Hokkaido colts and fillies tended to have higher T4 levels than Miyazaki colts and fillies throughout the periods under natural light. Our results indicated that horses raised in the colder north had a higher basal level of thyroid hormone than horses that dwelled in the milder south. Nevertheless, the higher level of T4 in Hokkaido horses did not conform to the growth profile. It can be suggested that Hokkaido horses adapted in response to a lower ambient temperature for maintenance of body homeostasis for long-term survival instead of growth. Regarding the limitations of cold resistance in horses, the lower critical temperature (LCT), the temperature at which metabolic heat production increases for body core temperature maintenance [12, 14], was estimated to be around 0°C in limit-fed for normal growth in yearlings [13, 14]. Typically, horses need 10–21 days to acclimatize to cold weather and required another 10–21 days for more diminished temperatures. When reaching

the LCT, physiological, metabolic, and behavioral changes occur to reduce heat loss and conserve energy [14].

Moreover, the current study did not show significant changes in T4 levels during the period of winter to early spring; only small fluctuations of T4 levels were shown, which was in agreement with other findings [14, 15, 18, 19]. This may suggest that chronic cold exposure as the season changed from winter to early spring did not affect the T4 concentration due to acclimatization of horses. Several studies about thermoregulation of horses in winter have clarified that T4 secretion is transiently elevated during acute or short cold exposure in adult horses [18, 26]. For chronically cold weather, serum thyroid hormone and metabolic rate did not change significantly from autumn to spring [28]. In the case of long-term exposure to low temperatures in other species, thyroid hormone levels increased and resulted in stimulation of metabolism and heat production in sows [1], sheep [34], and cattle [11]; horses, on the other hand, employed a state of hypometabolism to keep their bodies warm [5, 6, 26].

According to the present results, no significant differences in T4 levels were noted between the LS and control groups in Hokkaido colts and Miyazaki colts and fillies. However, T4 concentrations tended to be higher in LS Hokkaido fillies from March to April due to a decrease in T4 in control fillies. In other species, the photoperiod has been shown to affect thyroid activity; for example, the peak thyroid hormone concentrations in plasma were found in the increasing day length period of spring, while the lowest levels were recorded during decreasing daylight of early autumn in rams [33, 35] and in goats [40, 41]. We do not know the reason why the LS had no effects on T4 levels in yearling horses.

The present study showed that the increase in prolactin levels with light supplementation was not accompanied by an increase in T4. Also, there was no significant correlation between prolactin and T4 concentrations. Stimulation and suppression of prolactin secretion are regulated by TRH and dopamine, respectively. A previous study noted that mares pretreated with TRH followed by a mixture of a dopamine antagonist and growth hormone analog showed greater prolactin but lower TSH responses than mares not pretreated with TRH [17]. The results of the present study in conjunction with this previous report suggested that elevation of circulating prolactin might result from a decrease in dopamine caused by light supplementation rather than TRH stimulation. T4 may fluctuate mainly due to temperature changes, whereas prolactin alters due to the effects of light. We cannot deny the collective effect of these two climatic factors on physiological changes of horses.

Light supplementation in the present study did not result in any changes in circulating IGF-1 concentrations

in Hokkaido fillies and Miyazaki colts and fillies. Only Hokkaido LS colts had significantly higher IGF-1 levels than the controls. However, their IGF-1 levels were higher at the beginning than the controls before treatment, and the patterns of fluctuation were similar to those of the controls. A previous study reported that the localizations of IGF-1 and its receptor were stronger in postpubertal stallions than prepubertal stallions [43]. IGF-1 might not be obviously involved in the growth of the yearlings used in our research.

The present study showed that light supplementation helped to raise circulating prolactin concentrations and advanced the first ovarian activity in both Hokkaido and Miyazaki horses. Prolactin is secreted into the blood circulation at greater levels during a longer photoperiod and at lower levels during a shortened photoperiod. It is responsible for hair shedding and plays a role in reproduction in horses. Previous studies reported that prolactin and its receptor were found in equine follicular fluid, ovarian follicles, and the corpus luteum (CL) [21, 22, 32]. The periovulatory surge of prolactin in mare has been suggested to play a role around the time of ovulation [23]. In agreement with the results of previous studies, our results suggested that the light-induced increase in prolactin might hasten gonadal function in yearlings by increasing the number of LH receptors in granulosa and luteal cells of the ovary [20, 42].

According to growth results in the present study, the effects of LS tended to be higher in Hokkaido horses when compared with the Miyazaki horses. The basal levels of prolactin in Hokkaido horses prior to LS were lower than those of Miyazaki horses. The growth rates of Hokkaido horses were retarded due to lower temperatures during severely cold winters. These inferiorities might result in higher response to LS after the critical period in Hokkaido horses. On the other hand, the growth of Miyazaki horses might have already been accelerated as much as possible, so the increment of growth in response to LS became smaller. The present study suggested that prolactin was probably indirectly involved in the development of the body. Previous studies showed that calcium absorption in the intestine was promoted by prolactin [7, 8, 36], and expression of the prolactin receptor was found in epiphyseal plate [37].

The current study found that Hokkaido horses had higher cortisol levels than Miyazaki horses in February and April. This could suggest that the higher levels were presumably caused by the exercising program in Hokkaido, which tended to be harder than that in Miyazaki. In February, Hokkaido horses were trained on 800 m flat and slope courses, while in Miyazaki, the horses were only trained on a 1,600 m flat track. By April, the covering snow had melted, so the training program for Hokkaido horses changed to using 1,600 m flat and slope courses, whereas the 1,600 m flat



track continued to be used in Miyazaki. Furthermore, light supplementation did not show consistent effects on the cortisol levels in both Hokkaido and Miyazaki horses. The reason for this contradiction is unclear.

In conclusion, differences in climate, especially temperature, had influences on growth and reproductive development in yearling horses. Light supplementation tended to advance early gonadal development and promote body growth in Hokkaido yearlings to levels similar to those of Miyazaki horses. For further studies, the total and free T3, free T4, TSH, and basal and field metabolic rates should be investigated to improve understanding of growth and metabolism in young horses.

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