P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.098

Thyroxine, Cortisol and Testosterone Hormone Analysis to Overview the Adaptability of the Swamp Buffalo under Different Agroclimatic Conditions

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Article History: 23-255 Received: 18-Jul-23 Revised: 09-Aug-23 Accepted: 19-Aug-23

ABSTRACT

Different agroclimatic conditions affect the endocrine system and metabolism of animals. This study aimed to analyze the effect of different agroclimatic conditions on blood thyroxine, cortisol, and testosterone concentrations in Swamp buffaloes. For this study, hormonal profiles of 38 Swamp buffaloes, consisting of 18 buffalo bulls aged 3-5 years and 20 heifers aged 2-3 years, kept under different agroclimatic conditions were analyzed. These animals were sampled from three districts in West Sumatra, namely: Lubuk Basung district (representing lowland conditions), Akabiluru district (representing moderate-land conditions) and Matur district (representing highland conditions). Hormone analysis was performed at the integrated research laboratory of the Syahkuala University in Banda Aceh, using the ELISA method. The parameters measured were the ambient temperature, temperature-humidity index (THI) and concentrations of thyroxine, cortisol and testosterone hormones. The results showed non-significant differences in serum thyroxine, cortisol and testosterone concentrations in Swamp buffaloes of lowland, moderate land and highland. In conclusion, differences in agroclimatic conditions did not noticeably influence the endocrine system and metabolism of the Swamp buffaloes.

Keywords: Agroclimatic conditions, Hormonal profile, Swamp buffaloes.

INTRODUCTION

Buffalo is a valuable ruminant livestock species for meat production and is found in almost all provinces in Indonesia, because of its ability to live under various environmental conditions. Besides being a meat producer, the buffalo is also a good producer of milk, which is used as raw material for traditional fermented milk products like "dadiah" in West Sumatra (Putra et al. 2011; Hussain et al. 2020; Roza et al. 2021; Roza et al. 2022) and "dangke" in South Sulawesi (Yusuf et al. 2022). However, the sustainability of livestock production systems, especially in the tropics, is remarkably affected by climate changes (Susanty et al. 2018; Pereira et al. 2020; Yetmaneli et al. 2020; Pazla et al. 2021; Reswati et al. 2021).

Buffaloes are vulnerable to heat stress due to their specific morphological and anatomical characteristics (Hussain et al. 2017; Abdoon et al. 2020). Animals of this species have dark-colored skin that absorbs more heat and has fewer pores than cows, adversely affecting their thermoregulation ability (Mota-Rojas et al. 2020). Environmental stress can lead to changes in the endocrine and metabolic profiles of the livestock (Aggarwal and Singh 2010; Hussain et al. 2018), which affects their physiological well-being and productivity. Changes in biochemical and hormonal profiles are physiological responses of livestock to adapt to different climatic conditions so that they can survive and maintain productivity under changing climates (Maurya et al. 2015). The physiological profile of several hormones, including thyroxine, cortisol, and testosterone, are affected by the prevailing environmental conditions (Hilal and AL-Saeed 2020; Lendrawati et al. 2020; Perumal et al. 2021).

Thyroxine hormone is secreted by the thyroid gland (Khongdee et al. 2013). This hormone plays a crucial role in basal metabolism, supports thermogenesis (Yáñez-Pizaña et al. 2020), and regulates body temperature (Aggarwal and Singh 2010). Thyroxine levels in the body reflect changes in metabolism associated with variations in feed consumption (Aggarwal and Singh 2010).

Cite this Article as: Reswati R, Priyanto R, Manalu W, Arifiantini RI and Purwanto BP, 2024. Thyroxine, cortisol, and testosterone hormone analysis to overview the adaptability of the swamp buffalo under different agroclimatic conditions. International Journal of Veterinary Science 13(2): 211-217. https://doi.org/10.47248/journal.ijvs/2023.098

Heat stress usually results in decreased blood concentrations of thyroxine and triiodothyronine hormones (Aggarwal and Upadhyay 2013). Several studies have reported higher blood thyroxine levels in winter compared to summer (Singh et al. 2014; Perumal et al. 2021), while the study of Li et al. (2020) recorded the opposite results. The decreased blood thyroid hormone concentrations are an adaptation strategy of the animal body to reduce metabolic rate and heat production (Farooq et al. 2010).

Cortisol hormone is produced by the adrenal cortex, and its secretion is regulated by the hypothalamus and anterior pituitary gland (Katsu and Baker 2021). Environmental changes trigger the adrenal cortex to synthesize and secrete cortisol (Papadimitriou and Priftis 2009). This hormone serves as a stress marker, with its levels increasing significantly due to high environmental temperatures (Megahed et al. 2008; Aggarwal and Singh 2010). Cortisol is essential for organisms in maintaining homeostasis and managing physical and emotional stress (Papadimitriou and Priftis 2009; Hussain et al. 2022).

Testosterone is the male sex hormone produced by Leydig cells of the testis (Tran et al. 2016). This hormone plays a crucial role in the process of spermatogenesis and the emergence of sexual behavior (Maurya et al. 2015; Gohar et al. 2021). This hormone is the key determinant of male fertility (Qadarsina et al. 2019). Many factors, including climatic conditions, affect the blood concentrations of testosterone in the blood. According to Perumal et al. (2021), blood testosterone concentrations were highest in spring and lowest in summer, while Hilal and AL-Saeed (2020) found that testosterone concentrations were highest in winter and decreased in summer.

In Indonesia, farmers raise buffaloes in various regions, including lowland, moderate-land, and highland areas. Agro-climatic factors, such as ambient temperature, humidity, and solar radiation, vary in each region depending on its location above sea level. These factors directly affect the physiology of livestock, including the activity of a hormonal system. However, there is relatively little information in the literature on the hormonal profile of Swamp buffaloes, which are kept in different areas with different agroclimatic conditions. Therefore, the main aim of this study was to investigate the possible effects of different microclimatic conditions on the blood thyroxine, cortisol and testosterone hormone profiles of Swamp buffaloes. The results of this study are expected to serve as a valuable reference for the management of Swamp buffaloes in diverse regional conditions in Indonesia and worldwide.

MATERIALS AND METHODS

Location

The study was conducted in the West Sumatra province of Indonesia, and three locations (Lubuk Basung district, Akabiluru district, and Matur district) with different elevations were selected. The Lubuk Basung district is situated at a lowland location with an altitude of 20-35m above sea level (ASL), the Akabiluru district is in the moderate land area located 570-632m ASL, and the Matur district is a highland area with an altitude of 850-1190m ASL. Hormone analysis was carried out at the Integrated Research Laboratory, Faculty of Veterinary Medicine, Syiahkuala University, Banda Aceh, Indonesia.

Objects, Tools, and Materials

The study included 38 buffaloes, comprising 18 buffalo bulls aged 3-5 years (6 bulls per location) and 20 heifers aged 2-3 years (6 animals in the lowland, 7 each in the moderate land and the highland). Environmental parameters were measured using a dry-wet thermometer, and blood collection was performed using a clamp cage, coolbox, ice gel pack, venoject plain tube, syringe, microtube, micropipette, test tube rack, microtube rack, plastic tray, cotton, and tissue paper. The collected blood samples were stored in freezers until analysis. Hormone analysis was performed using ELISA equipment. The ingredients used for hormone analysis included blood serum, standard solution, quality control (QC) solution, conjugate enzyme, washing solution, substrate solution, and stop solution. ELISA kits for thyroxine, cortisol, and testosterone were purchased from DRG Instruments GmbH, Germany. Additional materials used included tissues, cotton, and 70% alcohol.

Sampling and Data Analysis

A 5mL blood sample was taken from the jugular vein of each animal, using a 10mL syringe at 6-8 AM local time. After collecting the blood, the syringe was placed horizontally on a tray to allow separation of serum. After 24 hours, the serum was transferred to microtubes using a micropipette and then stored at -20°C until used for hormone analysis. Hormone analysis was conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) kit method, following the guidelines provided with the respective kit.

The measured parameters included ambient temperature (Ta), Temperature Humidity Index (THI), and serum concentrations of thyroxine, cortisol, and testosterone. The THI was calculated using the formula: THI = 0.72 (DBT + WBT) + 40.6, where DBT represents Dry Bulb Temperature and WBT represents Wet Bulb Temperature (McDowell 1972). Data were analyzed using analysis of variance (ANOVA) with the SPSS software.

RESULTS AND DISCUSSION

Agroclimatic Conditions

As shown in Fig. 1, the highest mean ambient temperature was recorded in the lowland (29.94±2.77°C), while the lowest value was observed in the highland (25.87±4.15°C). Similarly, the highest THI was recorded in the lowland (82.15±3.45), while the lowest THI was observed in the highland (75.23±4.84). Both Ta and THI exhibited a gradual increase from the lowland to the moderate and the highland regions. The difference in ambient temperature among the three locations ranged from 1.84 to 4.07°C, while the difference in THI was between 3.16 and 6.92 points. Statistical analysis of the data revealed a significant difference ($P<0.05$) in ambient temperature between the lowland and highland, with the moderate region differing non-significantly from the other two regions. The mean THI value for the lowland was significantly higher than for the moderate and highland regions ($P<0.05$), but there was a non-significant difference in mean THI between the moderate land and the highland regions.

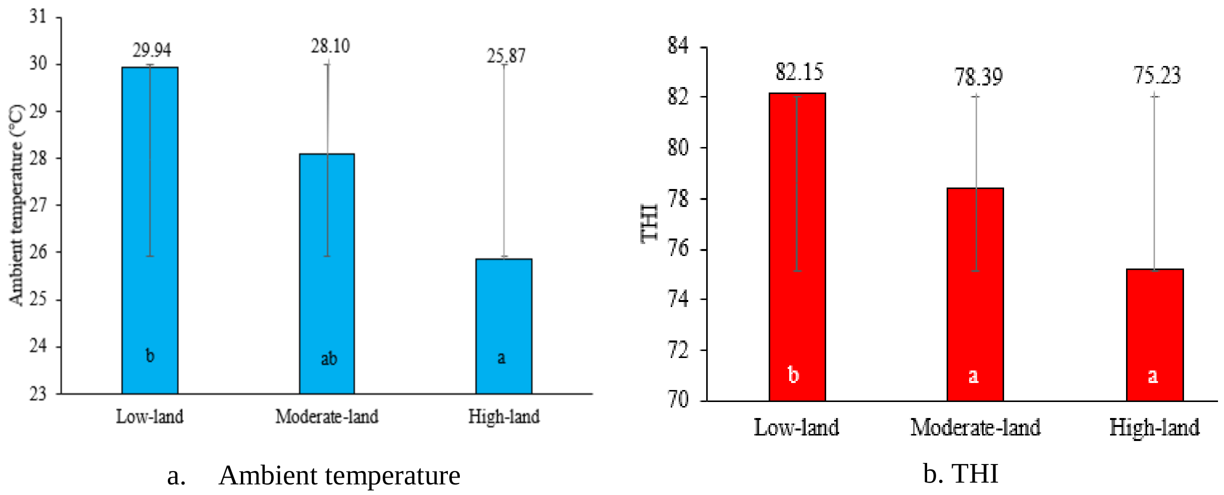

Fig. 1: Environmental temperature (a) and THI (b) in the lowland, moderate-land and highland areas.

Table 1: The average±SE concentration of the hormones thyroxine, cortisol, and testosterone of swamp buffalos in the lowland, moderate land, and highland

Hormones	Lowland	Moderate land	Highland	P Value*
Males				
Thyroxine (ng/mL)	63.02±5.34	64.18±5.57	80.90±13.94	0.334
Cortisol (ng/mL)	18.22±3.20	16.97±3.79	28.68±7.50	0.248
Testosterone (ng/mL)	1.71±0.29	2.18±0.38	2.29±0.31	0.439
Heifers				
Thyroxine (ng/mL)	64.98±6.76	71.50±8.24	99.89±26.54	0.337
Cortisol (ng/mL)	18.04±3.01	19.05±7.04	28.70±12.21	0.637

*P>0.05.

Buffaloes are affected by heat stress more quickly than cows. Animals of this species experience mild stress at THI 68-72, moderate stress at THI 73-76, and severe stress at THI ≥77 (Umar et al. 2021). Keeping in view this report, the results of this study showed that buffaloes in the lowland and moderate land experience severe heat stress, while those in the highland experience moderate heat stress. According to Reswati et al. (2022), the ambient temperature and THI attained their highest values at 3 PM in low, moderate, and highlands regions, while the lowest values were recorded at 6 AM, contributing to the fluctuating heat stress experienced by buffaloes.

Thyroxine

Table 1 shows data on serum thyroxine concentrations in buffalo bulls and heifers kept in the low, moderate, and highlands. The highest mean serum concentration of thyroxine hormone in buffalo males and heifers was found in the highland (80.90±13.94 and 99.89±26.54ng/mL, respectively), while the lowest concentrations were in animals kept in the lowland (63.02±5.34 and 64.98±6.76ng/mL, respectively). There was a gradual increase in mean serum thyroxine concentrations from the lowland to the moderate and highlands, but the difference was non-significant. This shows that ambient temperature and THI differences among low, moderate and highlands did not affect serum thyroxine concentrations in buffaloes.

These results are not supported by those of some previous studies (Wankar et al. 2014; Lakhani et al. 2018; Hilal and AL-Saeed 2020), where thyroxine concentrations were significantly lower ($P<0.05$) in buffaloes reared at high ambient temperatures than those kept under low temperatures. Opposite to these results, Mayahi et al. (2014) and Li et al. (2020), blood thyroxine concentrations in buffalo were higher in summer than those in winter. The non-significant differences in serum thyroxine concentrations among buffaloes kept at the three sites recorded in this study can be attributed to their ability to acclimatize to a changed environment. In this study, blood was taken from buffaloes that had been living in these sites for several years, enabling them to adapt to the prevailing environment.

The thyroid gland is highly sensitive to environmental changes (Sejian and Srivastava 2010). Thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4) play a crucial role in the metabolic *adaptation* and growth performance of animals (Sejian et al. 2018). The secretion of these hormones increases when cattle experience a lack of energy or are under cold stress, leading to increased heat production (Silva 2006). Heat stress leads to decreased production of Thyroid Releasing Hormone in the hypothalamus, resulting in reduced production of thyroid hormones (Habeeb et al. 2018).

Mean thyroxine hormone concentrations in buffaloes from the lowland, midland, and highland in both heifers and males were higher than those reported by Li et al. (2020) and Ingole et al. (2012). Differences in hormone concentrations are thought to be caused by variations in season and time of blood collection. Perumal et al. (2021) reported that the highest concentrations of thyroxine occurred at 08.00h in winter and spring, at 04.00h in summer, and at 24.00h in autumn. The age factor also contributes to such differences, where the highest thyroxine concentration is recorded in newborn buffalo calves and decreases with age (Ingole et al. 2012; Pandita et al. 2016). According to Habeeb et al. (2020), blood thyroxine concentration in Egyptian buffalo calves at birth was 120.2nmol/l and decreased to 95.9nmol/l at weaning. High blood thyroxine levels at birth are necessary to maintain a constant body temperature by increasing metabolism foradaptation to a cold environment. This hormone is shown to be negatively correlated with age, live weight and body weight gain of calves (Habeeb et al. 2020).

Cortisol

The concentrations of serum cortisol in buffalo males and heifers kept in lowland, moderate land, and highland are shown in Table 1. The highest cortisol concentrations were found in the serum of animals in the highland, both males (28.68±7.50ng/mL) and heifers (28.70±12.21 ng/mL), while the lowest value in the serum of male buffalos was in the moderate land (16.97±3.79ng/mL) and in the heifers it was in the lowland (18.04±3.01ng/mL). However, there were non-significant differences in serum cortisol concentrations among buffalo males and heifers kept at the three study sites.

Several researchers also reported non-significant differences in serum cortisol concentrations in buffaloes (Hafez et al. 2011; Das et al. 2014) and cattle (Romanello et al. 2018) raised under different environmental conditions. However, some other researchers (Habeeb et al. 2000; Wankar et al. 2014; Dayal et al. 2017; Shenhe et al. 2018; Hilal and AL-Saeed 2020; Li et al. 2020; Perumal et al. 2021) reported higher blood cortisol concentrations in summer than those in winter. The serum cortisol at each of the three study sites was higher than the average value of 14-16ng/mL reported earlier (Khan et al. 2003). Many factors can affect blood cortisol concentration, including the sampling time and age of the animal. According to Perumal et al. (2021), the highest cortisol secretion occurs in the morning when cattle wake up and reaches its lowest level at night when cattle are asleep. In the present study, buffalo blood at the three study locations was collected in the morning at 6-8 AM, when the animals woke up. Habeeb et al. (2020) reported that age also affects cortisol levels, the lowest levels were found at birth and the highest values were at weaning and after 24 months of age.

The non-significant differences in serum cortisol concentrations of buffaloes belonging to the three sites might be due to their ability to acclimatize while living in these areas. The buffaloes used in this study have lived in their respective area for several years. According to Niyas et al. (2015), plasma cortisol levels increase during acute heat stress and decrease during the chronic phase. The concentration of this hormone in the blood decreases during acclimation to reduce heat production (Stott and Robinson 1970). Alvarez and Johnson (1973) reported a 38% increase in glucocorticoids after 1 hour, 62% after 2 hours, and a peak of 120% at 4 hours after heat exposure, then decreased gradually to near normal after 48 hours and remained unchanged later. Provision of a body cooling system through puddles (Aggarwal and Singh 2010) and roof modifications (Khongdee et al. 2013) can help reduce cortisol levels in the blood. Similarly, vitamin C can also be used to effectively deal with heat stress (Ali et al. 2016).

Testosterone

The data presented in Table 1 also shows that the highest serum testosterone levels were observed in buffalo males raised at the highland (2.29±0.31ng/mL), while animals in the lowland exihibited the lowest levels (1.71±0.29ng/mL). However, there were non-significant differences in blood testosterone concentrations among the buffalo males from three locations.

These results indicate that differences in agroclimatic conditions among the three study sites had no effect on serum testosterone concentrations in buffalo bulls. The adaptability of animals to the prevailing climatic conditions seems to be the cause of these non-significant differences in the concentration of testosterone at the three study locations. This observation is supported by the report of Rhynes and Ewing (1973), who have stated that the endocrine function of the testes in bulls can adapt to a hot environment after a period of heat depression. This phenomenon was illustrated by 43% decrease in testosterone concentration from the control during the first two weeks after being subjected to heat stress, followed by an increase to control levels in the following weeks. The possibility of such adaptability may explain why serum testosterone concentrations did not differ among male buffaloes at the three study sites.

Serum testosterone concentration in the male buffaloes kept at three study sites was lower than that of Simeulue buffaloes (Qadarsina et al. 2019) but higher than that in the Gayo buffaloes (Ammar et al. 2021). According to Habeeb et al. (2020), blood testosterone concentrations were significantly positively correlated with an increase in age, live weight and body weight gain of buffalo calves. However, Osman et al. (2020) reported non-significant differences in blood testosterone levels among buffaloes of different age groups and in different seasons. Similarly, Mahmood et al. (2013) were unable to record any difference in serum testosterone concentration, reaction time and libido in Cholistani cattle of Pakistan kept under different environmental conditions in summer, autumn and winter. However, Hilal and AL-Saeed (2020) reported that serum testosterone concentrations in calves decreased in summer compared to winter and autumn seasons.

Conclusion

The results of this study indicated non-significant differences in the serum concentrations of thyroxine, cortisol and testosterone hormones among male buffaloes and heifers kept under different agroclimatic conditions, including lowland, moderate land and highland. This suggests that the agroclimatic conditions, including ambient temperature and the temperature-humidity index, had no effect on the activity of the endocrine system and metabolism of the Swamp buffaloes. However, this conclusion is based on the specific sample size and locations studied in this research. Further investigations with larger sample size and more diverse geographical regions may provide additional insights into the potential effects of agroclimatic conditions on the hormonal profile of animals, especially the Swamp buffaloes.

Authors' Contributions

Reswati Reswati conducted the experimental work, data collection and analysis, and drafted the manuscript. Bagus Priyo Purwanto provided guidance and oversight throughout the study, contributed to the study design and methodology, and reviewed and provided critical feedback on the manuscript. Rudy Priyanto also assisted in developing the research methodology, provided valuable insights and suggestions during data analysis, and reviewed the manuscript. Wasmen Manalu offered expertise in a specific area of the study, contributed to the interpretation of research findings, and reviewed and provided feedback on the manuscript. Raden Iis Arifiantini provided overall supervision and guidance throughout the research process, helped secure funding and resources for the research, and reviewed and approved the final version of the manuscript.

Ethical Approval

This research protocol was prepared keeping in view the welfare of animals. It was reviewed and approved by the Animal Ethics Committee, No. ACUC No: 152-2019 IPB of IPB University Bogor, Indonesia.

Competing Interest

All of the authors declare that they have no competing interests.

Acknowledgments

The authors would like to thank the Education Fund Management Institute (LPDP), Ministry of Finance of the Republic of Indonesia for their support in conducting this research. Thanks are also extended to Universitas Andalas for guiding the publication process of this article.

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