Research Article

# Physiological Response, Metabolic, Enzymatic, and Electrolytic Activities, and Milk Yield in Friesian and Friesian × Baladi Cows During Spring and Summer in Nile Delta of Egypt

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**Abstract** | This study aimed to assess the physiological responses, metabolic, enzymatic, and mineral activities, and milk yield of Friesian cows and their crossbreds with Baladi cows under various temperature-humidity indexes during spring and summer seasons in Nile Delta of Egypt. This study included a total of 10 cows (80-90 days in milk), 5 Friesian and 5 crossbred cows (Friesian x Baladi) aging 3.5-7 years and having 2-5 parities. Experimental cows were housed in semi-open sheds during spring and summer seasons. Rectal and skin temperatures, respiration (RR) and pulse (PR) rates, concentrations of cortisol and creatinine, and activity of AST and ALT in serum were significantly increased ($P<0.05$, 0.01 and 0.001), while TSH, T3, T4, total protein, glucose, cholesterol, Ca, P, Na, and K, ALP activity, and milk yield significantly decreased ($P<0.05$, 0.01 and 0.001) in summer than in spring in Friesian and crossbred cows. In summer season, Friesian cows showed significant increase ($P<0.05$, 0.01 and 0.001) in RR, PR, creatinine, K, ALT and AST, while T3, T4, glucose and milk yield showed significant reduction ($P<0.05$, 0.01 and 0.001) as compared to crossbred cows. In conclusion, Friesian×Baladi crossbred cows were more heat-tolerated than Friesian cows when they were housed loose in semi-open sheds during summer season in Nile Delta.

**Keywords** | Friesian; Crossbred, Season, Physiological measurements, Metabolism, Milk yield

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## INTRODUCTION

In Egypt, summer season normally extends from June to August (3 months) and with high ambient temperature (40°C), relative humidity (50-75%), and the solar radiation (4500 KJ/M$^2$) which induce greatest heat stress (Habeeb et al., 2020) because the temperature during summer is outside of the comfort zone of dairy cows (Hady et al., 2018). The production of farm animals is negatively affected by heat stress, leading to an effect on food supply chain of human and the economic efficiency of livestock (Bernabucci et al., 2010). Heat stress decreases milk and meat production with poor quality, reduces the reproductive efficiency, and vitality and health of animals (Sejian et al., 2010) leading to losses in dairy and beef industry (Nardone et al., 2010).

Lactating dairy cows respond to heat stress by exhibiting partially facilitate the thermal balance between heat gain and heat loss, and the most recognized impact of heat stress on dairy cow is the decrease in milk production (Tao et al., 2020). In Egypt, there are about 72247 cows from exotic breeds (mainly Friesian) and 95601 crossbred cows (mainly Baladi x Friesian crossbred) (EAS, 2020). Friesian cows were introduced to Egypt from about 70 years ago and they adapted to the local environmental conditions (Omran et al., 2011a). Under hot conditions, dairy cows can response to heat stress by behavioral, physiological, and cellular adaptations to reduce animal internal temperature (Dunshea et al., 2013). The heat tolerance in animals can be determine by the sweating rate (include morphology, density, and water transfer capacity of sweat glands), and the relative body surface area (Berman, 2005; Collier et al., 2008) metabolic heat production, and dissipation rates (Kadzere et al., 2002).

Animal adaptation to heat stress may be responsive to nutrition, management, and long-term targeted genomic selection which improve heat tolerance (Nguyen et al., 2016). The crossbreeding in dairy breeds may be a feasible avenue to achieve significant improvement more quickly in milk yield, fertility, and health characteristics than pure breeding (Dezetter et al., 2017). This process has been adopted for blending tropical cattle adaptability with the high milking potential of exotic breeds. In this respect, Egyptian environmental conditions can sustain only the composite genotypes of a moderate level of *Bos taurus* blood (Musa et al., 2008). Friesian crossbred noted to be suitable for their adaptability and the high milking capacity (Abdelatif and Alameen, 2012). Under heat stress, dry matter intake of cows was decreased and then milk yield was reduced in comparison with moderate-temperature conditions (Horowitz, 2002); cows endocrine and metabolic systems had important role in this case (Esposito et al., 2014), and breeds exhibit different reactions to unfavorable environmental thermal conditions (Pereira et al., 2008).

Therefore, the objective of this study was to determine the physiological responses, metabolic, enzymatic, and mineral activities, as well as milk yield of Friesian cows and their crossbreds with Baladi cows under various temperature-humidity indexes during spring and summer seasons in Nile Delta of Egypt.

## MATERIALS AND METHODS

The experimental work of the present study achieved the IACUC protocol Number (ARC/APRI/64/23) for the protection of animals used for scientific purposes and feed legislation at El-Gemmezah Animal Production Experimental Station (8.30 above sea level, 30.97 latitude-north and 30.97 longitude-east), Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

### Climatic condition

Throughout the experimental period, temperature humidity index (THI) was calculated for spring season (March, April, and May) and summer season (June, July, and August) of 2022 year. The THI was calculated according to Armstrong (1994) following this equation:

$$THI= (1.8\times AT+32) - (0.55-0.0055\times RH) \times (1.8\times AT-26).$$

Where, AT (ambient air temperature, °C) and RH (relative humidity %) were measured once weekly during the experimental period. Values of THI <72 indicated no heat stress, THI 72-79 mild stress; THI 80-90 high; THI 91-99 severe stress; THI > 99 death.

### Animals

Total of 10 multiparous lactating cows (2-5 parities; 3.5-7 years of age) at mid-lactation, 5 Friesian cows (84.8±13.02 day in milk, DIM) and 5 Friesian x Baladi cows (89.8±7.67 DIM) were used in this study. All cows were clinically healthy and physiologically sound. They were housed loose in semi-open sheds during the experimental periods. Cows in each group were fed on a ration of green berseem (*Trifolium alexandrinum*) in spring or corn silage in summer with concentrate feed mixture (CFM) and rice straw. The feed amounts were adjusted weekly according to milk yield, and live body weight. Feeds were offered to cows daily at 7 a.m. and 4 p.m., while clean drinking water was available all day times. By using milking machine, all cows were milked twice/day at 5 a.m. and 5 p.m. then daily milk yield was recorded during the experimental period.

### Physiological measurements

During the experimental period, rectal temperature (RT) was recorded using a clinical thermometer by inserting the thermometer in the rectum and the bulb should touch the rectum membrane mucous for 3 min. The respiration rate (RR) is visually determined by counting flank movements during 15 second-interval using a stopwatch without disturbing the animal and then multiplied by 4. The pulse rate (PR) was measured by palpating the femoral artery of the animal. Skin temperature (ST) was recorded using a non-contact infrared thermometer in the dorsal region. All physiological parameters were measured once weekly at 12 p.m

### Blood collection and the analytical methods

In 15 April of spring season and in 15 juley of summer season, blood samples were taken from all cows. Blood samples were collected from the jugular vein (Pugh, 2002) into clean test tubes, then samples were allowed to clot and serum was separated by the centrifugation of blood at 3000 rpm for 20 min then stored in clean dry Eppendorf tubes at -20°C till biochemical analysis of the concentrations of metabolites, minerals, and hormones as well as enzyme activities.

Concentration of thyroid stimulating hormone (TSH), thyroid hormones (T3 and T4) were assayed in blood serum of cows using ELISA kit (Immunospec corporation, USA, catalog No. PekinElmer-10304, PekinElmer-10301and PekinElmer-10302, respectively). Serum cortisol was determined by using ELISA kits (PekinElmer-10005;00000; DBC, Canada; catalog No. CAN-C-270 and SinoGene-Clon-SG-60105.

Using commercial kits, blood serum samples were analyzed for the concentrations of glucose (Tietz, 1986), total cholesterol (Anderson and Cockayne, 1993), total protein, and creatinine (Tietz, 1986). The activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT) in blood serum was assayed as the methods of Anderson and Cockayne (1993). Blood metabolic electrolytes including calcium (Ca), potassium (K), and sodium (Na) were determined by special kits using Tietz (1986) methods. As well, inorganic phosphorus (P) was determined according to Young et al. (1975) method.

### Statistical analysis

The experimental data, except for milk yield, were processed by T-test of SAS 9.0 package to compare each parameter between seasons (spring and summer) for each Friesian type, and to compare between Friesian types (Friesian and crossbred cows) within each season. One-way ANOVA was used to set the effect of month on average daily milk yield within each Friesian type or the effect of Friesian type within each month.

## RESULTS AND DISCUSSION

### THI during seasons

The mean values of ambient temperature (Ta), relative humidity (RH), and temperature-humidity index (THI) during different months of each season are shown in Table 1. The average monthly THI values was gradually increased from 56.18 in March (the minimum value) to 81.77 in August (the maximal value). The maximal THI value in spring was recorded at 31st March (69.07, 23.5°C Ta; 58.5% RH), and at 9th Aug in summer (85.32, 34.5°C Ta; 61% RH).

In dairy cows, the thermo-neutrality zone ranged between -5 and 24°C (Johnson, 1976). The THI is an excellent aid to dairy cattle farmers (Dimov et al., 2020) and can account for up to 78% of an animal's response to the climatic environment (Gaughan et al., 2002). The THI is classified into five categories based on air temperature and humidity. The ranges of THI represent the degree of heat stress in dairy cows: up to 71 as comfort; from 72 to 79 as mild; from 80 to 89 as moderate; more than 90 as severe and more than 100 as death (Armstrong, 1994). In the present study, average THI March was 56.2 (13.8°C Ta; 57.7% RH), which fits into the comfort range, while, it was moderate heat in August 81.8 (31.8°C Ta; 63.2% RH). In this respect, Vitali et al. (2009) considered that THI values of 77 being the upper minimum critical value and 87 is the upper maximum critical THI. In general, value of THI <77 was a critical value (Rosenberg et al., 1983; Hahn 1989; Silanikove 2000a) for ruminants.

### Physiological response

The physiological response in terms of rectal temperature (RT), skin temperature (ST), respiration rate (RR), and pulse rate (PR) of Friesian and crossbred cows in spring and summer are showed in Table 2. The obtained results revealed that RT and ST of Friesian and crossbred cows significantly increased in summer as compared to spring season, without significant differences between Friesian and crossbred cows. However, RR and PR of both Friesian and crossbred cows were significantly higher in summer than in spring, being significantly higher in Friesian than in crossbred cows only in summer.

The physiological response parameters are cardinal physiological measurements that help for maintaining heat balance and homeostasis in thermally stressful animals (Rashamol et al., 2018). The present results of RT values for Friesian and crossbred cows are in agreement with Yousef (1985), who indicated that under thermo-neutral conditions (16°C-25°C; air temperature), body temperature values ranged from 38.4 to 39.1°C. Generally, RT is considered a vital biomarker for quantifying heat stress conditions in livestock (Rashamol et al., 2018) and dairy cows are considered to have a normal body temperature only if can maintain a rectal temperature below 38.5°C (Igono et al., 1992). This was achieved for Friesian and crossbred cows in sparing, but the marked excess in RT and ST measured in summer was attributed to the exposing of the experimental cows to an environmental heat stress. A clear increase in RT, ST, RR, and PR was recorded under summer compared to spring conditions by 2.45, 5.48, 94.17 and 59.42% for Friesian cows, versus 2.25, 4.57, 133 and 57.78%, in crossbred cows, respectively. It is of interest to note that both Friesian and crossbred cows in thermally stressful conditions displayed heat stress symptoms by increasing RT more than 39°C and RR more than 60 beat/min (Kadokawa et al., 2012). These findings imply the inability of the animal to maintain the normal body temperature (Marai et al., 2007), whereas exposing dairy cows to thermal stress led to increase core temperature which activates heat loss mechanism through panting and sweating (El-Nouty, 1996).

Also, RR was affected significantly by heat stress in sum

**Table 1:** Changes in monthly means of ambient temperature (°C), relative humidity (%), and temperature-humidity index (THI) for spring and summer months

| **Month** | **Ambient temperature (°C)** | | | **Relative humidity (%)** | | | **THI** |
|---|---|---|---|---|---|---|---|
| | **Max.** | **Min.** | **Mean** | **Max.** | **Min.** | **Mean** | |
| March | 30 | 8 | 13.82 ±0.61 | 86 | 30 | 57.68 ±3.26 | 56.18 ±0.83 |
| April | 38 | 13 | 23.15 ±0.95 | 82 | 29 | 54.85 ±3.19 | 68.25 ±1.03 |
| May | 35 | 16 | 23.98 ±0.77 | 80 | 30 | 55.90 ±2.91 | 69.75 ±0.79 |
| June | 41 | 20 | 29.08 ±0.97 | 82 | 34 | 56.23 ±2.34 | 76.75 ±0.99 |
| July | 43 | 20 | 31.31 ±1.04 | 80 | 31 | 57.29 ±2.12 | 80.01 ±1.14 |
| August | 41 | 22 | 31.85 ±0.91 | 85 | 40 | 63.19 ±2.21 | 81.77 ±0.96 |

The values are the mean ± S.E.

**Table 2:** Physiological response of Friesian and crossbred cows in spring and summer.

| **Parameter** | **Season** | **Friesian type** | | ***P*-value** |
|---|---|---|---|---|
| | | **Pure** | **Crossbred** | |
| Rectal temperature (°C) | Spring | 38.32±0.10 | 38.20±0.10 | 0.4358 |
| | Summer | 39.26±0.15 | 39.06±0.17 | 0.4069 |
| | P-value | 0.0008*** | 0.0027** | - |
| Skin temperature (°C) | Spring | 36.14±0.29 | 35.86±0.17 | 0.4314 |
| | Summer | 38.12±0.31 | 37.50±0.23 | 0.1474 |
| | P-value | 0.0016** | 0.0005*** | - |
| Respiration rate (beat./min) | Spring | 41.20±4.63 | 30.80±3.94 | 0.1256 |
| | Summer | 80.00±1.84$^{a}$ | 71.80±2.44$^{b}$ | 0.0278 |
| | P-value | <.0001*** | <.0001*** | - |
| Pulse rate (pulse/min) | Spring | 55.20±3.38 | 50.20±3.23 | 0.3163 |
| | Summer | 88.00±2.19$^{a}$ | 77.20±2.73$^{b}$ | 0.0150 |
| | P-value | <.0001*** | 0.0002*** | - |

** and ***: Significant differences at P<0.01 and P<0.001, respectively.

**Table 3:** Hormonal profile in serum /plasma of Friesian and crossbred cows in spring and summer.

| **Hormone** | **Season** | **Friesian type** | | ***P*-value** |
|---|---|---|---|---|
| | | **Pure** | **Crossbred** | |
| TSH (μIU/ml) | Spring | 0.83±0.01 | 0.85±0.02 | 0.3577 |
| | Summer | 0.67±0.01 | 0.70±0.02 | 0.1814 |
| | *P*-value | 0.0001*** | 0.0003*** | - |
| $T_3$ (ng/ml) | Spring | 2.13±0.04 | 2.36±0.12 | 0.1106 |
| | Summer | 1.69±0.01 | 1.77±0.01 | 0.0017** |
| | *P*-value | 0.0001*** | 0.0013** | - |
| $T_4$ (μg/dl) | Spring | 7.10±0.13 | 6.90±0.13 | 0.3097 |
| | Summer | 5.60±0.04 | 5.77±0.03 | 0.0146* |
| | *P*-value | 0.0001*** | 0.0001*** | - |
| Cortisol (μg/dl) | Spring | 13.30±0.39 | 12.40±0.29 | 0.1005 |
| | Summer | 20.80±0.52 | 19.30±0.42 | 0.0547 |
| | *P*-value | 0.0001*** | 0.0001*** | - |

TSH: Thyroid stimulating hormone, $T_3$: Triiodothyronine, $T_4$: Thyroxine.
* Significant differences at P<0.05. ** Significant differences at P<0.01. *** Significant differences at P<0.001.

**Table 4:** Concentration of some metabolites in serum/plasma of Friesian and crossbred cows in spring and summer.

| Parameter | Season | Friesian type | | P-value |
|---|---|---|---|---|
| | | Pure | Crossbred | |
| Total protein (g/dl) | Spring | 7.70±0.07 | 8.04±0.24 | 0.2145 |
| | Summer | 6.10±0.11 | 6.50±0.23 | 0.1553 |
| | *P*-value | 0.0001*** | 0.0017** | - |
| Glucose (mg/dl) | Spring | 72.60±2.18 | 77.80±2.91 | 0.1902 |
| | Summer | 60.00±2.88 | 69.80±2.39 | 0.0309* |
| | *P*-value | 0.0082** | 0.0464* | - |
| Cholesterol (mg/dl) | Spring | 232.40±9.63 | 211.00±7.58 | 0.1188 |
| | Summer | 170.00±5.97 | 175.00±9.05 | 0.6570 |
| | *P*-value | 0.0006*** | 0.0158* | - |
| Creatinine (mg/dl) | Spring | 0.89±0.03 | 0.85±0.03 | 0.2943 |
| | Summer | 1.19±0.02 | 1.07±0.01 | 0.0010** |
| | *P*-value | 0.0001*** | 0.0001*** | - |

*,** and ***: Significant differences at P<0.05. P<0.01, and P<0.001, respectively.

**Table 5:** Enzymatic activity in serum/plasma of Friesian and crossbred cows in spring and summer.

| Parameter | Season | Friesian type | | P-value |
|---|---|---|---|---|
| | | Pure | Crossbred | |
| ALP U/l | Spring | 122.08±8.88 | 123.48±2.21 | 0.8822 |
| | Summer | 66.46±3.00 | 59.30±4.23 | 0.2048 |
| | *P*-value | 0.0003*** | 0.0001*** | - |
| ALT U/l | Spring | 19.90±1.03 | 19.38±0.73 | 0.6919 |
| | Summer | 30.28±2.38 | 21.40±0.86 | 0.0079** |
| | *P*-value | 0.0039** | 0.1122 | - |
| AST U/l | Spring | 61.68±0.35 | 55.98±0.94 | 0.0005*** |
| | Summer | 65.50±0.85 | 61.40±2.58 | 0.1693 |
| | *P*-value | 0.0032** | 0.0836 | - |

ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine transaminase.

** and ***: Significant differences at P<0.01 and P<0.001, respectively.

**Table 6:** Electrolytic concentrations in serum/plasma of Friesian and crossbred cows in spring and summer.

| Parameter | Season | Friesian type | | P-value |
|---|---|---|---|---|
| | | Pure | Crossbred | |
| Ca (mg/dl) | Spring | 8.10±0.27 | 7.90±0.23 | 0.5886 |
| | Summer | 5.70±0.07 | 5.54±0.25 | 0.5554 |
| | *P*-value | 0.0001*** | 0.0001*** | - |
| P (mg/dl) | Spring | 5.94±0.24 | 5.80±0.45 | 0.7912 |
| | Summer | 4.44±0.23 | 4.10±0.14 | 0.2455 |
| | *P*-value | 0.0020** | 0.0071** | - |
| Na (mEq/l) | Spring | 123.0±2.61 | 122.0±1.14 | 0.7344 |
| | Summer | 98.00±2.91 | 93.00±2.30 | 0.2152 |
| | *P*-value | 0.0002*** | 0.0001*** | - |
| K (mg/dl) | Spring | 4.70±0.29 | 4.50±0.07 | 0.5237 |
| | Summer | 4.10±0.14 | 3.40±0.16 | 0.0115* |
| | *P*-value | 0.1012 | 0.0002*** | - |

Ca: Calcium, P: Phosphorus, Na: sodium, K: Potassium.

*,** and ***: Significant differences at P<0.05. P<0.01, and P<0.001, respectively.

**Table 7:** Average daily milk yield (kg/d) of Friesian and crossbred cows in spring and summer.

| **Month** | **Friesian type** | | ***P*-value** |
|---|---|---|---|
| | **Pure (kg/d)** | **Crossbred (kg/d)** | |
| March | 20.26±0.34$^{a}$ | 20.80±0.34$^{a}$ | 0.2677 |
| April | 18.79±0.54$^{b}$ | 20.03±0.29$^{a}$ | 0.0469$^{*}$ |
| May | 15.89±0.46$^{c}$ | 18.04±0.30$^{b}$ | 0.0003$^{***}$ |
| June | 14.38±0.52$^{d}$ | 15.95±0.34$^{c}$ | 0.0077$^{**}$ |
| July | 13.82±0.35$^{de}$ | 14.20±0.21$^{d}$ | 0.3620 |
| August | 12.95±0.24$^{e}$ | 13.12 ±0.17$^{e}$ | 0.5628 |

a, b……e: Means with different superscripts within the same column are significantly different at P<0.05. $^{*}$, $^{**}$ and $^{***}$: Significant differences at P<0.05. P<0.01, and P<0.001, respectively.

mer and it is recognized universally as an early indicator for heat stress (Ji et al., 2020). It is of interest to observe that both Friesian and crossbred cows showed similar physiological response to heat stress in summer season in terms of increasing RT and ST. However, more response to heat stress in summer season was recorded in Friesian than crossbred cows in terms of significant increase in RR and PR during summer. Values of RR are more appropriate than RT, as a physiological an indicator of the physiological response (Brown-Brandl et al., 2005). In this respect, Indu and Pareek (2015) reported that increasing RR to be more than 80 beat/min in farm animals is an indicator of high heat stress quantum. Also, PR reflects primarily the homeostasis of circulation along with the general metabolic status (Sejian et al., 2010). The significant increase in PR in this study as affected by high-heat condition may be due to the adaptive mechanism of heat dissipation (Jones et al., 2013). Increasing PR under heat stress condition may be due to an increase in the muscular activity that controlling the RR, concurrent with elevated RR or by reducing resistance of peripheral vascular beds and arteriovenous anastomoses (Gupta et al., 2013). Furthermore, the increase in PR is followed by an increase in the flow of the blood from the core to the surface then increasing heat loss in terms of sensible (conduction, convection, and radiation) and insensible (through skin surface) methods (Marai et al., 2007). Cardiac output and cutaneous blood flow increase by heat stress by redistribution of the blood from core to more peripheral body surface (Silanikove, 2000b). The observed reduction in RR and PR of crossbred in comparison with Friesian cows in summer in our study may be due to variation in their skin structure that favors efficient evaporation and greater dissipation of heat (Fat-Halla, 1975).

## Hormonal profiles

Hormonal profiles of the thyroid activity and cortisol concentration in blood serum presented in Table 3 showed that, the exposure of either Friesian or crossbred cows to hot condition in summer significantly (P<0.001) reduced level of serum TSH, T3, and T4, and significantly (P<0.001) increased serum cortisol level. The reduction in TSH level and thyroid activity is in agreement with Omran et al. (2011a, b) in Egyptian buffaloes, Alameen and Abdelatif (2012) in crossbred dairy cows, and Abdel-Baki et al. (1995) in sheep. Reducing the level of TSH, T3, and T4 under heat stress condition can be explained by Khal et al. (2015), who reported that short-term heat stress imposed on growing steers by cycling environmental temperature between 32.2°C and 40°C resulted in pituitary–thyroid axis activity depression. During chronic stressful heat conditions, dairy cows seeking to acclimatization with this stress by decreasing fed intake that in association with reducing blood thyroid hormone levels in the circulation then rates of metabolic and thermo-genic were lowered and finally cow productivity were decreased (Zia-Ur-Rehman et al., 1982; Habeeb et al., 1992). It is of interest to note that metabolic hormones concentrations including T$_4$ correlated negatively to heat-stress condition (Bernabucci et al., 2010).

Regarding the differences between Friesian and crossbred cows, results in Table 3 showed significant decrease in serum T3 and T4 levels in Friesian than in crossbred cows although the level of TSH was not affected. These data indicated a mild thermal discomfort or proper genetic potential for crossbred cows, whereas, the metabolic heat production of Friesian cows reduced by a typical reaction emerging from the greater decrease in T$_3$ (Silanikove, 2000a). The reduction in thyroid function may be due to the impact of heat on the hypothalamo-pituitary-adrenal cortical axis which decreases thyrotrophic-releasing hormone that enable animal to reduce the basal metabolism (Johnson, 1987).

As presented in Table 3, a higher blood cortisol concentration was recorded in summer than in to spring season. A reliable physiological index for indicating the response of animals to stress is level of blood cortisol (Abilay et al., 1975). Under high-heat environmental conditions, cortisol level may reflect the metabolic rate, heat production, consequently tissue damage (Lu et al., 2021). It participates in various body functions including immune response and metabolism of fat, carbohydrate, and protein (He et al., 2019; Podder et al., 2022). Increased cortisol level during the thermal stressful condition in the present study was in accordance with Alameen and Abdelatif (2012), who found higher levels of plasma cortisol in dairy cows during summer season than the remained seasons. Also, Titto et al. (2013) reported a significant increase in serum cortisol levels during summer in Holstein dairy cows.

Serum cortisol level did not differ significantly between Friesian and crossbred cows in spring or summer. These results are in agreement with Ali and El-Tarabany (2019), who reported no significant differences in the serum cortisol levels between native and crossbred calves (Baladi-Brown Swiss) under hot summer climate.

## BLOOD BIOCHEMICAL METABOLITES

Serum metabolites assessed in Friesian and crossbred cows in spring and summer are presented in Table 4. Results revealed that level of total protein, glucose, and cholesterol significantly decreased in summer as compared to spring in both Friesian and crossbred cows. On the other hand, the crossbred cows had significantly higher serum glucose levels and lower creatinine level than to Friesian cows during summer season.

The observed reduction in serum total protein concentration in both cow types in summer was indicated in hyperthermic conditions in cattle (Dar et al., 2019; Gaafar et al., 2021; Joo et al., 2021). Under heat stress condition, changes in ruminal protein metabolism availability were reported by Bernabucci and Calamari (1998). They found that synthesis of the microbial protein in the rumen was decreased in cows under heat stress condition. Also, flow of the splanchnic blood may affect the absorption of amino acids in the animal body (Scharf et al., 2010). Furthermore, Ronchi et al. (1999) hypothesized an increasing in glucose pool with the contribution of amino acids in the circulation under heat stress condition as a result of reducing gluconeogenic amino acids. In ruminants, protein catabolism was increase by cortisol to supply regular energy for different functions (Sejian and Srivastava, 2010). In cattle and buffalo kept under heat stress conditions, an increase more than 200-fold was recorded in the level of heat shock protein 70 (HSP-70) in blood lymphocytes, which is a part of the response to thermal stress to prevent other proteins from heat-induced denaturalization (Collier et al., 2008), compared to controls (Mishra et al., 2011). Thus, the synthesis of HSP-70 may decrease the available total protein levels in the circulation (Gao et al., 2017).

The decrease in blood serum glucose as affected by hot summer condition (Koubkovà et al., 2002; Chaudhary et al., 2015) may be related to the decrease in nutrients availability as a consequence reduction in dry matter intake and ruminal contents of volatile fatty acids (Schneider et al., 1988) specifically the molar ratio of propionate: acetate (Lin et al., 2020). Also, increasing serum glucose utilization can provide fuel for muscular expenditure required for high muscular activity that associated with increasing the painting rate (Sejian et al., 2012) and the negative effect of the heat on gluconeogenesis as an endocrine adaptation to hot conditions (Abeni et al., 2007). Heat-stressed dairy cows showed significant decrease in blood glucose as affected by greater blood insulin activity (Wheelock et al., 2010; Baumgard and Rhoads, 2013). This diminishes were achieved despite the increase in the absorptive capacity of intestinal glucose (Garriga et al., 2006), poor reabsorption ability of the renal glucose (Ikari et al., 2005) and output of hepatic glucose (Febbraio, 2001). The differences in glucose level between both cow types under hot condition was reported by Ali and El-Tarabany (2019), who found a significant rise in blood serum glucose in native calve than in crossbred one. However, Khan et al. (2018) indicated that Holstein Friesian dairy cows had significant increase in glucose levels during high ambient temperatures as compared with local breeds.

Low ambient temperature led to increase in blood cholesterol level by stimulating the activity of thyroidal hormones to elevate basal metabolic rate for maintaining body temperature (Rasooli et al., 2004). The significant decrease in blood serum cholesterol during hot environmental conditions is in consistent with previous studies (Alberghina et al., 2013; Das et al., 2016; Joo et al., 2021) that may be due to the thyroid gland which exhibited more activity in spring than in summer as presented in Table 3. Also, low cholesterol level during summer could be explained by a reduction the liver activity (Ronchi et al., 1999).

Serum creatinine concentration of Friesian dairy cows had significantly greater ($P<0.05$) values than crossbred cows during summer season (Table 4). The effect of thermal stress could be indicated by a decrease in energy intake companies with hyperthermia is compensated by muscle proteolysis contributing to the creatinine increase for energy supply (Lamp et al., 2015), but not depend on the diet (Asai et al., 2005). Additionally, heat stress enhances the peripheral vasodilation to improve heat loss and blood flow to the internal organs (Srikandakumar et al., 2003).

The obtained results of creatinine are nearly similar to those of Gaafar et al. (2021) and Scharf et al. (2010). They found that creatinine concentration significantly lowered in winter as compared to summer season. The rate of excretion of creatinine is influenced by renal perfusion and glomerular filtration rate. A reduction in renal blood flow during heat stress might raise plasma creatinine concentration. In this context, Ronchi et al. (1999) recorded that heat stress caused a decrease in level of glucose and increase in the level of creatinine in the blood due to energy supply by muscular catabolism. Also, Abeni et al. (2007) observed that plasma creatinine significantly correlated with RT. Elevating the creatinine level in Friesian than in crossbred cows in thermally stressful conditions may suggest that crossbred cows were more heat-tolerated than Friesian cows. Generally, results of blood metabolites indicated that dairy cows experienced to summer condition did not show the same metabolic processes under low nutritional conditions, which has a direct impact on nutrient reduction in heat-stressed animals (Wheelock et al., 2010), to cope with the thermal stress.

### Liver function biomarkers

The activity of alkaline phosphatase (ALP) in the present study showed significant decreased ($P<0.01$) as affected by heat stress condition in both Friesian and crossbred cows compared with spring condition (Table 5). However, serum ALP activity did not differ significantly between Friesian and crossbred cows. The results of ALP are in agreement with Li et al. (2021), who found a reduction in activity of ALP in heat-stressed dairy cows. This finding may be associated with energy metabolism and endocrine acclimation responses under the hot environment, which resulting from reduced gut and liver activity (Abeni et al., 2007). In contrast to our results, Bhan et al. (2012) reported that the activity of ALP in growing and adult Sahiwal cattle was increased in summer as compared to spring values.

On the other hand, results in Table 5 reveled significant increase in AST and ALT activities in summer than in spring only in Friesian cows. Also, AST and ALT activities were significantly higher in Friesian than in crossbred cows. The elevation in ALT activity in summer (Bhan et al., 2012) may be due to an increase in gluconeogenesis (Cincovic et al., 2011) or may be related to thermal strain adverse effects on the thyroid gland, liver, and kidney functions (Marai et al., 1997a and b). In this respect, the higher AST activity in hyperthermia (Alameen and Abdelatif, 2012) may associate with negative energy status (Cebra et al., 1997). Generally, ALT activity may rise in normal conditions as affected by high metabolism concomitant with high milk production (Abo El-Nor et al., 2007; Sobiech et al., 2008).

### Blood electrolyte contents

Results in Table 6 showed that blood electrolyte contents of Ca, P, and Na were affected significantly ($P<0.001$) by season, being lower in summer than in spring, but exhibited insignificant differences between Friesian and crossbred cows. However, K content significantly ($P<0.001$) decreased in summer than in spring only for crossbred cows, and in crossbred than in Friesian only in summer. These results are similar to Joo et al. (2021), who observed a clear reduction in serum Ca, K, and Na concentrations in Holstein and Jersey cows under heat stress conditions. Alameen and Abdelatif (2012) found significant reduction in serum P level of crossbred cows in winter season, while serum Ca level was not affected by season. Scharf et al. (2010) reported that serum P and Na levels did not show any changes between Angus and Romosinuano steers under heat stress.

There was an increase in mineral loss and body fluid excretion in heat-stressed dairy cows. Extreme environmental stress resulted in a marked increase in the rate of loss of ionic substances such as Na, K, and Ca (Collier et al., 1982). A lower serum Na level is likely during thermal stressful conditions (El-Nouty et al., 1980). Such a decline perhaps resulted from increasing observed in the excretion of urinary Na due to increasing total urinary output or increasing blood volume by increasing water consumption, while the increase in Na concentration may be due to dehydration (Chaudhary et al., 2015). The reduction in serum K concentrations in crossbred cows experiencing heat strain over the long term may be attributed to the increasing the loss of K through sweating (El-Nouty et al., 1980; Garcia et al., 2015). In cattle, sweat of the apocrine glands contains 4-5 folds of the K level compared with Na (Johnson, 1970). The genetic basis of heat tolerance is due the sensible heat transfer ability and, mostly, to the higher sweating rate of crossbred than Friesian cows (Finch 1985; Burns et al., 1988). It could be an interpretation of the capacity of crossbred cows to maintain a normal body temperature without exerting much more thermoregulation mechanisms than Friesian cows under climatic condition in Egypt. Increasing levels of P in both Friesian and crossbred cows in spring than in summer may be attributed to the variation in the availability of green fodder (Berseem) during spring season. In this respect, Yokus and Cakir (2006) found differences in blood P level as affected only by seasonal variation in dairy cows thrived under subtropical conditions.

### Milk yield

Average daily milk yield (ADMY) during different months in spring (March, April, and May) and summer (June, Juley, and August) seasons showed significantly ($P<0.05$) a gradual reduction by advancing month of each season in Friesian and crossbred cows. Significant increase in milk yield was observed in crossbred than between Friesian cows in April, May, and June (Table 7).

In accordance with our results, Ali et al. (2023) reported a reduction in milk production by 36 and 36.9% for Friesian and crossbred cows under heat stress condition. When the environmental temperature exceeds the zone of thermo-neutrality, the milk production declines significantly (Zheng et al., 2009). Dairy cattle have a sensitivity to heat stress (Berman, 2005) and high-producing cows are more sensitive to heat stress than low-producing cows (Silanikove, 2000a) due to the zone of thermal neutrality shifts to lower temperatures as milk production, feed intake, and metabolic heat production increases. According to Bouraoui et al. (2002), THI value over 69 led to decrease daily milk yield by 0.41 kg/cow. It is of interest to note that the highest ADMY in Friesian and crossbred cows was recorded with the lowest THI value (56.18) in March, while the lowest one was recorded with the highest THI (81.77) in August (Table 1). Milk synthesis and secretion are complicated processes that are governed by several hormones and are sensitive to both physiological and environmental cues (Rhoads et al., 2009). The observed decrease in ADMY in both Friesian and crossbred cows during summer than in winter was attributed to increasing RT values. In this respect, McDowell et al. (1976) suggested that the rise in RT by 1°C or less is enough to reduce animal performance by reduce milk production by about 10-20%. Also, the reduction of 15% in milk yield occurred when animals were transferred from location with 18°C air temperature to another location with 30°C. Moreover, the increase in body temperature by 1°C increases basal metabolic rate by 20-30% (Brody, 1945). Also, the significant increase in cortisol concentration with heat stress is in association with a decrease in milk yield due to the deviation of availability of energy for coping mechanisms to challenges heat stress (Silanikove, 2000a).

Increasing ADMY significantly in crossbred than in Friesian cows during April, May, and June could be explained by the faster response of Friesian cows to the worsening of thermal conditions than crossbred cows in terms of increasing RR and PR significantly in Friesian than in crossbred cows. This early negative slope of means DMY values for FR cows could be attributed to the abruptly imposed cows to high ambient temperatures (38, 35, and 41° C) in April, May, and June, respectively. The upper critical temperature for Holsteins was from 25 to 26 (Berman et al., 1985). Also, Bouraoui et al. (2002) claimed that the decrease in milk yield started at a THI value of 69 in mediterranean climate. The increase in ADMY of crossbred as compared to Friesian cows was associated with increasing blood glucose level because milk lactose is synthesized from blood glucose, thus blood concentration of glucose is a limiting factor in lactose synthesis and consequently milk production (Herbut et al., 2019).

Friesian cows in the current study become adapted to Egyptian environmental conditions by the impact of heat stress on genome improvement plasticity and DNA modification which may lead to better postnatal protection against the negative effects of high ambient temperature (Baumgard et al., 2011). Despite these well-adapted Friesian cows to the Egyptian climate, they exert greater efforts to reach homeorhetic adaptation compared with their crosses with native cows.

## CONCLUSION

Despite Friesian cows being well adapted to the Egyptian climate, they exerted more effort to reach homeorhetic adaptation compared to their crossbreds with native cows because crossbred cows displayed a milder response to heat stress than Friesian cows. Cross-breeding dairy cows showed a light depression in hormones responsible for metabolism indicating mild thermal discomfort reflecting appropriate genetic potential. This may be beneficial to the body by reducing its metabolic rate and heat production, thus reducing or preventing tissue damage, which resulted in a less precipitous decrease in milk production than in pure Friesian cows. It has been concluded that Friesian × Baladi crossbred dairy cows are more heat-tolerated than Friesian dairy cows when they were kept loose in semi-open sheds during summer season in Nile Delta of Egypt.

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## CONFLICT OF INTEREST

No conflicts of interest.

## NOVELTY STATEMENT

This study conducted to evaluate the differences between raising Friesian or Friesian×Baladi crossbred dairy cows in semi-open sheds under Egyptian Nile Delta conditions during summer season to identify the perfect breed for small breeders.

## AUTHOR'S CONTRIBUTION

All authors were contributed to suggest, design, and achieving the experimental work. Shaarawy, A.B.M. and Wafa, W.M. conducted the experimental procedures and collected data. Rezk, R.A.A. performed the sample preparations and chemical analysis. Genena, S. K. and El-Sawy, M.H. conducted the statistical analyses. Wafa, W.M. critically revised the manuscript.

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