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Biochemical and physiological responses of Nili-Ravi Buffalo (*Bubalus bubalis*) to heat stress

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Abstract: The present study was designed with an objective to evaluate the impact of various strategies to alleviate heat stress on certain physiological attributes viz. respiratory rate (RT), body surface temperature (BST), pulse rate (PR), and respiratory rate (RR); biochemical attributes viz. glucose, total protein (TP), and cholesterol; and endocrinological parameters (tri-iodothyronine and tetra-iodothyronine) of the Nili-Ravi buffaloes during early summer season. Lactating Nili-Ravi buffaloes (n = 20) with close production and parity stage were divided into four treatment groups as group A (control group under roof shades only), B (experimental group under roof shades with antistress supplementation), C (experimental group under roof shades with ceiling fans), and D (experimental group under showers and ceiling fans) at the Buffalo Research Institute, Punjab, Pakistan. All the physiological study parameters, that is, RT, BST, PR, and RR, were significantly ($P \leq 0.05$) higher for Group A and B as compared to Group C and D, respectively. The results revealed that all the biochemical study attributes were significantly ($P \leq 0.05$) higher in Group C and D as compared to Group A and B. Regarding endocrinological parameters (T3 and T4), both these hormones were significantly ($P \leq 0.05$) different between the four study groups, being higher for Group D as compared to the other groups. Nili-Ravi buffaloes showed a resistant behavior in terms of RR, PR, and serum biochemical components, which implies that the fan strategy could be an alternative to showering.

Key words: Buffalo, heat stress, rectal temperature, thyroid

1. Introduction

The geographical location of Pakistan is in the subtropics as it is situated 23.6° above the line of the equator. Therefore, summer season prevails for a longer duration with high ambient temperatures and relative humidity. Environmental temperatures may rise up to 45 °C in hot dry conditions [1]. The climatic conditions of Pakistan during March and April have been found to be hot for lactating animals and these months have been termed as spring season [2]. However, the THI level was found to be 77 and 85 with a maximum temperature (29.3 °C and 34.6 °C) and humidity (51.3% and 54.6%) March and April, respectively, in 2012 as seen in Figures 1 and 2.

Lactating dairy cows begin to suffer from mild heat stress at a THI of 72. They become moderately stressed at a THI > 80 and severely heat-stressed at a THI > 90. A significant ($P < 0.001$) increase in the RT and RR was found in young and adult Murrah buffaloes after an exposure of animals to 40 °C, 42 °C, and 45 °C for 4 h against thermo-neutral temperature [3]. By using various cooling methods like fans, sprinklers, and foggers, a decline can be achieved in PR, RR and RT [4,5]. Aggarwal and Singh [6] reported

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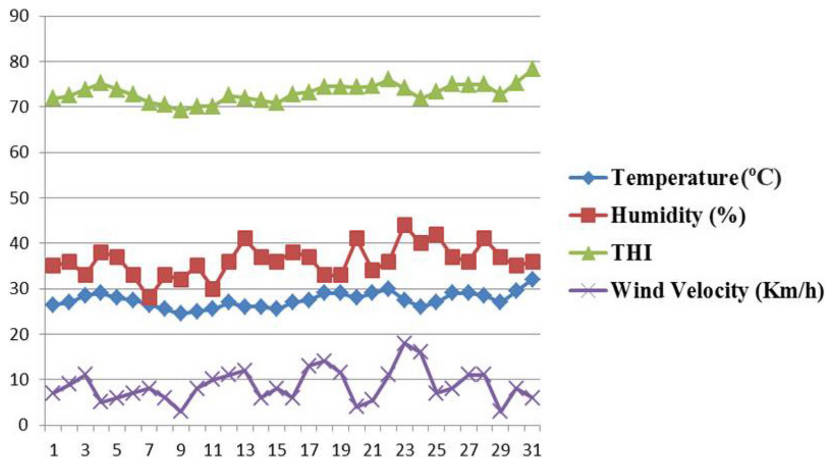

Figure 1. Meteorological indices for March during peak hours (noon, 2 pm).

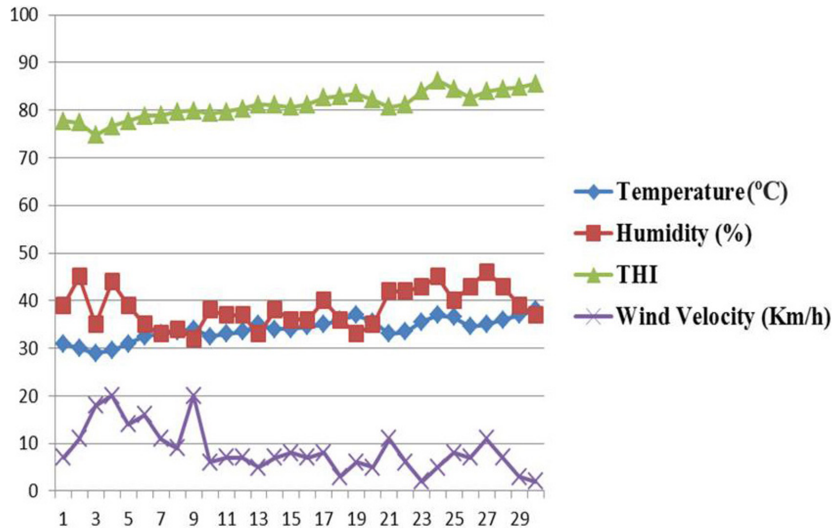

Figure 2. Meteorological indices for April during peak hours (noon, 2 pm).

changes in the circulating T_3 hormone when heat-stressed cattle are given provision of fans and cooling measures. Similarly, Aggarwal and Upadhyay [7] reported that the thyroid level was low in heat-stressed animals compared to the animals provided with various cooling measures. This study was designed to evaluate the effect of hot dry conditions in early summer on the biological performance in terms of physiochemical and endocrinological attributes (T3 and T4) of the Nili-Ravi buffaloes under various housing strategies.

2. Materials and methods

2.1. Animals and treatment

The research was conducted at Buffalo Research Institute (BRI), Livestock Experiment Station (LES), Pattoki, Punjab, Pakistan. The experimental station is located in the central irrigated area of Punjab (31 °N, 73 °E), Pakistan. The approval for this project was granted by the Committee on Use of Animals in Research and Experimentation of University of Veterinary and Animal Sciences. Lactating multiparous (3rd, 4th, 5th, and 6th parity) Nili-Ravi buffaloes (n = 20) were incorporated in the study. The stage of lactation and the level of milk production were kept the same for all the animals. The animals were selected from the herds maintained at BRI, LES, Pattoki, Punjab, Pakistan and were raised during March and April (early summer). The buffaloes were randomly assigned to 4 different groups (n = 5/group) according to various heat stress alleviating strategies as Group A (control group under roof shades only), Group B (experimental group under roof shades with antistress supplementation), Group C (experimental group under roof shades with ceiling fans), and Group D (experimental group under showers and ceiling fans) (Table 1). Silage was

Table 1. Treatments and experimental layout.

Sr. No.	Group	No. of animals	Description
1	A	5	Buffaloes under roof shades only (control)
2	B	5	Roof shades with antistress supplement
3	C	5	Roof shades with ceiling fans
4	D	5	Roof shades with showers and ceiling fans

offered ad libitum to meet the maintenance requirements at 11:00 am daily and was accessible till milking in the next morning, whereas concentrate was offered along maize silage (as such basis) as 1 kg for every 2 L of milk [8] produced (Table 2).

2.2. Meteorological data

Ambient environmental temperature (°C) and relative humidity (%) was recorded during the peak hours of the day (12:00 pm to 2:00 pm) using a hygrometer (Thermo-Hygro; TH-208 B). Wind velocity (km/h) was determined during the peaks hours of the day (12:00 pm to 2:00 pm) with the help of a digital anemometer (Intel Smart Sensor; AR-816). The THI was calculated by collecting data on air temperature and relative humidity, which was then calculated using the following formula as described in a previous study by Mader et al. [9].

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb - 14.4)] + 46.4$$

where THI: Temperature humidity index, RH: Relative humidity, T_{db}: Dry bulb temperature.

2.3. Physiological attributes

All the buffaloes were observed for their PR in the peak hours of the day (01:00 pm to 02:00 pm). While pulse was taken from the coccygeal artery on the sides of the ventral part of the tail [10], the researcher's hand grasped the tail from the dorsal aspect and the fingers readily perceived the pulsations. The RR was observed (01:00 pm to 02:00 pm) in terms of counting the flank movement, one inward and one outward movement taken as one breath [11]. Respiration count was taken for 1 min The RT of the Nili-Ravi buffaloes was taken (01:00 pm to 02:00 pm) by inserting a digital thermometer approximately 2–2.5 inches deep per rectum [10] and the RT value was recorded once it became stable on digital display. The BST of the buffaloes was taken (01:00 pm to 02:00 pm) by using a noncontact infrared temperature measuring instrument and pointing the laser towards the targeted skin part with approximately 6 inches. Middle neck, middle back, and rump site were selected for the determination of BST on a weekly basis for a period of 2 months.

2.4. Blood collection and biochemical and endocrinological analysis

Blood samples were taken from individual buffaloes for the assessment of thyroid functions by determining the thyroid hormones (tri-iodothyronine (T_3) and thyroxine (T_4)) as well as the serum biochemical profile including glucose (mg/dL), total proteins (g/dL), and cholesterol (mg/dL). Blood analysis was conducted in Quality Operation Laboratory (QOL), UVAS, Lahore.

The blood samples were collected from the jugular vein in a sterile container (20 mL) without anticoagulant. The blood was initially allowed to rest for 2 h at room temperature and then centrifuged at 750 *g* for 15 min. The supernatant was collected and stored in a freezer at −20 °C until use for serological test [12]. Serum biochemical analyses were conducted for glucose, total protein, and cholesterol by using a serum chemistry analyzer. The concentration of T_3 and T_4 was assessed using Accubind ELISA kits (Monobind Inc.).

Table 2. Ingredients and chemical composition of concentrate ration fed to lactating Nili-Ravi buffaloes.

Ingredients	Inclusion level (%)
Cotton seed cake	22
Maize	8
Wheat bran	32
Rape seed cake	3
Maize gluten	20
Molasses	14
Mineral mixture[a]	1
Crude Protein (CP) %	18.0
TDN	76.0
ME (Mcal/kg)	2.6

[a]Mineral mixture included DCP 70.81 %, NaCl 18.91 %, $MgSO_4$ 8.64 %, $FeSO_4$ 0.89 %, $ZnSO_4$ 0.22 %, $CuSO_4$ 0.03 %, KI 0.00877 %, $CoCl_2$ 0.0089 %, and $NaSiO_3$ 0.00150 %.

2.5, Statistical analysis

The statistical analysis of the recorded data was performed using a statistical software (SPSS). The impact of using various strategies to alleviate heat stress in lactating buffaloes was analyzed through the analysis of variance technique (ANOVA) under completely randomized design

(CRD). The differences of the means among the treatment groups were determined by with Duncan's multiple range test [13].

3. Results

The RT (mean ± SE) in lactating Nili-Ravi buffaloes in the treatment groups A, B, C, and D was found as 101.12 ± 0.06 °F , 100.92 ± 0.09 °F, 100.37 ± 0.05 °F, and 100.08 ± 0.07 °F, respectively (Table 3). The RT was higher ($P < 0.05$) in Group A buffaloes kept under roof shades as compared to the control group. The lowest RT ($P < 0.01$) was noticed among the buffaloes given a treatment of showers and fans (Group D) and then fans alone (Group C; Table 3). Nonsignificant results ($P > 0.05$) were found between Group A and Group B.

The mean PR (pulses per minute) of the Nili-Ravi buffaloes was found as 44.8 ± 0.51, 45.3 ± 0.41, 51.9 ± 0.90, and 50.6 ± 0.90 in the treatment groups A, B, C, and D, respectively. The PR was higher ($P < 0.05$) in Group A and B with buffaloes kept under roof shade without fans and/ or showers. A lower PR ($P < 0.05$) was noticed in Group C and D (Table 3). The findings suggested a substantial ($P < 0.01$) decrease in PR when animals were cooled with fans with or without showers.

The mean RR (breaths per minute) of animals raised under different housing strategies was observed and found as 19.0 ± 0.31, 19.9 ± 0.32, 16.8 ± 0.30, and 16.6 ± 0.21, for Group A, B, C and D, respectively. The highest RR was observed in buffaloes of Group B supplemented with an antistress product and a lower RR was observed in animals of Group C and D treated with fans with or without showers. There was a significant difference in terms of RR between the antistress group (B) and Group C and D, which were given housing treatment of fans and showers.

The mean skin temperature (middle neck) of the buffaloes was observed and found as 27.3 ± 0.56 °C, 27.4 ± 0.53 °C, 25.3 ± 0.57 °C, and 24.9 ± 0.57 °C for Group A, B, C, and D, respectively (Table 3). The highest ($P < 0.05$) skin temperature in the middle neck region was observed in Group B, followed by that in the control group, whereas the lowest middle neck temperature was noted in Group D animals (Table 3) treated with fans and showers. The control group buffaloes and the buffaloes treated with an antistress product displayed a significant difference ($P < 0.05$) in terms of skin temperatures compared with the skin temperatures of the buffaloes treated with fans and showers (Group C and D).

The highest mean skin temperature measured in the middle back of lactating Nili-Ravi buffaloes was observed in Group B (29.9 ± 0.65 °C) followed by that in the control group (A) (29.8 ± 0.69 °C), Group C (26.5 ± 0.57 °C) ,and Group D (26.3 ± 0.55 °C; Table 3). Nonsignificant ($P > 0.05$) variations were noticed among the treatment groups A and B, and similarly between Group C and D for middle back temperature. The buffaloes raised under fans with or without showers showed lower temperatures and the buffaloes kept under shade (A and B) showed higher skin temperatures.

The rump temperature of buffaloes under various housing schemes was observed and found to be 28.8 ± 0.66 °C, 29.1 ± 0.63 °C, 26.3 ± 0.59 °C, and 26.2 ± 0.59 °C for Group A, B, C, and D, respectively. Nonsignificant ($P > 0.05$) differences were observed between Group A and B, and between Group C and D for rump temperature (Table 3). The buffaloes raised under fans with or without showers showed lower temperatures ($P < 0.05$) and the buffaloes kept under shade (Group A and B) showed higher skin temperatures ($P < 0.05$).

The buffalo blood samples were examined to evaluate the T_3 and T_4 levels as a measure to observe the activity of thyroid gland. The results revealed a significant variation ($P \leq 0.05$) among different treatment groups. The mean T_3 level of the Nili-Ravi buffaloes was found as 182.9 ± 0.81 ng/dL, 184.9 ± 0.77 ng/dL, 189.2 ± 0.79 ng/dL, and 197.0 ± 0.71 ng/dL for the treatment groups A, B, C and D, respectively (Table 4). The T3 level was higher (P <

Table 3. Physiological performance of Nili-Ravi buffaloes during early summer.

Groups	RT (°F)	BST (°C)			RR	PR
		Mid. Neck	Mid. Back	Rump	(per min.)	(per min.)
A (Control)	101.12±0.06^a	27.3±0.56^a	29.8±0.69^a	28.8±0.66^a	19.0±0.31^a	50.6±0.90^a
B	100.92±0.09^a	27.4±0.53^a	29.9±0.65^a	29.1±0.63^a	19.9±0.32^b	51.9±0.90^a
C	100.37±0.05^b	25.3±0.57^b	26.5±0.57^b	26.3±0.59^b	16.8±0.30^c	45.3±0.41^b
D	100.08±0.07^c	24.9±0.57^b	26.3±0.55^b	26.2±0.59^b	16.6±0.21^c	44.8±0.51^b

A: roof shade; B: antistress product; C: fans; D: shower and fans; RT: rectal temperature; BST: body surface temperature, RR: respiration rate, PR: pulse rate
Means having different superscript in column are significantly different ($P < 0.05$).

Table 4. Biochemical constituents and thyroid hormones in Nili-Ravi buffaloes during early summer.

Groups	Serum biochemical components			Thyroid hormones	
	Glucose (mg/dL)	Total protein (TP; g/dL)	Cholesterol (mg/dL)	T_3 (ng/dL)	T_4 (μg/dL)
A (Control)	62.2±0.76^{c}	6.59±0.07^{c}	101.6±1.32^{c}	182.9±0.81^{c}	5.72±0.04^{c}
B	63.5±0.52^{b}	6.54±0.09^{b}	109.0±1.26^{b}	184.9±0.77^{c}	5.79±0.06^{c}
C	66.7±0.84^{a}	6.96±0.12^{a}	113.9±1.51^{a}	189.2±0.79^{b}	6.01±0.05^{b}
D	70.9±0.89^{a}	7.36±0.08^{a}	116.1±0.99^{a}	197.0±0.71^{a}	6.03±0.04^{a}

A: roof shade; B: antistress product; C: fans; D: shower and fans; T3: tri-iodothyronine, T4: tetra-iodothyronine; Means having different superscript in column are significantly different (P < 0.05).

0.05) in Group D buffaloes treated with fans and showers, whereas it was low (P < 0.05) in the control group A. The results indicated significant differences (P < 0.05) for the control group with animals treated with fans with or without showers. A nonsignificant variation (P > 0.05) was observed between Group A and Group B.

The mean T_4 level of buffaloes assigned to various treatment groups was 5.72 ± 0.04 μg/dL, 5.79 ± 0.06 μg/dL, 6.01 ± 0.05 μg/dL, and 6.03 ± 0.04 μg/dL for the treatment groups A, B, C and D, respectively (Table 4). The buffaloes raised under fans with or without showers displayed higher T_4 levels (P < 0.05), whereas the buffaloes kept under shade (Group A and B) showed lower values of the T_4 level in blood. Nonsignificant variations (P > 0.05) were noticed between Group A and B, whereas Group D had the highest value (P < 0.05) followed by that in Group C for the T_4 levels.

The blood chemistry of the Nili-Ravi buffaloes was examined to evaluate the effect of heat stress on the levels of glucose, total proteins, and cholesterol. The blood glucose level was examined for various treatment groups and found as 62.2 ± 0.76 mg/dL, 63.5 ± 0.52 mg/dL, 66.7 ± 0.84 mg/dL, and 70.9 ± 0.89 mg/dL for the treatment groups A, B, C and D, respectively. Nonsignificant (P > 0.05) differences were observed between Group A and B, whereas a significant variation was observed between Group C and D. The buffaloes raised under showers showed higher blood glucose levels (P < 0.05), the buffaloes raised under fans showed moderate levels, and the buffaloes kept under shade (Group A and B) displayed lower values of glucose in blood.

The values of total protein in blood were analyzed and the values were found to be 6.59 ± 0.09 g/dL, 6.54 ± 0.07 g/dL, 6.96 ± 0.12 g/dL, and 7.36 ± 0.08 g/dL for Group A, B, C and D, respectively. A high value of total protein (P > 0.05) was observed in Group D buffaloes given the treatment of showers with fans, while the control group A and B showed a lower value (P > 0.05). The values of cholesterol (mg/dL) in blood were analyzed and detected as 101.6 ± 1.32 mg/dL, 109.0 ± 1.26 mg/dL, 113.9 ± 1.51 mg/dL, and 116.1 ± 0.99 mg/dL for Group A, B, C and D, respectively. A higher value of cholesterol (P > 0.05) was observed in Group D, a moderate value in Group C, and a lower value in control groups A and B (P > 0.05).

4. Discussion

The RT (mean ± SE) in lactating Nili-Ravi buffaloes in the treatment groups was noticed to be higher for Group A, followed by that in Group B, C, and D as 101.12 ± 0.06 °F, 100.92 ± 0.09 °F, 100.37 ± 0.05 °F, and 100.08 ± 0.07 °F, respectively. An earlier study by Singh et al. [14] investigated the effect of the environment on the physiology of Murrah buffaloes and it was stated that RT has a positive correlation with environmental temperature. The lowest RT was found in buffaloes kept under fans and showers. The results are similar to the earlier findings of Jadoa [15], who reported that RT increases with increasing ambient temperatures. Similar results were presented by Tao et al. [16] and Monteiro et al. [17], who stated that animals suffering from heat stress adapt themselves through various physiological mechanisms.

The buffaloes kept under roof shades showed a high PR (pulses/minute) compared to the lower PR in the group treated with fans and showers with fans. Our findings are in line with those of the the earlier study by Aggarwal and Upadhyay [7], who stated that buffaloes showed decreased PR and RR when provided with a cool environment as compared to cattle. The RR was noticed to be high for the treatment group B, but a lower RR was detected in Group D animals treated with fans and showers. The results are in accordance with those of West [18], who reported that cows that received cooling showed a sharp decline in RR, *i.e.* 57 vs. 95 breaths/min; which might be a result of decreased expenditure of energy for body cooling. A significant increase in RT and RR was recorded in the Egyptian buffaloes from the spring season to summer and similarly from summer to the winter season, indicating that the animals were heat-stressed [19]. Similarly, Farooq et al. [20] and Gaughan [21] reported high RRs in heat-stressed animals.

The highest ($P < 0.05$) skin temperature (°C) in the middle neck region was observed in Group B, followed by the control group, whereas the lowest middle neck temperature was noted in the animals of Group D, which were treated with fans and showers. Similarly, the middle back and rump temperatures were the highest for those buffaloes kept under shade but lower in the groups treated with fans and shower. This might be a result of a black pigment in the thickened skin of buffaloes that assists in the absorption of more heat, leading to disproportionate radioactive and convective heat loss from the extremities when exposed to solar radiation. A similar finding has been reported by Chaudhari and Singh [22], who reported high skin temperatures in the control group Murrah buffaloes as compared to the buffaloes that had provision. Due to high wind velocity for buffaloes under fans, the convection process helped to cool off buffaloes as wind helps in evaporative heat loss and is suggested as optimum for buffaloes [23]. Similarly, Gudev et al. [24] and Aggarwal and Singh [4] stated that the availability of mist and fans as measures of cooling significantly decreased the udder skin and skin temperature.

The blood glucose level (mg/dL) was examined for various treatment groups and significant differences were found among the treatment groups A, B, C, and D in the present study. The results are in line with the study by Das et al. [25], who reported a decrease in the glucose level in heat-stressed animals and attributed this decrease in blood glucose mainly to RR, which causes increased utilization of respiratory muscles [19]. A decrease in feed intake also contributes to a decrease in glucose.

The values of total protein in blood were analyzed and significant differences were found in the treatment groups A, B, C, and D. The results are in line with the findings of El-Masry and Marai [26], who reported serum total proteins as 44 g/L in summer and 51 g/L in winter. Similarly, Das et al. [25] reported a variation in total proteins levels in heat-stressed cows.

The cholesterol in serum was analyzed and the values were found as 101.6 ± 1.32 mg/dL, 109.0 ± 1.26 mg/dL, 113.9 ± 1.51 mg/dL, and 116.1 ± 0.99 mg/dL for Group A, B, C, and D, respectively. A high value of cholesterol ($P > 0.01$) was observed in Group D, while the control group A and Group B showed a lower value ($P > 0.05$). The results of our study are in accordance with those of Garcia et al. [27], who reported high cholesterol levels in heat-stressed cows [27]. However, the results of our study are contradictory to those of Verma et al. [28], who reported a decreased level of cholesterol in lactating Murrah buffaloes in response to heat stress.

The mean ± SE value for T_3 in the Nili-Ravi buffaloes was found as 182.9 ± 0.81 ng/dL, 184.9 ± 0.77 ng/dL, 189.2 ± 0.79 ng/dL, and 197.0 ± 0.71 ng/dL for the treatment groups A, B, C, and D, respectively. The results of our study are similar to the findings of Aggarwal [29] as they reported that an increasing environmental temperature brings out a reduction in the level of thyroid hormone as compared to thermo-neutral zone. Aggarwal and Upadhyay [7] reported that plasma hormones are suitable indicators regarding the physiological status of cows and buffaloes. A reduction in the level of thyroid hormone as well as a decreased concentration of plasma GH (growth hormone) exerts a synergistic effect to lower heat production. Similarly, Das et al. [25] reported hypofunctioning of the thyroid gland in heat-stressed animals, which leads to lower circulating levels of T_3 and T_4 in blood.

In conclusion, Nili-Ravi buffaloes showed a resistant behavior in terms of RR, PR, and serum biochemical components, which implies that fan strategy could be an alternative to showering. However, the control group did not show a promising biological response by any means; thus, strategic measures are necessary for buffaloes to cope with heat stress during early summer.

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