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RESEARCH ARTICLE

Modified Technique for Estimating Total Body Water in Live Animals Using Antipyrine Substance for Measuring Thermal Tolerances

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ABSTRACT

Estimating the total body water (TBW) in the live animals using the antipyrine substance as a modified technique was the first objective of this research. TBW was estimated *in vivo* in 10 native bovine calves using the conventional method (extrapolation technique) and also by the suggested modified method (equilibration technique). The averages of TBW in native bovine calves were 136.5 ± 16 and 133.1 ± 16 L by convention and modified technique, respectively, without significant differences between the two techniques. The accuracy of the modified technique was 97.5% as compared with the convention method, and at the same time, the new method is an easy, simple, accurate, and quick technique and more reliable. Estimation of heat adaptability of animals to heat stress conditions was the second objective of this research. Animals when exposed to high ambient temperature the TBW increases and consequently TBS (live body weight-TBW) decreases with different percentages according to the animal response to stressful conditions. TBW or TBS values were estimated before and after heat stress exposure and the percentage change in TBW or TBS in the animal due to heat stress may be used for evaluating the animal's adaptability to heat stress. The percentage increase in TBW or the percentage decrease in TBS due to heat stress conditions may be used as an index for heat tolerance coefficient (HTC). The most heat tolerance animals are those with the highest HTC values.

Key words: Body water, Calves, Body solids, Heat tolerance, Antipyrine

INTRODUCTION

The total body water (TBW) pool is all the water in the animal including the alimentary tract, which has a large volume, particularly in ruminants. Body water is the water content of an animal body that is contained in the tissues, the blood, the bones, and elsewhere (Hansard, 1964). Estimation of TBW in live animals is important for research whether the research involves nutrition, physiology, genetic, disease, and meat production (Alexander and Gerken, 2010). However, estimation of TBW in animals using slaughter and chemical analysis of the whole body's organs is tedious processing, time consuming, and high expensive operation (Kamal and Habeeb, 1984). Besides that, the high cost of animal analysis has created an interest in indirect methods of estimating TBW. This indirect method or *in vivo* also can provide repeated estimates of TBW for the same animal whereas slaughter and chemical analysis obviously can only be done once (Habeeb *et al*., 2001). Moreover, the live bodyweight of the animal alone provides a poor index of the metabolically active tissue due to that bodyweight is including body solids and body water, consequently using live body weight (LBW) for estimating body weight gain of animals is a misleading index of growth performance, since it may be due to the increase in water retention and not to the increase in body protein and fat. In other words,

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a unit of body weight gain in one animal may be due to the increase in body water at the expense of body tissue loss, while in the other animal, maybe due to the increase in body solids (Habeeb *et al*., 2020).

Most methods for measuring the TBW *in vivo* have been based on the degree of dilution of a foreign substance after its intravenous injection. This substance should possess rapid distribution throughout body water; non-toxicity in required doses; slow transformation in, and excretion from the body; accurate and convenient estimation of slow its concentration in the plasma. Antipyrine (ANP) may be used in the estimation of TBW in live animals. Measuring the TBW of the animal *in vivo* by ANP has been developed by Brodie *et al*. (1949). This conventional method of Brodie involves the use of ANP (l-phenyl-2, 3-dimethylpyrazolone-5-one) for estimating TBW by injection 1 g/100 kg body weight of ANP in distilled water intravenously from a calibrated syringe and five blood samples are withdrawn at 1, 2, 3, 4, and 5 h subsequently and protein precipitation for plasma. ANP is measured in the filtrate from the ultraviolet absorption of 4-nitroso-antipyrine by the addition of sodium nitrite and sulfuric acid to the plasma filtrate. The plasma concentration at zero time i.e., at the time of ANP injection was carried out by plotting the plasma levels on semi-logarithmic paper and extrapolating the straight portion of the time-concentration curve back to the time of injection by the method of least squares (extrapolation technique).

Estimating TBW content in a live animal using ANP by single blood sample at ½ h after ANP injection as a modified technique and comparison between the two methods for estimating body water in 10 calves was the objective of this research. Besides, using TBW or total body solid (TBS) (bodyweight-body water) (TBS) in live animals for evaluation of the animal's adaptability to heat stress was the second objective of this study.

MATERIALS AND METHODS

Location

The experimental work was carried out in the Bovine Farm of Biological Application Department, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, at Inshas, Egypt (latitude 31° 12' N to 22° 2' N, longitude 25° 53' E to 35° 53' E).

Ethics

Experimental animals were cared for using husbandry guidelines derived from the Egyptian Atomic Energy Authority standard operating procedures. This work was reviewed and approved by the Animal Care and Welfare Committee of the Egyptian Atomic Energy Authority. These ethics contain relevant information on the endeavor to reduce animal suffering and adherence to best practices in veterinary care according to the International Council for Laboratory Animal Science guidelines.

Animals and feeding

The present study was conducted in the bovine farm project, Experimental Farms Project, Biological Application Department. Ten bovine calves after weaning at 8 months of age were used in this research. Animals were fed the ration consisted of concentrate feed mixture (CFM), clover hay (CH), and rice straw according to their requirements (NRC, 1981). Ingredients of the CFM are 35.0, 30.0, 30.0, and 5.0% for undecorticated cottonseed meal, yellow maize, wheat bran, and soybean meal, respectively. The chemical composition of CFM (on a dry matter basis %) is 17.7, 14.5, 2.9, 47.2, and 6.0 for crude protein, crude fiber, ether extract, nitrogen-free extract, and ash, respectively. The corresponding values for CH are 14.2, 25.1, 2.6, 34.6, and 12.5. Calculated nutritive values of the CFM are 4.0 for net energy (MJ/kg DM), 60.8 for total digestible nutrients (%), and 115.0 for digestible crude protein (g/kg DM). The respective values for CH are 2.6, 48.0, and 80.0. Each 100 kg concentrates was supplemented with 100 g minerals mixture (each kg contains 40g Mn, 3 g Cu, 0.3g I, 0.1g Si, and 30g Fe from Pfizer-Co., Egypt), 100 g vitamins mixture (AD3 E), 2 kg dicalcium phosphate, and 1 kg coarse refined iodized kitchen salt (El-Nasr Saline's Co., Egypt).

Experimental procedure

Ten healthy native bovine calves were used in the experiment. The experiment was carried out under comfortable conditions during the winter season since the average ambient temperature (AT) and

relative humidity (RH%) in the farm were 20 ± 2°C and 65 ± 2.5 RH %, respectively. The same calves were entered in a separate room (20 × 50 m) for 1 week. The room was provided with electrical heaters and the calves were exposed to thermal stress conditions using electrical heaters for 7 h daily from 9.0 am to 4.0 pm, since the average AT and RH% were 35.0 ± 2°C and 60 ± 3%, respectively. At 4.0 pm, the electrical heaters were set off and the calves returned to comfortable conditions from 4.0 pm to 9.0 am. The room was provided individually with troughs and a source of fresh drinking water to be available automatically to each calf at any time. Each calf was weighted during comfortable conditions and after the thermal stress period and during weighting the experimental animals; each calf was injected in the left jugular vein with ANP at the rate of 1 g per 100 kg LBW in both the end of comfortable and thermal stress periods to determining TBW. One blood samples were withdrawn from the write jugular vein of each calf after ½ h for estimating TBW using modified technique and four blood samples were withdrawn from the write jugular vein of each calf after 1, 2, 3, and 4 h from the injection of ANP to be distributed in the animal body for estimating TBW using convention technique. Consequently, TBSs were estimated by subtracting TBW from LBW.

Chemical reagents required for ANP estimation are zinc reagent solution (10%), sodium hydroxide (0.75N), sodium nitrite (0.2%), and H_2SO_4 acid with different normality (6 N, 4 N, and 0.07 N). Precipitation of plasma proteins in plasma samples was carried out using zinc sulfate and centrifuged at the rate of 2000 rpm for 20 min. ANP concentration in the supernatant was estimated by a computerized spectrophotometer at 350 UV. TBW, ml in animals, was determined by dividing the concentration of ANP injected (µ)/concentration of ANP in the plasma sample (µ/ml). TBS values were estimated by subtracting TBW from LBW (Habeeb, 2019).

Estimation of TBW *in vivo* in animals

Injection dose of ANP

The standard dose is 1 g ANP each 100 kg LBW. Each animal injects with 5 ml (contains 1 g ANP) in the left jugular vein and blood samples were withdrawn in tubes containing anticoagulant from the right jugular vein after ½ and 1, 2, 3, and 4 h from the injection. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored at –20°C until the estimation of ANP.

Preparation of standard ANP

One milliliter from the injection dose was put and completes the solution with H_2SO_4 (0.07N) to reach 50 ml. Two milliliters from this standard were put in the tube and 0.1 ml sodium nitrite was added and then mixed by the vortex and incubates the tube at 22°C for 20 min and then read this solution using a spectrophotometer to obtain the optical density (O.D.) of the standard.

Precipitation of plasma proteins in plasma samples

One milliliter from each plasma sample was put in one tube and 1 ml distilled water plus 1 ml zinc reagent plus 1 ml NaOH was added. The containing tubes were mixed using vortex for ½ min and centrifuged the sample tubes at 3500 rpm for 15 min to obtain the supernatant.

Estimation of ANP in the supernatant of samples

Two milliliters from supernatant solution (contains ½ ml plasma) were put in one tube and 0.1 ml sodium nitrite and one drop (50 µ l) H_2SO_4 (4N) was added and incubated the tubes at 22°C for 20 min. Optical densities of all tubes were reading using the spectrophotometer. The concentration of ANP (µg/ml) in each sample was determined as follows:

ANP concentration= (Optical density of sample/ Optical density of std.) × concentration of standard (8 µg/ml.) = µg. In a recent spectrophotometer, the standard tube put in the spectrophotometer and O.D. of the standard was fixed and the concentration of ANP in each sample was determined directly without the equation.

Estimation of body water

Estimation of body water (ml) in the animal was carried out by dividing the concentration of ANP Injected (µg) by concentration of ANP in the plasma sample (µg).

Body water = ANP injected (µg)/ANP in plasma sample (µg/ml).

Estimation was carried out in ½ ml plasma (2 ml from supernatant/4 ml during precipitation of plasma proteins). Therefore, multiplied concentration in

dilution factor (2) and also multiplied in 100/93 (percentage of water content in plasma) as following: Body water = [ANP injected (μg)/ANP in plasma sample (μg/ml)] × 2 × 100/93= liter.

Statistical analysis

Data of TBW in the 10 calves by two methods were analyzed statistically using a t-paired test according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Estimation of body water using antipyrine substance

Estimation of body water in native calves using extrapolation technique

In this conventional method, five samples must withdraw in each calf after 1, 2, 3, 4, and 5 h from ANP injection. Make ANP standard curve and extrapolated back to the time of injection by the method of least squares (extrapolation technique). O.D. is plotted against the corresponding concentrations of ANP (μg/ml) on a semi-logarithmic paper. Clear supernatant of plasma samples after protein precipitation by centrifugation was added one drop (0.05 ml) of 4 N H_2SO_4 followed by two drops (0.1 ml) of 0.2% sodium solution and then read optical density of samples at different times after dosing. From the standard curve, the concentrations of ANP in plasma samples (μg/ml) at different hours after dosing were known.

Plasma levels of ANP at various intervals after intravenous injection were plotted on semi-logarithmic paper against time in hours. To correct for the metabolism of the ANP during the time required for uniform distribution, the curve for the plasma level is extrapolated of the logarithm of the plasma concentrations to 0 time. The plasma ANP concentration (μg/ml) at 0 time is calculated by plotting the plasma levels of ANP. The straight portion of the time concentration curve was extrapolated back to the time of injection (ANP μg/ml at 0 time) by the method of least squares. The plasma water level of ANP is calculated by dividing the plasma level ANP by the water content of the plasma. The calculation for body water is made as follows: TBW, ml = amount of ANP injected (μg)/amount of ANP in plasma (μ/ml). Body water was estimated as shown in Table 1. Table 1 shows ANP concentrations at 0 time and estimation of body water in the 10 calves using extrapolation technique.

Estimation of body water using the modified technique

Estimating of body water content in a live animal using ANP was carried out by a single blood sample at ½ h after ANP injection as a modified technique in the same calves. The O.D. of one sample (½ h after injection) and also ANP concentration in one sample at ½ h after the injection of 2 g ANP in each calf were estimated. Standard tube put in the spectrophotometer and O.D. of the standard was fixed and concentration of ANP in each sample determined directly according to this equation: TBW = {(2×1000×1000)/ANP at 0 time or ½ h after dosing}×2×(100/93) = liter as presented in Table 2. Comparable between convention and modified methods in estimation TBW in 10 calves is shown in Table 3.

Data show that averages of TBW in 10 calves were 136.5 and 133.1 L in the extrapolated method and modified method, respectively. In the present study, the average TBW in 10 calves determined by the modified method was 3.4 L (2.5%) less than that obtained from the extrapolation method. This means that the modified method measures about 97.5 of the TBW in calves. However, the values for TBW obtained by the two methods did not differ significantly. The lower TBW values by the modified method than that obtained by the convention method may be due to the fact that ANP takes at least 4–5 h to equilibrate within rumen water (Kamal and Habeeb, 1984). Although the modified method underestimates body water only 2.5% in calves, it has more advantages than the conventional method. Because in estimation TBW by the modified method, the animals not depriving from feed and drinking water for at least 5 hours after injection ANP. Besides, animals do not lose water by vaporization during such a time and their physiological systems are not disturbed by convention method measurement. Besides, the modified method is 5 times faster than the conventional method. Kamal and Habeeb (1984) studied the comparison between methods of estimating TBW using ANP and desiccation in Friesian cattle and found that estimating body water using ANP was an accurate technique with relation to the desiccation method.

Table 1: ANP concentrations at 0 time and estimation of body water in the 10 calves using extrapolation technique

Calf No.	Bodyweight of calves, kg	ANP (µg/ml) at 0 time	Total body water, liter
1	210	33.0	TBW = {(2×1000×1000)/33}×2×(100/93)=130.3
2	235	26.5	TBW = {(2×1000×1000)/26.5}×2×100/93)=162.3
3	235	27.0	TBW={(2×1000×1000)/27}×2×(100/93)=159.3
4	185	34.0	TBW={(2×1000×1000)/34}×2×(100/93)=126.5
5	168	37.0	TBW={(2×1000×1000)/37}×2×(100/93)=116.2
6	210	30.0	TBW={(2×1000×1000)/30}×2×(100/93)=143.4
7	200	35.0	TBW={(2×1000×1000)/35}×2×(100/93)=122.9
8	189	33.0	TBW={(2×1000×1000)/33}×2×(100/93)=130.3
9	220	28.0	TBW={(2×1000×1000)/28}×2×(100/93)=153.6
10	172	36.0	TBW={(2×1000×1000)/36}×2×(100/93)=119.5

Table 2: Estimate the total body water in the 10 calves using the modified technique (½ h after injection)

Calf No.	O.D.	ANP concentration	TBW, liter (using modified technique)
1	0.095	33.5	TBW={(2×1000×1000)/33.5}×2×(100/93)=128.4
2	0.075	27.5	TBW={(2×1000×1000)/27.5}×2×(100/93)=156.4
3	0.075	27.5	TBW={(2×1000×1000)/27.5}×2×(100/93)=156.40
4	0.099	34.5	TBW={(2×1000×1000)/34.5}×2×(100/93)=124.67
5	0.131	38.0	TBW={(2×1000×1000)/38}×2×(100/93)=113.2
6	0.093	30.5	TBW={(2×1000×1000)/30.5}×2×(100/93)=141.0
7	0.099	35.5	TBW={(2×1000×1000)/35.5}×2×(100/93)=121.2
8	0.095	33.5	TBW={(2×1000×1000)/33.5}×2×(100/93)=128.4
9	0.078	29.0	TBW={(2×1000×1000)/29}×2×(100/93)=148.3
10	0.12	37.0	TBW={(2×1000×1000)/38}×2×(100/93)=113.2

Table 3: Estimate the body water in the 10 calves using convention and modified techniques

Calf No.	Bodyweight of calves, kg	Convention method		Modified method		Differences
		µg/ml ANP at 0 time	Total body water, l	µg/ml ANP at 0 time	Total body water, l	
1	210	33.0	130.3	33.5	128.4	–1.90
2	235	26.5	162.3	27.5	156.4	–5.90
3	235	27.0	159.3	27.5	156.4	–2.90
4	185	34.0	126.5	34.5	124.7	–1.80
5	168	37.0	116.5	38.0	113.2	–3.30
6	210	30.0	143.4	30.5	141.0	–2.40
7	200	35.0	122.9	35.5	121.2	–1.70
8	189	33.0	130.3	33.5	128.4	–1.90
9	220	28.0	153.6	29.0	148.3	–5.30
10	176	36.0	119.5	38.0	113.2	–6.30
X±SE	202.8±7.4		136.5±16.0		133.1 ±5.2	–3.4 L ^{NS}

Accuracy % 97.5%

NS: Not significant

Estimation of heat adaptability (heat tolerance coefficient [HTC]) in animals

When the animals are exposed to high environmental temperature, most of the physiological and biochemical parameters are disturbed. The heat-induced changes may be used for evaluating the animal's adaptability to heat stress or may be used as an index for HTC as suggested by Habeeb *et al*. (2001). Estimating the TBW using modified techniques in the 10 calves after exposure to heat stress conditions for 1 week is found in Table 4. Data in Table 4 showed that TBS in 10 calves during 1 week under heat stress conditions loosed about 15 kg including a decrease of about

Table 4: Total body water using ANP with modified technique under normal (comfortable) and heat stress conditions

Calf no.	Under comfortable conditions (20±2°C, 65±2.5 RH %)			Under heat stress conditions (35±2°C, 60±3.0 RH %)		
	Bodyweight of calves, kg	Total body water, L	Total body solids, kg	Bodyweight of calves, kg	Total body water, L	Total body solids, kg
1	210	128.4	81.6	206	135	71
2	235	156.4	78.6	230	168	62
3	235	156.4	78.6	231	163	68
4	185	124.7	60.3	182	134	48
5	168	113.2	54.8	165	119	46
6	210	141.0	69.0	207	149	58
7	200	121.2	78.8	195	132	63
8	189	128.4	60.6	186	141	45
9	220	148.3	71.7	216	166	50
10	176	113.2	62.8	174	136	38
X±SE	202.8±7.4	133.1 ±5.2	69.7±3.0	199.2±7.2	144.3±5.2	54.9±3.5

4.0 kg in LBW and an increase of about 11 liters in TBW compared with comfortable conditions.

When exposed the animals to high AT, water intake increases and consequently TBW content increases with different percentages according to animal response to stressful conditions (Habeeb *et al*., 2001). The percentage change (heat-induced changes) in TBW or TBS contents in each animal may be used for evaluating the animal's adaptability to heat stress. The heat-induced changes may be used as the index for HTC. The heat-induced changes in each of TBW and TBS in live animals by ANP dilution technique were used previously as HTC for detection of heat adaptability in farm animals (Kamal and Habeeb, 1999; Habeeb and Gad, 2018).[1-10] Estimation of the HTC or heat adaptability (heat stress index) based on TBW and TBS or TBW/100 kg TBS ratios are presented in the following:

Estimation of the HTC using TBW

The TBW is determined using ANP dilution technique under comfortable conditions (TBW_1) and heat stress exposure (TBW_2). The percentage increase in TBW due to heat stress conditions may be used as the index for HTC as following: $HTC=100–[TBW_2–TBW_1/TBW_1×100]$ where TBW_1 and TBW_2 are TBW values under comfortable and hot conditions, respectively. The most heat tolerance animals are those with the highest values as presented in Table 5.

In Table 5, data showed that calves no. 1, 3, 5, and 6 are the best calves in heat tolerance while calves no. 9 and 10 are the worst calves in heat tolerance. Consequently, calves no. 2, 4, 7, and 8 are moderate in heat tolerance. The most heat-tolerant animals are those with the highest values of HTC and the less heat-tolerant animals are those with the lower values of HTC.

Habeeb (2010) estimated this coefficient (HTC) in sheep and goats and concluded that the most heat-tolerant animals are those with the highest values. Results are similar to that obtained perversely by Kamal, 1982; Kamal and Habeeb, 1999; and Habeeb *et al*., 2001.

Estimation of the HTC using TBS

It is well known that bodyweight including TBS and TBW. TBS = LBW - TBW. Estimation of the TBW using ANP by modified methods under each of comfortable (TBW_1) and heat stress (TBW_2) and each value was subtracted from the corresponding LBW ($weight_1$ and$weight_2$) to obtain body solids under comfortable (TBS_1) and under heat stress (TBS_2).

TBS loss due to heat stress may be used as HTC as following: HTC = 100 der ea_2 BS l_1/TBS_1 x 100] where TBS_1 and TBS_2 are the TBS during comfortable and heat stress, respectively, as presented in Table 6. The most heat-tolerant animals are those with the highest values of HTC and the less heat-tolerant animals are those with the lower values of HTC.

Data in Table 6 showed that calves no. 1, 3, 5, and 6 are the best calves in heat tolerance while calves no. 9 and 10 are the worst calves in heat tolerance.

Table 5: Estimation of the HTC using TBW

Calf no.	TBW_1, L under comfortable conditions	TBW_2, L under heat stress conditions	Change, %	*HTC (100-change %)	Adaptability grade
1	128.4	135	5.1	94.9	Best
2	156.4	168	7.4	92.6	Moderate
3	156.4	163	4.2	95.8	Best
4	124.7	134	7.5	92.5	Moderate
5	113.2	119	5.1	94.9	Best
6	141.0	149	5.7	94.3	Best
7	121.2	132	8.9	91.1	Moderate
8	128.4	141	9.8	90.2	Moderate
9	148.3	166	11.9	88.1	Worst
10	113.2	136	20.1	79.9	Worst
X±SE	133.1 ±5.2	144.3±5.2	8.6±1.5		

Change %=(TBW_2–TBW_1)/TBW_1×100. *HTC=100–Change%. HTC: Heat tolerance coefficient, TBW: Total body water

Table 6: Estimation of the HTC using TBS

Calf no.	TBS_1, kg under comfortable conditions	TBS_2, kg under heat stress conditions	Change, %	*HTC (100-change %)	Adaptability grade
1	81.6	71	13.0	87.0	Best
2	78.6	62	21.0	79.0	Moderate
3	78.6	68	13.5	86.5	Best
4	60.3	48	20.4	79.6	Moderate
5	54.8	46	16.0	84.0	Best
6	69.0	58	15.9	84.1	Best
7	78.8	63	20.1	79.9	Moderate
8	60.6	45	25.7	74.3	Moderate
9	71.7	50	30.3	69.7	Worst
10	62.8	38	39.5	60.5	Worst
X±SE	69.7±3.0	54.9±3.5	21.5±2.6		

Change %=(TBS_1–TBS_2)/TBS_1×100, *HTC=100–Change%. HTC: Heat tolerance coefficient, TBS: Total body solids

Consequently, calves no. 2, 4, 7, and 8 are moderate in heat tolerance.

Kamal and Habeeb (1999) in Friesian calves and Habeeb and Gad (2018) in growing native and crossing bovine calves determined this HTC using the change in TBS and found that the most heat-tolerant animals are those with the highest values. In buffaloes and Friesians, the TBS decreased by 11.42% when the AT increased from 16°C, 50% RH to 32°C, 50% RH, constantly for 1 week, in the climatic chamber (Kamal and Seif, 1969). Kamal and Habeeb (1999) found a heat stress-induced significant decrease in TBS in both male and female Friesian calves. In Friesian calves, the average TBS content decreased by 16.0% with the increase in AT in the climatic chamber (Kamal, 1982). The same authors determined TBS as kg/100 kg body weight in 12 Friesian calves under low (19.0°C) and high (36.0°C) temperatures of 6 h daily for 2 weeks and found that the heat-induced percentage decrease in TBS was negatively correlated significantly with the growth rate during the 4 months of the hot summer season and concluded that the destruction of body tissues as a result of heat exposure is considered to be a serious stage of heat stress in animals. The tissue damage estimated by TBS losses may be attributed to an increase in glucocorticoids and catecholamines and a decrease in insulin secretion in heat stressed animals (Alvarez and Johnson, 1973; Habeeb *et al*., 1992). Besides, exposure to a hot environment can affect digestibility in a time-dependent fashion (Bernbabucci *et al*., 1999).[11-17]

Estimation of the HTC using TBW, L/100 kg TBS

The heat-induced changes in TBW, L/100 kg TBS in each of comfortable and heat stress conditions

Table 7: HTC using TBW, L/100 kg TBS ratio

Calf no.	TBW/100 kg TBS under comfortable conditions	TBW/100 kg TBS under heat stress conditions	Change, %	*HTC (100-change %)	Adaptability grade
1	157.4	190.1	20.8	79.2	Best
2	199.0	271.0	36.2	63.8	Moderate
3	199.0	239.7	20.5	79.5	Best
4	206.8	279.2	35.0	65.0	Moderate
5	206.6	258.7	25.2	74.8	Best
6	204.3	256.9	25.7	74.3	Best
7	153.8	209.5	36.2	63.8	Moderate
8	211.9	313.3	47.9	52.1	Moderate
9	206.8	332.0	60.5	39.5	Worst
10	180.3	357.9	98.5	1.5	Worst
X±SE	192.6±6.7	270.8±16.6	40.7±7.6		

Change %=(TBW/100 kg TBS under heat stress–TBW/100 kg TBS under comfortable)/TBW/100 kg TBS under comfortable×100, *HTC=100–Change %. HTC: Heat tolerance coefficient, TBW: Total body water

may be used as heat tolerance index in animals. It is clear from the data in Table 7 that each 100 kg solids in animals need 192.6 and 270.8 L water under comfortable and heat stress conditions, respectively, with the difference of 78.2 L water. The ratio between solids and water is 1:1.9 under comfortable and is 1: 2.7 and heat stress conditions. These data indicated that the water presents about 2/3 of the body weight under comfortable conditions while the water presents about ¾ of the bodyweight under heat stress conditions. The most heat-tolerant animals are those with the highest values of HTC and the less heat-tolerant animals are those with the lower values of HTC. Table 7 data showed that calves no. 1, 3, 5, and 6 are the best calves in heat tolerance while calves no. 9 and 10 are the worst calves in heat tolerance. Consequently, calves no. 2, 4, 7, and 8 are moderate in heat tolerance. Habeeb *et al*. (2001) estimated the HTC using TBW/100 kg TBS in Friesian calves and found that TBW/100 kg TBS had highly significantly negative correlated with daily body weight gain (DBWG) as follows: DBWG = 920.4–252.2×TBW, l/100 kg TBS (r=–0.8925, $P < 0.002$).

CONCLUSION

It is concluded that estimate body water using ANP by the new method is a simple, easy, accurate, and quick technique and more reliable and the accuracy of the modified technique was 97.5% as compared with the convention method. Besides, the heat-induced changes in each of TBW and TBSs in live animals using ANP dilution technique may be used as HTC for detection of heat adaptability in live animals.

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CONFLICTS OF INTEREST

No potential conflicts of interest were reported by all the authors. All authors decided that no acknowledge any financial interest or benefit we have arising from the direct applications of our research.

INTEREST STATEMENT

The direct benefits from the subject of this manuscript are that estimate body water using ANP by the new method is simple, easy, accurate, and more reliable and the accuracy of this technique was 97.5% and may be used as a quick technique for the estimation the heat tolerance in farm animals.

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DISCLOSURES AND DECLARATIONS

Our study-specific approval by the appropriate ethics of the Egyptian Atomic Energy Authority committee for research involving animals and a statement on the welfare of animals. Our work submitted for publication does not have any implication for public health or general welfare.

DATA TRANSPARENCY

All authors confirmed that the availability of data and materials as well as a software application or custom code support their published claims and comply with field standards.

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