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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 (ANP) substance as a modified technique was the first objective of this research. TBW was estimated in vivo in ten 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 liters 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.

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Introduction

The total body water 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[1]. Estimation of total body water (TBW) in live animals is important for research whether the research involves nutrition, physiology, genetic, disease and meat production[2]. 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[3]. 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 [4]. Moreover, the live body weight 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 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, 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[5].

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 **Brodieet al.**[6]**.** 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 hours 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 (the concentration at the time of injection) 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 hour after ANP injection as a modified technique and comparison between the two methods for estimating body water in ten calves was the objective of this research. Besides, using TBW or total body solids (Bodyweight-body water) (TBS) in live animals for evaluation of the animal's adaptability to heat stress was the second objective of this study.

Material and methods

Location*:* The experimental work was carried out in Bovine Farm of Biological Application Department, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, at Inshas, Egypt (latitude 31º 12' N to 22 º 2' N, longitude 25 º 53' E to 35º 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 Endeavour 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 bovine farm project, Experimental Farms Project, Biological Application Department. Ten bovine calves after weaning with 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 (RS) according to their requirements[7]**.** Ingredients of the concentrate feed mixture (CFM) are 35.0, 30.0, 30.0 and 5.0 % for un-decorticated 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 (AD3E), 2 kg AliphosDical 18 (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 were20 ± 2°C, 65 ± 2.5 RH %, respectively. The same calves were entered in a separate room (20 x 20 meters) for one week. The room was provided with electrical heaters and the calves were exposed to thermal stress conditions using electrical heaters for 7 hours 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 the 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 antipyrine (ANP) at the rate of 1g per 100 kg live body weight 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 hour for estimating TBW using modified technique and 4 blood samples were withdrawn from the write jugular vein of each calf after 1, 2, 3 and 4 hours from the injection of ANP to be distributed in the animal body for estimating TBW using convention technique. Consequently, total body solids (TBS) were estimated by subtracting TBW from live body weight.

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 (6N, 4N and 0.07N). Precipitation of plasma proteins in plasma samples was carried out using zinc sulfate and centrifuged at the rate of 2000 rpm for 20 minutes. 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 plasma sample (μ/ml). Total body solids (TBS) values were estimated by subtracting TBW from LBW[8]**.**

Estimation of TBW in vivo in animals:

Injection dose of ANP: The standard dose is 1 gram ANP each 100 kg live body weight (LBW). Each animal (weight 100 kg) inject with 5 ml (contains 1g 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 hours from the injection. Plasma was separated by centrifugation at 3,000 rpm for 15 min and stored at -20º C until estimation of ANP.

Preparation of standard ANP: One ml from injection dose (contains 400 µg ANP) was put and complete the solution with H_2SO_4 (0.07N) to reach 50 ml. Two ml from this standard was put in the tube and add 0.1 ml sodium nitrite, vortex and incubate the tube at 22ºC for 20 minutes. 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 ml from each plasma sample was put in one tube and adds 1 ml distilled water plus 1 ml zinc reagent plus 1 ml Na OH. Mixing the containing tubes using vortex for ½ minute and centrifuge the sample tubes at 3500 rpm for 15 minutes to obtain the supernatant.

Estimation of ANP in the supernatant of samples: Two ml from supernatant solution (contains ½ ml plasma) was put in one tube and add 0.1 ml sodium nitrite and one drop (50 µl) H_2SO_4 (4N) and incubate the tubes at 22ºC for 20 minutes. 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.) x concentration of standard (8µg/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 any animal 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/4ml 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)] x 2 x 100/93= liter.

Statistical analysis: Data of total body water in ten calves by two methods were analyzed statistically using a t-paired test according to **Snedecor and Cochran[9].**

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 hours 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 (0.05ml) of 4N 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 zero time. The plasma ANP concentration (µg/ml) at zero 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 zero 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 in Table (1).

Estimation 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 hour 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 hour after the injection of 2 g ANP in each calf was 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 x1000 x1000)/ANP at zero time or at ½ hr. after dosing} x 2 x (100/93) = liter as presented in Table (2).

Data shows that averages of total body water in ten calves were 136.5 and 133.1 liters in the extrapolated method and modified method, respectively. In the present study, the average total body water in 10 calves determined by the modified method was 3.4 liters (2.5 %) less than that obtained from the extrapolation method. This means that the modified method measures about 97.5 of the total body water 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 hours to equilibrate within rumen water[3]. Although the modified method underestimates body water only 2.5% in calves, it has more advantages than the conventional method. Because in the modified method not depriving the animals of feed and water for 5 hrs. 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 convention method. **Kamal and Habeeb[3]**studied the comparison between methods of estimating total body water 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.

Estimation of heat adaptability (Heat Tolerance Coefficient) 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 heat tolerance coefficient (HTC)[4].

Estimating the TBW using modified techniques in the ten calves after exposure to heat stress conditions for one week was found in Table (4). Data in Table (4) showed that TBS in ten calves during one week under heat stress conditions loosed about 15 kg including a decrease of about 4.0 kg in LBW and an increase of about 11 liters in TBW compared with comfortable conditions.

When exposure the animals to high ambient temperature, water intake increases and consequently TBW content increases with different percentages according to animal response to stressful conditions[4]**.** 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 heat tolerance coefficient (HTC). The heat-induced changes in each of TBW and TBS in live animals by ANP dilution technique were used previously as heat tolerance coefficient for detection of heat adaptability in farm animals[10, 11]**.** 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 the 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 x 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**[12]estimated this coefficient (HTC) in sheep and goats and concluded that the most heat tolerant animals are those with the highest values. Similar to that obtained perversely by **Kamal**[13]**.**

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 live body weight ($weight_1$ and$weight_2$)to obtain body solids under comfortable (TBS_1) and under heat stress (TBS_2).

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.

In Table (6) 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.

Kamal and Habeeb[10] in Friesian calves and **Habeeb and Gad**[11]in growing native and crossing bovine calves determined this heat tolerance coefficient (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 ambient temperature increased from 16°C, 50% RH to 32°C, 50% RH, constantly for one week, in the climatic chamber[13]**. Kamal and Habeeb**[10] 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 ambient temperature in the climatic chamber[14]. 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 hours daily for two weeks and found that the heat-induced percentage decrease in TBS was negatively correlated significantly with the growth rate during the four 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[15, 16]. Besides, exposure to a hot environment can affect digestibility in a time-dependent fashion[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 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 liters water under comfortable and heat-stress conditions, respectively with the difference of 78.2-liter 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 bodyweight under comfortable conditions while the water presents about ¾ of the body-weight 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. In 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.** [4] 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 x TBW, l / 100 kg TBS [r = -0.8925, P < 0.002.

Table 1: ANP concentrations at zero time and estimation of body water in the ten calves using extrapolation technique.

Calf No	Bodyweight of calves, Kg	ANP (µg/ml) at 0 time	Total body water, liter
1	210	33	TBW = { (2 x1000 x 1000) / 33 } x 2 x (100/93) =130.3
2	235	26.5	TBW = { (2 x1000 x 1000) / 26.5} x 2 x 100/93)=162.3
3	235	27	TBW = { (2 x1000 x 1000)/ 27} x 2 x (100/93) = 159.3
4	185	34	TBW = { (2 x1000 x 1000) / 34 } x 2 x(100/93) = 126.5
5	168	37	TBW = {(2 x1000 x 1000) / 37} x 2 x (100/93) = 116.2
6	210	30	TBW = { (2 x1000 x 1000) / 30 } x 2 x (100/93) =143.4
7	200	35	TBW = { (2 x1000 x 1000) / 35 } x 2 x (100/93) =122.9
8	189	33	TBW = { (2 x1000 x 1000) / 33 } x 2 x (100/93) =130.3
9	220	28	TBW = { (2 x1000 x 1000) / 28 } x 2 x (100/93) =153.6
10	172	36	TBW = { (2 x1000 x 1000) / 36} x 2 x (100/93) =119.5

Table 2: Estimate the total body water in the ten 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 ={ (2x1000x1000)/33.5} x 2 x (100/93) =128.4
2	0.075	27.5	TBW ={(2x1000x1000)/27.5} x 2 x (100/93) = 156.4
3	0.075	27.5	TBW = { (2 x 1000 x 1000) / 27.5 } x 2 x (100/93) = 156.40
4	0.099	34.5	TBW = { (2 x 1000 x 1000) / 34.5} x 2 x (100/93) = 124.67
5	0.131	38	TBW = { (2 x 1000 x 1000) / 38} x 2 x (100/93) = 113.2
6	0.093	30.5	TBW ={ (2x1000x1000)/30.5} x 2 x (100/93)=141.0
7	0.099	35.5	TBW ={ (2x1000x1000)/35.5} x 2 x (100/93)=121.2
8	0.095	33.5	TBW ={ (2x1000x1000)/33.5} x 2 x (100/93)=128.4
9	0.078	29	TBW ={ (2x1000x1000)/29} x 2 x (100/93) =148.3
10	0.12	37	TBW ={ (2x1000x1000)/38} x 2 x (100/93) =113.2

NS= not significant

Comparable between convention and modified methods in estimation TBW in ten calves were in Table (3).

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

Calf no	Bodyweight of calves, kg	Convention method		Modified method		Differences
		µg/ml ANP at zero time	Total body water, l	µg/ml ANP at zero time	Total body water. l	
1	210	33	130.3	33.5	128.4	-1.9
2	235	26.5	162.3	27.5	156.4	-5.9

3	235	27	159.3	27.5	156.4	-2.9
4	185	34	126.5	34.5	124.7	-1.8
5	168	37	116.5	38	113.2	-3.3
6	210	30	143.4	30.5	141	-2.4
7	200	35	122.9	35.5	121.2	-1.7
8	189	33	130.3	33.5	128.4	-1.9
9	220	28	153.6	29	148.3	-5.3
10	176	36	119.5	38	113.2	-6.3
X±SE	202.8±7.4		136.5±16.0		133.1 ±5.2	-3.4 L NS
Accuracy %					97.5%	

NS= not significant.

Table 4: Live body weight, total body water and total body solids 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	69	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

Table 5: Estimation of the heat tolerance coefficient (HTC) using total body water (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 % = $(TBW_2 - TBW_1)/ TBW_1 x100$

*Heat tolerance coefficient (HTC) =100 – Change%.

TBS loss due to heat stress may be used as HTC as following:

HTC = 100 – [TBS_2 –TBS_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).

Table 6: Estimation of the heat tolerance coefficient (HTC) using total body solids (TBS)

Calf no	TBS1, kg under comfortable conditions	TBS2, kg under heat stress conditions	Change, %	*HTC (100-change %)	Adaptability grade
1	81.6	71	13	87	Best
2	78.6	62	21	79	Moderate
3	78.6	68	13.5	86.5	Best
4	60.3	48	20.4	79.6	Moderate
5	54.8	46	16	84	Best
6	69	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 % = $(TBS_1 - TBS_2)/ TBS_1$ x100,

*Heat tolerance coefficient (HTC) =100 – Change%.

Table 7. Heat Tolerance Coefficient (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 x100.

*Heat tolerance coefficient (HTC) =100 – Change %.

Conclusion

It is concluded that estimate body water using the ANP by the new method is simple, easy, accurate and quickly 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 total body water and total body solids in live animals using ANP dilution technique may be used as heat tolerance coefficient for detection of heat adaptability in live animals.

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Interest statement: The direct benefits from the subject of this manuscript is 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 estimation the heat tolerance in farm animals.

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

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