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Research Paper

Estimating Animal Body Water and Solids *in Vivo* to Measure the Heat Adaptability

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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 the suggested modified method (equilibration technique). The averages of TBW 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 during the hot summer season TBW increases and consequently TBS (Live body weight-TBW) decreases with different percentages according to animal response to stressful conditions. TBW or TBS values were estimated before and after heat exposure and the percentage change in TBW or TBS in each 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.
KEYWORDS: *Total body water, antipyrine, calves, total body solids, heat tolerance.*

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I. 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 **(Hansard, 1964).** Body water is the water content of an animal body that is contained in the tissues, the blood, the bones and elsewhere (**Macfarlane et al., 1972).** Estimation of body water in live animals is important for research whether the research involves nutrition, physiology, genetic, disease and meat production. However, estimation of body water in animals using slaughter and chemical analysis of the whole body's organs is tedious processing, time- consuming and expensive operation **(Kamal and Habeeb, 1984).** Besides that, the high cost of animal analysis has created an interest in indirect methods of estimating body water. This indirect method or in vivo also can provide repeated estimates of body water for the same animal whereas slaughter and chemical analysis obviously can only be done once **(Habeeb et al., 2001).** Moreover, 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 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 **(Habeeb et al., 2020).**

Most methods for measuring the total body water (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. **Holleman et al., 1982, Johnson et al., 1989, Speakman et al., 2001, Habeeb (2009) and Alexander and Gerken (2010**) estimating TBW content by radioisotopes dilution technique while **Andrews et al., (1997)** estimating of TBW content using

deuterium oxide dilution. Antipyrine (ANP) as non-radioactive substance may be used in the estimation of body water in live animals. Measuring the total body water 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 total body water 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 body water 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, how theoretically using total body water or total body solids in live animals for evaluation of the animal's adaptability to heat stress was the second objective of this study.

II. MATERIALS AND METHODS

Location and ethics: 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 31º 12' N to 22 º 2' N, longitude 25 º 53' E to 35º 53' E). 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, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, Inshas, Cairo, Egypt. 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 (RS) according to their requirements (**NRC 1981**). 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 (AD3 E) , 2 kg Aliphos Di-Cal 18 (Di-calcium phosphate) and 1 kg coarse refined iodized kitchen salt (El-Nasr Saline's Co. Egypt).

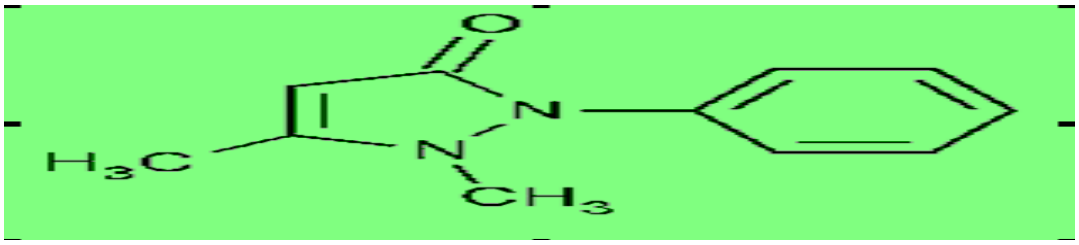
Experimental procedure: Ten native bovine calves healthy were used in the experiment. The experimental calves were under comfortable conditions during the winter season since the average ambient temperature and relative humidity were 20±2°C, 65±2.5 RH %. The same calves were exposed to thermal stress conditions using electrical heaters for 7 hours daily from 9.0 am to 4.0 pm for one week, since the average ambient temperature and relative humidity 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 experimental calves during both comfortable and thermal stress were in a separate room (20 x 20 meters). The room was provided with electrical heaters during the thermal stress period. 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 total body water (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 the plasma sample (µ/ml). Total body solids (TBS) or dry body weight was estimated by subtracting TBW from LBW.

Water consumption was measured by subtracting the residuals of water from that offered in the previous day for each calf under both comfortable and thermal stress conditions.

Estimation of total body water in vivo in animals:

1- Estimation of body water using ANP dilution technique: Before clarifying the accurate and quick technique for estimating body water using ANP. Chemical composition of ANP: Chemical of ANP is1, 2-Dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one or 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, Molecular Formula: $C_{11}H_{12}N_2O$, Molecular Weight: 188.23, Percent Composition: C 70.19%, H 6.43%, N 14.88%, O 8.50%. Colorless crystal or white crystalline powder, dissolved in water, alcohol, chloroform, ethyl ether-soluble and solution neutral. ANP is a nontoxic substance and is completely diluted in the body water of the animal. ANP is not found in the animal body for more than 5-6 hours. ANP structure as following:



1-Injection dose of ANP:

The standard dose is 1 gram ANP each 100 kg live body weight (LBW). Any animal weighted 100 kg needs 1 gram ANP for injection. The standard dose is 1g/100 kg live body weight because assume TBW percentage = 50% i.e. 50 liter # 1g/50 liter →1000000µg ANP/50000 ml i.e.100µg/5 ml → 20 µg/ml→inter reading of Spectrophotometer).

For the preparation of the injection dose of ANP, dissolve 20-gram ANP in physiological saline solution and complete the final solution to 100 ml→20 g/100 ml →1g/5 ml. Each animal or person (weight 100 kg) inject with 5 ml (contains 1g ANP) in the left jugular vein and one blood sample from each animal was withdrawn from the writing jugular vein after ½ to 1 hour from injection.

2-Chemical reagents required for ANP estimation:

- Zinc reagent solution: Ten g hydraulic zinc sulfate ($ZnSO_4.7\ H_2O$) was dissolved and 4 ml H_2SO_4 (6N) in distilled water and complete the solution with distilled water to reach 100 ml. This solution is used in protein precipitation in the plasma samples.

10 $ZnSO_4.7\ H_2O$ + 4 ml H_2SO_4 (6N) $\xrightarrow{\text{dis. water}}$100 ml zinc reagent solution.

- Sodium hydroxide (0.75N): Three g recently dry sodium hydroxide was dissolved in distilled water and completes the solution with distilled water to reach 100 ml. 3g NaOH $\xrightarrow{\text{dis.water}}$100 ml Na OH (0.75 N).
- Sodium nitrite (0.2%): Dissolve 0.1 g sodium nitrite was dissolved in distilled water and completes the solution with distilled water to reach 50 ml. 0.1 g Sodium nitrite $\xrightarrow{\text{dis.water}}$50 ml of Sodium nitrite solutions (0.2%).
- H_2SO_4 acid with different normality as following in Table 1.

H_2SO_4 concentration analar, Molecular weight = 98.08, Specific gravity = 1.84 i.e. 1 Liter = 1.84 kg # Concentration = 95-97% (96 %).

Table 1. H_2SO_4 acid with different normality and volume for ANP analysis

H_2SO_4(6N)	H_2SO_4(4N)	H_2SO_4(0.07 N)
10 ml	10 ml	250 ml
(6 x 98.08)/2 = 494.2 g / liter (494.2g) /100 = 4.94 g/ 10 ml 4.94 g / 1.84 = 2.68 ml, Conc.96%,(2.68x100)/96= 2.79 ml. Take 2.8 ml H_2SO_4 con. and complete with distilled water to reach the final volume of 10 ml. →10 ml H_2SO_4(6N)	4 x 98.08) /2 =196.2 g / liter (196.2) / 100 =1.96 g / 10 ml 1.96 g / 1.84 =1.06 ml Conc.96% (1.06 x100)/96 =1.1 ml. Take 1.1 ml H_2SO_4 con. and complete with distilled water to reach the final volume of 10 ml. →10 ml H_2SO_4(4N)	0.07 x 98.08) / 2 =3.43 g / liter (3.43) / 4 = 0.858 g / 250 ml 0.858 g / 1.84 = 0.466 ml Conc.96%, (0.446 x100) / 96= 0.49 ml.Take ½ ml H_2SO_4 con. and complete with distilled water to reach the final volume of 250 ml. →250 ml H_2SO_4(0.07N)

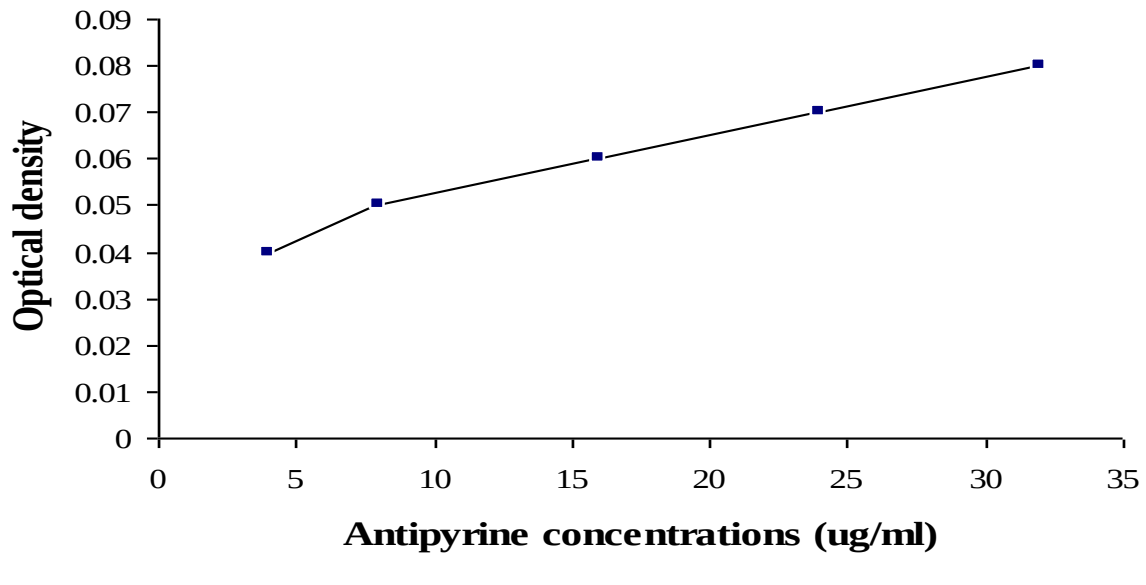
3-Preparation of standard ANP: One ml from injection dose (contains 400 µg ANP) was put and complete the solution with $H_2 SO_4$ (0.07N) to reach 50 ml, i.e. 400µg/50 ml →8 µg/ml. (Standard). Two ml from this standard was put in the tube and add 0.1 ml sodium nitrite, vortex and incubate the tube at 22ºC for 20 minutes. Then read this solution using a spectrophotometer to obtain the optical density of the standard.
4-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 NaOH. Mixing the containing tubes using vortex for ½ minute and centrifuge the sample tubes at 3500 rpm for 15 minutes to obtain the supernatant.
5- 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) $H_2 SO_4$ (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 optical density of standard was fixed and the concentration of ANP in each sample was determined directly without the equation.
6- Estimating 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.

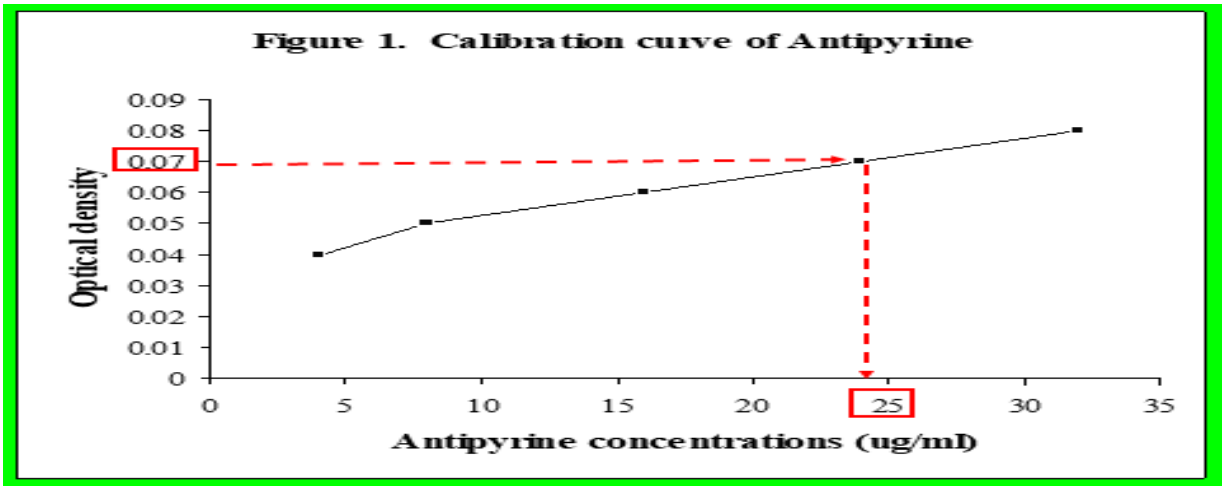
7-Statistical analysis:
Data of total body water in ten calves by two methods were analyzed statistically using a t-paired test according to **Snedecor and Cochran (1989).**

III. RESULTS AND DISCUSSION

1- Estimation of body water using the Antipyrine substance:
Estimation of body water in ten calves using extrapolation technique:
1- In this conventional method, five samples must withdraw after 2 g ANP injection in each calf and make ANP standard curve and extrapolated back to the time of injection by the method of least squares (extrapolation technique). Optical density is plotted against the corresponding concentrations of ANP (µg/ml) on a semi-logarithmic paper as following in Figure (1a and 1b).

Figure 1. Calibration curve of Antipyrine





2- 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 (Table 2).

Table 2. The optical density of ANP samples after dosing.

Calf No	The optical density of ANP at different hours after dosing, hour			
	After 1st hour	After 2nd hour	After 3rd hour	After 4th hour
1	0.090	0.080	0.072	0.070
2	0.069	0.056	0.042	0.034
3	0.073	0.063	0.046	0.044
4	0.086	0.066	0.042	0.032
5	0.115	0.090	0.069	0.044
6	0.093	0.082	0.074	0.071
7	0.068	0.056	0.040	0.033
8	0.075	0.064	0.045	0.043
9	0.087	0.067	0.044	0.031
10	0.114	0.093	0.068	0.043

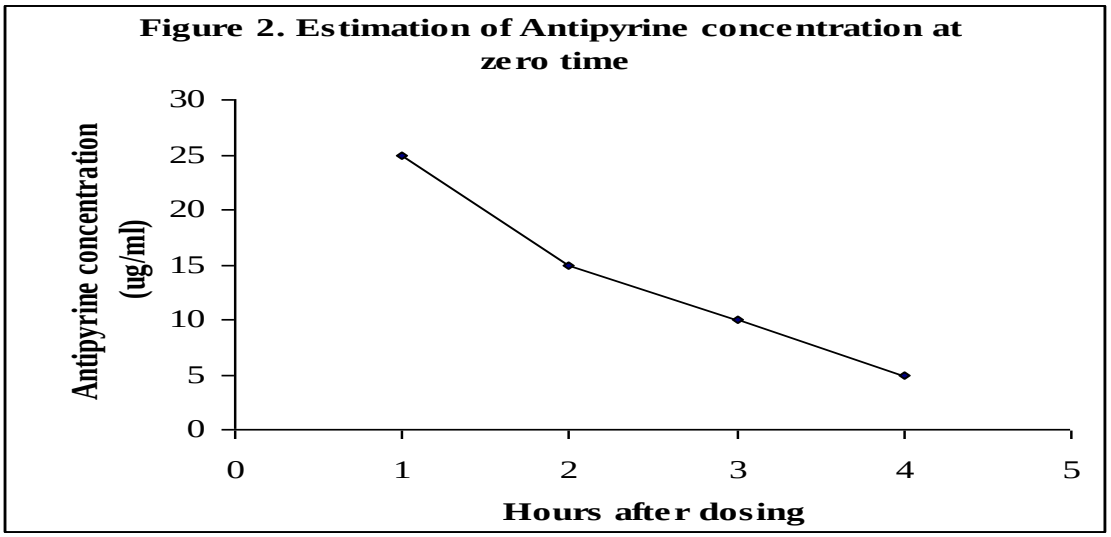
From the standard curve, the concentrations of ANP in plasma samples (µg/ml) at different hours after dosing were known as the following in Table (3).

Table 3. ANP concentrations (µg/ml) in plasma samples at different hours after dosing

Calf No	ANP concentrations (µg/ml) at different hours after dosing, hour			
	After 1st hour	After 2nd hour	After 3rd hour	After 4th hour
1	29	26	23	20
2	22	18	14	11
3	24	21	15	12
4	27	21	14	10
5	37	29	23	15
6	22	18	14	11
7	27	21	14	10
8	29	26	23	20
9	24	21	15	13
10	22	18	14	10

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 (Figure 2).



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: Body water, ml = Amount of ANP injected (µg)/ Amount of ANP in plasma (µ/ml). Body water was estimated as in Table (4).

Table 4. 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	33.0	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	27.0	TBW = { (2 x1000 x 1000)/ 27} x 2 x (100/93) = 159.3
4	185	34.0	TBW = { (2 x1000 x 1000) / 34 } x 2 x(100/93) = 126.5
5	168	37.0	TBW = { (2 x1000 x 1000) / 37} x 2 x (100/93) = 116.2
6	210	30.0	TBW = { (2 x1000 x 1000) / 30 } x 2 x (100/93) =143.4
7	200	35.0	TBW = { (2 x1000 x 1000) / 35 } x 2 x (100/93) =122.9
8	189	33.0	TBW = { (2 x1000 x 1000) / 33 } x 2 x (100/93) =130.3
9	220	28.0	TBW = { (2 x1000 x 1000) / 28 } x 2 x (100/93) =153.6
10	172	36.0	TBW = { (2 x1000 x 1000) / 36} x 2 x (100/93) =119.5

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 hour after ANP injection as a modified technique in the same calves. The optical density 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 optical density of 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 (5).

Table 5. 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 ={ (2 x 1000 x 1000)/33.5 } x 2 x (100/93) = 128.4
2	0.075	27.5	TBW ={(2 x 1000 x 1000)/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	38.0	TBW = { (2 x 1000 x 1000) / 38} x 2 x (100/93) = 113.2
6	0.093	30.5	TBW ={ (2 x 1000 x 1000)/30.5 } x 2 x (100/93)= 141.0
7	0.099	35.5	TBW ={ (2 x 1000 x 1000)/35.5 } x 2 x (100/93) = 121.2
8	0.095	33.5	TBW ={ (2 x 1000 x 1000)/33.5 } x 2 x (100/93) = 128.4
9	0.078	29.0	TBW ={ (2 x 1000 x 1000)/29 } x 2 x (100/93) = 148.3
10	0.12	37.0	TBW ={ (2 x 1000 x 1000)/38} x 2 x (100/93) = 113.2

Comparable between convention and modified methods in estimation body water in five calves were in Table (6).

Table 6. 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	33.0	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.

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 body water obtained by the two methods did not differ significantly. The lower body water 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 (**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 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 conventional method. However, **Kamal and Habeeb (1984)** studied the comparison between methods of estimating total body water using Tritiated water, 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) (**Habeeb et al., 2001 and 2007**).
Estimate total body water using modified techniques in the same ten calves after exposure to heat stress conditions for one week. The data are found in Table (7)

Table7. 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

Data in Table (7) showed that total body solids in ten calves during one week under heat stress conditions loosed about 15 kg including a decrease of about 4.0 kg in body weight and an increase of about 11 liters in body water compared with comfortable conditions.

Estimation of the Heat Tolerance Coefficient (HTC)

Detection of such phenomena in animals could be achieved by different indices. Body water varies considerably in animals during growing due to differences in the rate of accumulation of the less hydrated, fat, collagen and fibrous tissues in replacement of the more hydrated functioning protoplasmic mass (**Habeeb et al., 1992**). The body water concentration in an animal is also different due to the difference in response to nutritional and climatic factors. Animals when exposed to high ambient temperature during the hot summer season, water intake increases and consequently body water content increases with different percentages according to animal response to stressful conditions (**Habeeb et al., 2001).** The percentage change (heat-induced changes) in body water or body solids (Live body weight- body water) contents in each animal may be used for evaluating the animal's adaptability to heat stress **(Habeeb, 2010).** The heat-induced changes may be used as the index for heat tolerance coefficient (HTC). Detection of such phenomena in the animals could be achieved by body water and body solids as presented. The heat-induced changes in each of total body water and total body solids in live animals by ANP dilution technique were used previously as heat tolerance coefficient for detection of heat adaptability in farm animals (**Kamal and Habeeb, 1999; Habeeb et al., 2001 and Habeeb and Gad, 2018).**

Estimation of the heat tolerance or heat adaptability (heat stress index) based on body water and body solids (Live body weight-body water) or body water/body solids ratios which are presented in the following:

1- Estimation of the Heat Tolerance Coefficient (HTC) using total body water (TBW):

The body water is determined using ANP dilution technique under comfortable conditions (TBW_1) and heat stress exposure (TBW_2). The percentage increase in body water due to heat stress conditions may be used as the index for heat tolerance coefficient (HTC) as following:
HTC =100 - [TBW_2 - TBW_1 / TBW_1 x 100] where TBW_1 and TBW_2 are body water during comfortable and hot conditions, respectively. The most heat tolerance animals are those with the highest values as assuming in Table (8).

Table 8. 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%.

Table (8) 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. TBW as an absolute value in liters or relative to LBW increased more significantly in summer than in winter in buffaloes and Friesian cows (**Kamal and Seif, 1969**). Results similar to that obtained by **Kamal and Habeeb (1999)** and **Habeeb et al. (2001)** showed that exposure of the Friesian calves to heat stress increased significantly TBW. The same was true in water buffaloes and Red Danish cattle whether the animals were heifers, pregnant, or lactating (**Kamal et al., 1978).** Holstein and Friesian calves also showed the same response of TBW under heat stress (**Kamal and Johnson, 1971 and Kamal, 1982).**

The water requirements are determined by the amount needed to give the proper osmotic concentration to body fluids and to compensate for total water loss. On the other hand, to maintain total body water content at a relatively constant level a continuous supply must be provided. In a steady-state (comfortable conditions), water intake including free water, feed water and metabolic water, which is derived from the oxidation of fat, carbohydrates and proteins must balance water output including urine, sweating, skin vaporization and respiratory vaporization (**Abdel-Samee, 1982 and Aggarwal and Upadhyay, 1998).** Under hot climate, water plays a major role in heat dissipation through evaporative cooling including sweating (skin vaporization), and respiratory vaporization and thermal conduction between consumed water and both of body core and excreted water through urine, feces, milk, salivation, tears and nasal tract secretions but vaporization at high temperature is considered the main route of heat dissipation **(Kamal, 1976 and Kamal et al., 1984).** The increase in water vaporization in heat-stressed animals stimulates the peripheral thermal receptors to transmit suppressive nerve impulses to the thirst center in the hypothalamus causing the increase in water consumption **(Habeeb et al., 1992).**

2. Estimation of the Heat Tolerance Coefficient (HTC) using total body solids:

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

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 (9) 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 as presented in Table (9).

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Table 9. Estimation of the heat tolerance coefficient (HTC) using total body solids (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 x100, *Heat tolerance coefficient (HTC) =100 – Change%.

Kamal and Habeeb (1999) in Friesian calves and **Habeeb and Gad (2018)** in growing native and crossing bovine calves determined this heat tolerance coefficient (HTC) using the change in body solids and found that the most heat tolerant animals are those with the highest values. Several variations resulted in a decrease of TBS from spring to summer (110.9 to 59.5 kg) and from summer combined with solar radiation (59.5 to 58.6 kg) in buffalo (**Kamal et al. 1978)**. In Friesian cows, TBS was found to decrease significantly from winter (118.0 kg) or spring (126.5 kg) to summer (91.0 kg), under a naturally hot climate (**Kamal and Seif, 1969).** In growing buffaloes, TBS was similarly lower at 32°C and 50% RH than at 18°C and 50% RH (100 and 124 kg, respectively) (**Kamal et al., 1972).** In Holstein's calves, the heat caused a 15% decrease significantly in TBS (**Kamal and Johnson, 1971)**. In Friesian calves, the average body solid content decreased by 16% with the increase in ambient temperature in the climatic chamber (**Kamal, 1982).** In buffaloes and Friesians, the TBS decreased by 11.42% at each level of temperature, when the ambient temperature increased from 16°C, 50% RH to 32°C, 50% RH, constantly for one week, in the climatic chamber (**Kamal and Seif, 1969). Kamal (1982)** 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 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. Results similar to that obtained by **Kamal and Habeeb (1999) and Habeeb et al. (2001)** showed that exposure of the Friesian calves to heat stress decreased significantly TBS and found a heat stress-induced significant decrease in TBS in both male and female Friesian calves.

The tissue damage estimated by TBS losses may be attributed to an increase in glucocorticoids and catecholamines (**Alvarez and Johnson, 1973)** and a decrease in insulin secretion in heat-stressed animals (**Habeeb et al. 1992**). Besides, exposure to a hot environment can affect digestibility in a time-dependent fashion (**Bernbabucci et al., 1999**).

3. Estimation of the Heat Tolerance Coefficient (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 (10) 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.

This data indicated that the water presents about 2/3 of the body weight 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.

Table (10) 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., 2019).**

Habeeb et al. (2001) estimated the heat tolerance coefficient (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:

$$DBWG = 920.4–252.2 \times TBW, l / 100 \text{ kg TBS } [r = -0.8925, P < 0.002.$$

Table 10. Heat Tolerance Coefficient (HTC) using TBW, L /100 kg TBS ratio

Calf no	Water consumption under comfortable conditions	Water consumption under heat stress conditions	Change %	*HTC (100-change %)	Adaptability grade
1	31.5	37.0	17.5	82.5	Best
2	33.0	42.0	27.3	72.7	Moderate
3	33.5	40.0	19.4	80.6	Best
4	28.0	34.5	23.2	76.8	Moderate
5	26.0	30.5	17.3	82.7	Best
6	31.5	37.0	17.5	82.5	Best
7	30.5	39.0	27.9	72.1	Moderate
8	29.0	36.5	25.9	74.1	Moderate
9	32.5	45.5	40.0	60.0	Worst
10	28.5	40.0	40.4	59.6	Worst
X±SE	30.4±0.77	38.2±1.31	25.6±2.8		

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 %.

4- Estimation of the Heat Tolerance Coefficient (HTC) using the water intake (WI):

Practically on the scale of farmers without injection, any substance may be used water intake as heat-tolerant index by estimating the water intake under each of comfortable and heat-stress conditions and used the percentage increase in water intake as a heat-tolerance index as following: HTC = 100--[WI_2-WI_1/ WI_1x 100] where $WI_1$and WI_2 are the water intake during comfortable and hot climates, respectively. The most heat tolerant animals are those with the highest values of HTC (Table 11).

Table 11. Estimation of the heat tolerance coefficient (HTC) using the water intake

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 % = (WC under heat stress - WC under comfortable) / WC under comfortable x100.
*Heat tolerance coefficient (HTC) =100 – Change %.

It is clear from the data in Table (11) that the average water intake in the ten calves increased by 7.8 liter (+25.6%) due to exposure the calves to thermal stress conditions for one week as comparable with comfortable conditions.

IV. 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 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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