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Simple Methods to Estimate Total Body Water in Live Animals Using Antipyrine with Detection of Heat Adaptability

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Abstract

Estimating body water content in live animal using Antipyrine substance as a new and modified technique was the objective of this research. Body water was estimated in five calves with average body weight of 183.6 ± 30.3 kg using conventional method (extrapolation technique) and in the same time by modified method. The averages of total body water in five calves were 135.8 ± 11.8 and 132.3 ± 11.35 liters by convention and modified technique, respectively, without significant difference between the two techniques. The corresponding summations of body water in all calves were 679.2 and 661.4 liters. The accuracy of modified technique was 97.4 % as compared with convention method.

It is concluded that estimate body water using Antipyrine by new method is easily, accurate and quickly technique and more reliable. Animals when exposed to high ambient temperature during hot summer season, water intake increase and consequently body water content increases with different percentage according animal response to stressful conditions. The percentage change in body water or body solids (Live body weight- body water) contents in each animal may be used for evaluation the animal's adaptability to heat stress.

Keywords: Body water; Antipyrine; Calves; Body solids

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. About 99% of all molecules in the body are water, which forms about 70% of the body weight of a tropical ruminant. About 45% of bodyweight is intracellular water and 25% is extracellular, divided between plasma (5%) and interstitial fluids (20%) [1].

Body water in live animals is important for research whether the research involves nutrition, physiology, genetic, disease and meat production. Estimation of body water in animals using slaughter and chemical analysis of the whole bodies organs is a tedious processing, time-consuming and expensive operation [2]. 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 same animal whereas slaughter and chemical analysis obviously can only be done once [3].

Moreover, body weight of animal alone provides a poor index of the metabolically active tissue due to that body weight 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, may be due to the increase in body solids [4].

Most methods for measuring the total water content of the body *in vivo* have been based on the degree of dilution of a foreign substance or radioisotope after its intravenous injection. This substance or radioisotope should possess the following characteristics: even and 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. Application of the isotopes materials using tritium or deuterium for measuring total body water after counting ß-activity in Liquid Scintillation Counting was carried out by different authors [5-10].

There are many disadvantages in using isotopes in estimation of total body water in live animals. This technique needs special instrument like liquid scintillation counting with liquid scientillator solution to counting ß-activity. In addition, these animals after injection with radioisotopes must be culled. Therefore, antipyrine (ANP) as nonradioactive substance may be used in 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. [11]. 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 gm/100 kg body weight of antipyrine in distilled water intravenously from calibrated syringe and five blood samples are withdrawn at 2, 3, 4 and 5 hours subsequently and protein precipitation for plasma. ANP is measured in filtrate from the ultraviolet absorption of 4-nitroso-antipyrine by the addition of sodium nitrite and sulphuric acid to plasma filtrate.]

The plasma concentration at zero time (the concentration at the time of injection) by plotting the plasma levels on semilogarithmic 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 live animal using ANP by single blood sample at ½ hour after ANP injection as modified technique and comparison between the two methods for estimating body water in five calves was the objective of this research. In addition, how theoretically using total body water or total body solids in live animals for evaluation the animal's adaptability to heat stress was the second objective of this study.

Materials and Methods

Location and ethics

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).

This work was reviewed and approved by the Animal Care and Welfare Committee of Zagazig University, Egypt (ANWD-206). 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 (ICLAS) guidelines. Experimental animals were also cared using husbandry guidelines derived from Egyptian Atomic Energy Authority standard operating procedures.

Estimation of body water using antipyrine (ANP) using modified method

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

For preparation 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 →1 g/5 ml. Each animal or person (weight 100 kg) inject with 5 ml (contains 1 g ANP) in the left jugular vein and one blood sample withdrawn from write jugular vein of animal after ½-1 hour from injection.

Chemical reagents required for antipyrine estimation: Zinc reagent solution: 10 g hydrolic zinc sulphate ($ZnSO_4.7H_2O$) was dissolved and 4 ml H_2SO_4 (6 N) in distilled water and complete the solution with distilled water to reach 100 ml. This solution used in protein precipitation in the plasma samples. $10ZnSO_4.7H_2O$ + 4 ml H_2SO_4 (6 N) distilled water→100 ml zinc reagent solution.

Sodium hydroxide (0.75 N): 3 g recently dry sodium hydroxide was dissolved in distilled water and completes the solution with distilled water to reach 100 ml. 3 g NaOH 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 dis.water→50 ml 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	4 x 98.08) /2=196.2 g /liter	0.07 x 98.08)/2=3.43 g /liter
(494.2 g)/100=4.94 g/10 ml	(196.2) / 100 =1.96 g /10 ml	(3.43)/4=0.858 g/250 ml
4.94 g / 1.84 = 2.68 ml	1.96 g / 1.84 =1.06 ml	0.858 g/1.84=0.466 ml
Conc.96%	Conc.96%	Conc. 96%
(2.68 x 100)/96=2.79 ml	(1.06 x 100) / 96 = 1.1 ml	(0.446 x 100)/96= 0.49 ml
Take 2.8 ml H_2SO_4 con and complete with distilled water to reach the final volume 10 ml.	Take 1.1 ml H_2SO_4 con. and complete with distilled water to reach the final volume 10 ml.	Take ½ ml H_2SO_4 con. and complete with distilled water to reach the final volume 250 ml.
→10 ml H_2SO_4 (6 N)	→10 ml H_2SO_4 (4 N)	→250 ml H_2SO_4 (0.07 N)

Preparation of standard ANP: One ml from injection dose (contains 400 µg ANP) was put and complete the solution with H_2SO_4 (0.07 N) to reach 50 ml, i.e. 400 µg/50 ml →8 µg/ml. (Standard). 2 ml from this standard was putted in tube and add 0.1 ml sodium nitrite, vortex and incubate the tube on 22°C for 20 minutes. Then read this solution using spectrophotometer to obtain the optical density of standard.

Precipitation of plasma proteins in plasma samples: 1 ml from each plasma sample was put in one tube and add 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 samples tubes on 3500 rpm for 15 minutes to obtain the supernatant.

Estimation of ANP in supernatant of samples: 2 ml from supernatant solution (contains ½ ml plasma) was putted in one tube and add 0.1 ml sodium nitrite and one drop (50 µl) H_2SO_4 (4N) and incubate the tubes on 22°C for 20 minutes. Optical densities of all tubes were reading using Spectrophotometer. Concentration of ANP (µg/ml) in each sample was determined as following: ANP concentration= (Optical density of sample/ Optical density of std.) x concentration of standard (8 µg/ml) = µg. Standard tube put in spectrophotometer and optical density of standard was fixed and concentration of ANP in each sample determined directly without the equation.

Estimating of body water: Estimation of body water (ml) in any animal or person is given by concentration of ANP Injected (µg) to concentration of ANP in 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)] x 2 x 100/93= liter.

Statistical analysis

Data of total body water in five calves by two methods were analyzed statistically using t-paired test according to Snedecor and Cochran [12].

Results and Discussion

Estimation body water in 5 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 densities are plotted against the corresponding concentrations of ANP (µg/ml) on a semilogaithmic paper as following **(Figure 1).**

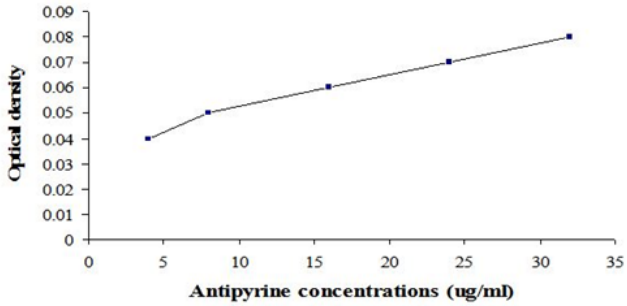

Figure 1: Calibration curve of antipyrine.

2: 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 **(Table 2).** From standard curve, the concentrations of ANP in plasma samples (µg/ml) at different hours after dosing were known as following in **Table 3.** Plasma levels of antipyrine at various intervals after intravenous injection were plotted on semilogarthmic 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 antipyrine is calculated by dividing the plasma level of antipyrine 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.**

Estimation body water using modified technique: Estimating body water content in live animal using ANP was carried out by single blood sample at ½ hour after ANP injection as modified technique in the same calves. Optical density of one sample (½ h after injection) and also ANP concentration in one sample at ½ hour after injection of 2 g ANP in each calf was estimated.

Standard tube put in spectrophotometer and optical density of standard was fixed and concentration of ANP in each sample determined directly according this equation: TBW = {(2 x 1000 x 1000)/ANP at zero time or at ½ hr after dosing} x 2 x (100/93) = liter as presented in **Table 5.** Comparable between convention and modified methods in estimation body water in five calves was in **Table 6.** Data shows that averages of total body water in five calves were 135.8 and 132.3 liters in extrapolated method and modified method, respectively.

In the present study, average total body water in 5 calves determined by the modified method was 3.5 liters (2.6%) less than that obtained from the extrapolation method. This means

that the modified method measures about 97.4 of the total body water in calves. However, the values for body water obtained by the two methods did not differ significantly. Because in modified method not depriving the animals of feed and water for 5 h.

In addition, animals do not lose water by vaporization during such a time and their physiological systems are not disturbed by convention method measurement. Besides, modified method is 5 times faster than the convention method. However, Kamal and Habeeb studied the comparison between methods of estimating total body water using tritiated water, Antipyrine and desiccation in Friesian cattle and found that estimating body water using Antipyrine was accurate technique with relation to desiccation method.

Estimation of heat tolerance coefficient: Body water is vary considerably in animal during growing due to difference in the rate of accumulation of the less hydrated, fat, collagen and fibrous tissues in replacement of the more hydrated functioning protoplasmic mass [13]. In addition, body water concentration in animal is also differed due to difference in response to nutritional and climatic factors.

Animals when exposed to high ambient temperature during hot summer season, water intake increase and consequently body water content increases with different percentage according animal response to stressful conditions [3]. 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 evaluation the animal's adaptability to heat stress [10]. The heat-induced changes may be used as 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 antipyrine dilution technique were used previously as heat tolerance coefficient for detection of heat adaptability in farm animals [3,14,15].

Estimation of Heat Tolerance Coefficient (HTC) using total body water: The body water is determined using ANP by modified technique under each of comfortable conditions (BW1) and under heat stress exposure (BW2). The percentage increase in body water due to heat stress conditions may be used as index for heat tolerance coefficient (HTC) as following:

HTC=100-[BW2-BW1/BW1 x 100] where BW1 and BW2 are body water during comfortable and hot conditions, respectively. The most heat tolerance animals are those with the highest values as assuming in **Table 7.**

Data in **Tables 7 and 8** are assuming values to clear how HTC and adaptability grade were estimated. Habeeb [14] estimated actually this coefficient (HTC) in sheep and goats and concluded that the most heat tolerant animals are those with the highest values.

Estimation of Heat Tolerance Coefficient (HTC) using total body solids: It is well known that body weight including body solids and body water. Body solids (BS) = body weight - body water.

Table 2: Optical density of ANP samples after dosing.

Calf no.	Time after dosing, hr			
	1	2	3	4
1	0.09	0.08	0.072	0.07
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	0.09	0.069	0.044

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

Calf no.	Time after dosing , hr			
	1	2	3	4
1	29	26	23	22
2	22	18	14	11
3	24	21	15	14
4	27	21	14	10
5	37	29	23	15

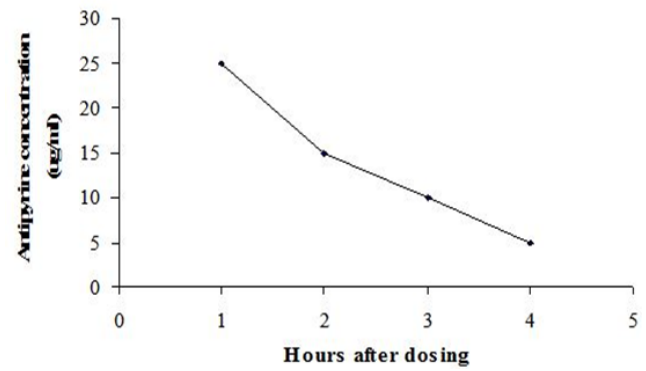

Figure 2: Estimation of antipyrine concentration at zero time.

Estimation of the body water using ANP by modified methods under each of comfortable (BW1) and heat stress (BW2) and each value was subtracted from the corresponding body weight (weight1 and weight2) to obtain body solids under comfortable (BS1) and under heat stress (BS2). Body solids loss due to heat stress may be used as HTC as following:

HTC = 100 – [BS2 –BS1 / BS1 x 100], where BS1 and BS2 are the BS during comfortable and heat stress, respectively, as assuming in **Table 8.**

Table 4: ANP concentrations at zero time and estimation of body water in five calves using extrapolation technique.

Calf no.	Body weight of calves, kg	ANP (µg/ml) at zero time	Total body water, liter
1	200	33	TBW = { (2 x 1000 x 1000)/33 } x 2 x (100/93) = 130.34
2	230	26.5	TBW = { (2 x 1000 x 1000)/26.5} x 2 x (100/93)=162.30
3	235	27	TBW = { (2 x 1000 x 1000)/27} x 2 x (100/93) = 159.30
4	185	34	TBW = { (2 x 1000 x 1000)/34 } x 2 x(100/93) = 126.50
5	68	44	TBW = { (2 x 1000 x 1000)/44} x 2 x (100/93) = 97.75

Table 5: Estimate total body water in five calves using modified technique.

Calf no.	O.D. of one sample (½ h after injection)	ANP concentrations in one sample (½ h after injection)	Total body water, liter (Modified technique)
1	0.095	33.5	TBW ={(2 x 1000 x 1000)/33.5 } x 2 x (100/93) =128.39
2	0.075	27.5	TBW ={(2 x 1000 x 1000)/27.5} x 2 x (100/93) = 156.40
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	45	TBW = {(2 x 1000 x 1000)/45} x 2 x (100/93) = 95.58

Table 6: Estimate body water in calves using convention and modified techniques.

Calf no.	Body weight of calves, kg	Convention method		Modified method		Differences
		ANP at 0 time	Total body water, l	ANP at 0 time	Total body water. l	
1	200	33	133.34	33.5	128.39	-4.95
2	230	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	124.67	-1.83
5	68	44	97.75	45	95.58	-2.17
Sum	918		679.2		661.4*	-17.8
X ± SE	183.6 ± 30.3	32.9 ± 3.16	135.8 ± 11.8	33.6 ± 3.20	132.3 ± 11.35	-3.5

Accuracy %		97.4
*NS= Not Significant		

Table 7: Estimation of heat tolerance coefficient (HTC) using total body water (BW)

Animal no.	BW1 under comfortable conditions	BW2 under heat stress conditions	Change %	*HTC (100-change %)	Adaptability grade
1	100	120	20	80	Best
2	100	130	30	70	Moderate
3	100	140	40	60	Worst
*Heat tolerance coefficient (HTC) =100 - [BW2 - BW1 / BW1 x 100]					

Table 8: Estimation of heat tolerance coefficient (HTC) using total body solids (BS).

Under comfortable			Under heat stress			Change % in BS	*HTC (100-% change)	Adaptability grade
Weight 1	BW1	BS1	Weight 2	BW2	BS2			
160	100	60	180	120	60	0	100	Best
160	100	60	180	130	50	17	83	Moderate
160	100	60	180	140	40	33	67	Worst
*Heat tolerance coefficient (HTC) =100 - [BS2 - BS1 / BS1 x 100]								

Kamal and Habeeb [4] in Friesian calves and Habeeb and Gad [15] in growing native and crossing bovine calves determined actually this heat tolerance coefficient (HTC) using change in body solids and found that the most heat tolerant animals are those with the highest values.

Conclusion

It is concluded that estimate body water using ANP by new method is simple, easily, accurate and quickly technique and more reliable and the accuracy of modified technique was 97.4 % as compared with convention method. In addition, the heat-induced changes in each of total body water and total body solids in live animals using antipyrine dilution technique may be used as heat tolerance coefficient for detection of heat adaptability in live animals

References

1. Macfarlane WV, Howard B, Maloiy GMO, Hopcraft D (1972) Tritiated water in field studies of ruminant metabolism in Africa. Proceeding symposium on use of isotopes in studies on the physiology of domestic animals with special reference to hot climate. In: Proceedings of the 7th Pan-African Ornithological Congress.
2. Kamal TH and Habeeb AAM (1984) Comparison between methods of estimating total body water using tritiated water, antipyrine and desiccation in Friesian cattle. Proceedings of the First Egyptian-British Conference on Animal and Poultry Production, Zagazig University, Zagazig , Egypt.
3. Habeeb AAM, Aboulnaga AI, Kamal TH (2001) Heat-induced changes in body water concentration, T3, cortisol, glucose and cholesterol levels and their relationships with thermoneutral body weight gain in Friesian calves. Proceeding of the Second International Conference on Animal Production & Health in Semi-Arid Areas, Suez Canal University, Faculty of Environmental Agricultural Sciences, El-Arish, North Sinai, Egypt.
4. Kamal TH and Habeeb AAM (1999) The effect of sex difference in Friesian calves on heat tolerance using the heat-induced changes in total body water, total body solids and some blood components. Egypt J Applied Sci 14: 1-15.
5. Hansard SL (1964) Total body water in farm animals. Am J Physiol 206: 1369-1372.
6. Andrews FM, Nadeau JA, Saabye L, Saxton AM (1997) Measurement of total body water content in horses, using deuterium oxide dilution. Am J Vet Res 58: 1060–1064.
7. Holleman DF, White RG, Luick JR (1982) Application of the isotopic water method for measuring total body water, body composition and body water turnover. In: International Atomic Energy Agency, editor. Use of tritiated water in studies of production and adaptation in ruminants. Nairobi, Kenya: International Atomic Energy Agency 9–32.
8. Habeeb AAM (2009) Estimating the adaptability of farm animals to hot climatic conditions using radioisotopes. The 12th Scientific conference on animal nutrition organized by the egyptian society of nutrition and feeds, held at Sharm El-Shiekh, Egypt. Egyp J Nutr Feeds.
9. Speakman JR, Visser GH, Ward S, Krol E (2001) The isotope dilution method for the evaluation of body composition. In: Speakman JR, editor, Body composition analysis of animals. A hand book of non-destructive methods. Cambridge: Cambridge University Press pp: 56–98.

10. Alexander R, Gerken M (2010) Estimating total body water content in suckling and lactating llamas (Lama glama) by isotope dilution. Trop Anim Health Prod 42: 1189–1193.

11. Brodie BB, Axelrod J, Soberman B, Levy BB (1949) The estimation of antipyrine in biological materials. J Biol Chem 179: 25-29.

12. Snedecor GW, Cochran WG (1989) Statistical Methods, 8th edn., Ames, Iowa State University Press, Iowa, USA pp: 503.

13. Habeeb AAM, Marai IFM, Kamal TH (1992) Heat Stress. In: farm animals and the environment. Edited by C. Phillips and D. Piggins, Commonwealth Agricultural Perau International, UK, pp. 27-47.

14. Habeeb AAM (2010) Estimation of total body water in sheep and goats using antipyrine for detection of heat adaptability coefficient. 3rd International Scientific Conference on Small Ruminant Development, Organized by Egyptian Association for Sheep and Goats (EASG) in Hurghada, Egypt.

15. Habeeb AAM, Gad AE (2018) Effect of genetic crossing between native bovine cows with holstein friesian bull on body water and solids content in growing calves under winter and summer seasons. CPQ Medicine 2: 01-15.