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Estimation of Water Metabolism in Freisian Cows

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TOTAL water intake averages of dry pregnant cows, late lactating cows were 27.25 ± 0.33 and 34.21 ± 1.88 respectivel whereas, water turnover rate averages were 28.78 ± 0.49 and 35.96 ± 1.39, respectively. The difference between the averages was significant ($P < 0.05$).

Pulmocutaneous water loss averages as L/day extimated by subtraction technique in dry pregnant and late lactating cows were 11.47 ± 1.00 and 16.37 ± 2.08, respectively whereas those as estimated by TOH dilution technique were 12.40 ± 0.98 and 18.12 ± 1.58, respectively. The difference between the averages of two techniques was significant ($P < 0.05$).

TOH dilution technique gives higher averages than the convetional technique by 8 - 10 %. At 18° skin vaporization water loss averages were 0.36 ± 0.05 and 0.37 ± 0.05 L/day estimated by conventional and TOH dilution techniques, respectively.

Under hot conditions (at 38°) the average of skin vaporization was 0.61±0.07 L/day by the conventional technique compared with 0.65 ± 0.07 L/day that estimated by TOH dilution technique.

TOH dilution technique was higher average than the conventional technique for about 4.03 - 6.64% and the difference was found insignificant.

The average counts for plasma net activity was 970 ± 150 cpm/ml increased significantly ($P < 0.01$) in milk to 1276 ± 167 cpm/ml.

The averages of quenching were 2.15 ± 0.06 and 1.63 ± 0.03 for plasma and milk samples, respectively. The differences were found significant ($P < 0.01$). When the samples were corrected for their quenching, their averages were 2159 ± 333 and 2307 ± 357 in plasma and milk, respectively.

Milk samples can be used for total body water and water turnover rate determinations, however, the averages of TBW L/100 kg LBW were 74.78 ± 0.61 and 73.77 ± 0.27 that estimated by plasma and milk respectively. WTR average was 36.16 ± 0.26 L/day by plasma decreased to 35.3 ± 0.83 L/day by milk samples.

The direct estimation of water intake in a large number of animals especially under grazing condition is practically impossible. Therefore, it was attempted in this study to estimate water intake with a simpler and practical method for such conditions using the tritiated water dilution technique as the major objective. In this technique blood samples, which are collected once a day for 4-5 days, were replaced by milk or urine samples, which are easier to collect, and this modification was tested for accuracy of water intake determination as another objective in this study.

Under hot climate, where heat dissipation through conduction, convection and radiation become ineffective the evaporative cooling renders the most efficient system of heat dissipation through skin and respiratory vaporization. The estimation of pulmocutanous vaporization by the conventional subtraction technique encounters some errors especially under hot climate due to keeping the animals deprived of feed and water for 12-18 hr which would decrease the rate of skin vaporization. In this study it was attempted, as another objective, to use the tritiated water dilution technique in determining the pulmocutanous water loss while providing the animals with feed and water *ad libitum*.

Material and Methods

A. Animals, feed, water and management

Six 3 years old lactating Friesian cows and six 3 years old pregnant cows belonging to ARE USA-NSF Project of "Bovine Adaptation to the Sahara" in the Atomic Energy Authority were used in this study. The animals were fed individually in metabolic cages, a ration consisted of rice straw, pelleted concentrates. The concentrates consisted of undecorticated cotton seed cake 60%, wheat bran 10%, rice milling 25%, limestone 2%, sodium chloride 1% and molasses 2%. The concentrates and roughages were given to the animals according to their body weight and milk yield, as recommended by Morrison (1957). They were watered freely *ad libitum* using automatic water bowls. The animals were stanchioned on the cages for 10 days in the shade. The minimum and maximum averages of ambient temperature at 6 a.m. and 4 p.m. ranged from 25-33°. Urine was separated from feces partially by plastic seive through which the urine passed to a plastic container.

B. *Experimental design and statistical analysis*

6 dry pregnant cows and 6 late lactating cows were used in the present study. The goats were maintained in the climatic chamber laboratory for 7 days, 7 hr daily at 18° and 65% R.H. followed by 7 days, 7 hr daily at 38° and 50% R.H. The raise of temperature in climatic chamber was gradually and reaches to the maximum point (38°) of temperature through one hour. During such time live body weight measurements were carried out before eating and watering on the 1st and on 7*th* days in each of mild and hot climates.

The components of water metabolism were determined by the TOH dilution and the conventional techniques. The physiological parameters which were measured consisted of total body water, water turnover rate, biological half time of TOH, total body solids and heat tolerance coefficient. Milk and urine samples were used for total body water and water turnover rate determinations by extrapolation technique. The differences between the averages of the two treatments were tested by means of the "t" test of significance, according to Snedecor and Cochran, (1967).

C. Tracer techniques for the estimation of water metabolism

1. *Total body water (TBW)*

a. *Radiation dose preparation* : Tritiated water (TOH), in which one hydrogen atom is replaced by radioactive tritium was used for determining total body water and water metabolism in this study. The radioactive tritiated water was purchased from the Radiochemical center Amersham, Buckinghambshire, England.

b. *Dose injection and sampling* : The animals were deprived of water and feed for about 12 hr after which TOH was injected intravenously in jugular vein on the first day of the mild climate and on the 2*nd* day of the hot climate exposure with a dose rate of 1 mc at 18° and 2 mc at 38° 100 kg body weight as described by Kamal and Johnson (1971). The second dose was higher so as to minimize the residual activity of the first dose. Following each dose seven blood samples were collected from the opposite jugular vein in vaoutainer test tubes containing 2 drops of 30% Na_2EDTA (Ethylenediaminetetra acetic acid disodium salt) as an anticoagulant. Blood, urine samples were collected during the 7 days of each climatic exposure. All samples were stored frozen at 18° in order to determine the total body water as described by Kamal and Seif (1969).

c. *Counting of radioactivity* : One ml aliquot of plasma and urine was transferred into the liquid scintillation counting vial which contained 10 ml of the scintillation fluor solution. The teagents of the fluor solution consisted of : 0.1 g POPOP (2, 2-p-phenylene bis (5-phenyloxazole) 4 g PPO (2, 5-diphenyloxa-zole) and 60 Naphthalene, which were dissolved in 1 L, 4-dioxane. The dioxane is a primary solvent which the particles of the ^3H atoms transfer their energy. The POPOP acts as wavelength shifter to change the solvent energy to photons of a shorter wavelength. The naphthalene increases the efficiency of dioxane. The vials were stored in complete darkness overnight as 4° into the regregerating cabient of the automatic liquid scintillation spectrometer and were counted between 0.2 and 9.9 on the energy dial, thus covering practically the whole tritium spectrum.

An aliquot of the dose solution was diluted to (1:1000) in distilled water and 0.1ml of this diluted standard solution was transferred into the vial which contained 10 ml fluor mixture. Each sample was counted for one minute, after which, 0.1 ml of tritiated water standard was added to each vial using an

automatic pipette. All vials were thereafter counted again for one minute. This second counting was used for internal standardization process. The samples and internal standard, however, were made in doublicates. The value of using an internal standard was to correct for the quenching due to colour, chemical composition and physical strucutre of the sample. The corrected activity was plotted on a semilogarithmic grid to obtain the activity of plasma milk or urine samples at zero time of dosing by using the least square method. The zero time activity was then used in calculating body water.

d. Calculation of results

$$TBW = \frac{S \times V \times D}{A \times 1000}$$

TBW = Total body water (L).

S = Standard net activity (cpm/ml).

V = Administered dose volume.

D = Standard dilution factor.

A = Plasma, milk or urine activity at zero time.

2. *Water intake*

Water intake as determined by tracer technique *i.e.*, water turnover rate. Water turnover rate was determined from the tritiated water exponential disappearance constant and total body water in the animals as follows :

$$WTR = TBW \times 24 \text{ hr}$$

$$= 0.693/T_{1/2}$$

$T_{1/2}$ = Biological half life time of tritiated water.

Water intake as determined by the conventional technique

a. Free water : The water provided to the animals in buckets was weighed before and after being offered to the animals.

b. Metabolic water : The amounts of metabolic water were estimated based on the assumption that 1 mg of digestible protein, fat and carbohydrate gave 0.4, 1.07 and 0.6g of metabolic water, respectively (Leitch and Thomson, 1944).

c. Feed water : The samples of concentrates, hay and straw were analyzed for water, the samples dried at 105° for 24 hr in a 250 g glass weighing bottle, and water content was calculated as percent of fresh weight.

3. *Water output*

a. *Urinary water* : The urine was collected daily quantitasvelyusing polyethelene funnels and the samples were taken for water analysis.

b. Fecal water: Feces were collected daily quantitatively using hind bags made of polyethelene. The funnels and hind bags were attached to the animals using harness. A composed sample of 7 days was collected from each animal. The samples were dried at 105° for 24 hr, in a 250 g glass weighing bottle and water content was calculated as percent of fresh weight.

c. *Pulmocutaneous water loss* : Pulmocutaneous water loss as determined by water turnover rate using tritiated water dilution technique =

Water turnover rate-(urinary water + fecal water) pulmocutaneous water loss as determined by the conventional technique =

Total water intake - (urinary water + fecal water).

In this technique it is assumed that, as long as the live body weight of the animal is constant, then, total water intake must equal the water output (Temor *et al.*, 1969, Wilson, 1970, Maloiy and Taylor, 1970 and Maloiy, 1972).

d. Respiratory vaporization water : The method that used for measuring the water output from the respiratory tract by evaporation and suitable for use on animals confined in climatic chamber has been described by Kibler and Yeck (1962).

This method is adaptable for either sheep or goats and readings are taken over a short period (generally not more than ten minutes) (Yeats *et al.*, 1975).

Respiratory water loss = apparatus weight (Mask + Tubes + Calcium chloride battary) after measuring-apparatus weight before measuring. Respiratory water output was measured twice during heat exposure period (7 hr daily).

e. Skin water loss : Skin water output by water turnover rate using tritiated water dilution technique = Water turnover rate - (Urinary + fecal water + respiratory water).

The general equation of skin water output by the conventional technique is as follows :

1. Skin water output = Total water intake - (urinary water + fecal water + respiratory water output).

Under hot conditions, the equation of skin water output is as follows :

2. Skin water output = Total water intake - (Urinary water + fecal water + respiratory water + retained water).

4. *Water balance*

Water balance by tracer technique = Water turnover rate - (Urinary water + fecal water + respiratory water + skin water).

5. *The retained water, l/day* Water retention =

$$\frac{\text{(Total body water, L at 38°)-(Total body water, L at 18°)}}{\text{Heat exposure period (days)}}$$

(D) *Tracer technique for estimation of heat Tolerance coefficient (HTC)*

This technique was carried out on goats based on the estimation of total body solids (TBS) under mild and hot climates, then the percentage decrease in total body solids content due to heat exposure was subtracted from 100 to obtain the coefficient of heat tolerance as described by Kamal and Johnson (1971) in cattle. The total body solids estimated by subtraction the total body water (TBW) from the live body weight in each of climatic conditions. The equation used for the TBS HTC is as follows :

$$\text{TBS HTC} = 100 - \frac{\text{TBS\% at 18°-TBS\% at 38°}}{\text{TBS\% at 18°}} \times 100$$

Results and Discussion

A) TOH dilution and conventional techniques for determining total water intake and Pulmocutaneous and Skin water loss

Feasibility of the water turnover rate technique for water balance studies, emerges from the fact that tritiated water as a tracer marker is distributed uniformly in the water pool of the body after equilibrium is attained and behaves physiologically as ordinary water (Ernest and Pinson, 1952; Best and Taylor, 1966).

Table 1 shows that total water intake average of dry pregnant and lactating cows 27·25 ± 0·33 and 34·21 ± 1·88 L/day respectively. Whereas water turnover rate averages were 28·78 ±0·49 and 35·96 ± 1·39 L/day, respectively. The difference between the two averages was significant ($P < 0.05$).

It is worthnoting, however, that water turnover rate overestimated total body water by 5·61 - 5·11%. This can be mainly attributed to the fact that tritiated water space overestimates total body water (Pace and Schachan, 1947; McManus and Prichard *et al.*, 1969) because the tritium atom of the tritiated water exchanges with exchangeable hydrogen atoms present in the body. The rapidly exchangeable hydrogen atoms in the organic constituents of body are estimated to correspond to a water equivalent 0·5 to 0·2% of the body weight in man (Heresy and Jacobson, 1940). For carbohydrates 6% of carbohydrates is hydrogen and that 35% of hydrogen is exchangeable (Boxer and Steeten, 1944). The rapidly exchangeable H atoms in fat are assumed negligible in amount.

TABLE 1. TOH dilution and conventional techniques for determining total water intake and pulmocutaneous water loss.

Dry pregnant cows		Late lactating cows		Pulmocutanous water loss/1/day			
Conventional techniques L/day	TOH techniq.-ues L/day	Convent-ional techniques L/day	TOH techniq-ues L/day	Jn day pregnant convention-al tecnique	cows ToH technique	In lactating Conventional techniques	cows TOH technique
X- 27.25	28.78	37.21	35.96	11.47	12.40	16.37	18.12
SE 0.33	0.49	1.88	1.39	1.00	0.98	2.08	1.58
C.V. 2.98	4.19	13.51	9.50	21.44	19.51	31.18	21.39
"T" 3.46*		3.30*		3.99*		3.28*	

*Significant at ($P < 0.05$)

The comparison between total water intake and water turnover rate have been studied by many investigators. Baiely and Broster (1958) found that water turnover rate of grazing animals was similar to water consumption recorded by them in dairy heifers well fed in stalls. Water turnover rate was also similar to the water consumption of grazing Holstein X Zebu cows in Jamica (Wilson and Barrat, 1962). In three sheep, which were accustomed to consume water containing 1·3 salt the daily water intakes were 8·3, 10·84 and 8·35 L/day as compared to water turnover rate of 8·26, 10·6 and 8·40 L/day, respectively, (Potter and Jones, 1970).

In this connection, water turnover rate was similar to or, slightly above the amount of water consumed from the trough. This is consistent with a small of moisture from the vegetation (Brown and Lynch, 1972). Moreover, the calculated water entry rate values were comparable to the corresponding estimate water consumption in sheep by Kamal (1977) who stated that water consumption average was 3·79 $\pm$0·14. Also El-Fouly *et al.* (1979) found that total water intake L/day was 2·71 compared with water turnover rate that was 2·67 L/day.

Pulmocutaneous water loss was measured by subtraction technique which depend on the subtraction of output sensible water loss (urinary and fecal water from total water intake (Free, metabolic and feed water), (Temor *et al.*, 1969), Wilson, 1970 and Maloiy, 1972). This was based on the assumption that water output equals water intake when the animals are in a steady state with a constant body weight.

The tritiated water turnover rate as a new technique proposed and used in this study for estimating the pulmocutaneous water loss measures the rate of water output whether or not the animal is in a steady state, *i.e.* whether or not retaining water in its body.

The difference between water turnover rate and urinary and fecal water output represented the pulmocutaneous water loss, where :

Pulmocutaneous water loss =

Water turnover rate - (Urinary water + fecal water).

If respiratory water loss is estimated and subtracted from the pulmocutaneous water loss, the skin water loss can be obtained :

Skin water loss = Pulmocutaneous water loss-respiratory water loss

= Water turnover rate-(Urinary water + Fecal water + respiratory water loss).

Data presented in Table 1 shows that pulmocutaneous water loss averages as L/day estimated by conventional technique in dry pregnant and lactating cows were 11.47 $\pm$ 1.00 and 16.37 $\pm$ 2.08 respectively, whereas those as estimated by tritiated water dilution technique were 12.40 $\pm$ 0.98 and 18.12 $\pm$ 1.58 respectively. The difference between the averages of the two techniques was found significant ($P<0.05$).

From the above results it is indicated that tritiated water dilution technique give higher averages for the pulmocutaneous water loss because this technique depends on estimating the disappearance rate constant of TOH and the tritiated water space. The latter as mentioned previously overestimates total body water (Pace and Schacham, 1947: McManus and Prichard, 1969).

The averages in the present study, however, are in accordance with those found by Kibler and Brody, (1950),

Kellaway and Colditz (1975) and Siebert *et al.*, (1978) for pulmocutaneous water loss as estimated by other different techniques.

B) Plasma, milk and plasma, urina activities after dosing the tritiated water in animals

Comparisons between plasma and milk net activity at 24, 48, 72, 96 and 120 hr after dosing the tritiated water in lactating Friesian cows are presented in Table 2. The average for plasma net activity was 970 $\pm$ 150 cpm/ml increased significantly ($P<0.01$) in milk to 1276 $\pm$167 cpm/ml. Milk was higher than plasma net activity by 33.55%.

The differences between plasma and milk net activity were attributed to the fact that part of the energy in different biological samples may be dissipated without production photons. Such loss in activity is known as quenching (Horrocks, 1964). This quenching is due to the differences in chemical composition colour and other physical properties of these fluids. For estimating the quenching value for all samples, 0·1 ml of tritiated water internal standard was added to the 1ml of plasma and milk samples and the samples activity was corrected as described by Kamal and Seif (1969). Moreover, the facility of the internal standard addition process is the only limitation of this method, which involved correction for all types of quenching (Stubbs, 1973).

The averages of quenching were 2·15 $\pm$ 0·06 and 1·63 $\pm$0·03 for plasma and milk samples, respectively, the differences were found significant ($P > 0·01$) as shown in Table 2.

The corrected sample activities cpm/ml were 2159 $\pm$ 333 and 2307 $\pm$ 357 in plasma and milk, respectively (Table 2).

The higher activity of milk than plasma was due to the fact that the milk was synthesized at earlier time from plasma of a higher activity than that used for counting.

The previous discussion revealed that there are no significant differences between corrected sample activity for plasma and milk so, milk samples can be used for total body water and water turnover rate determinations by extrapolation technique.

The average $\pm$ SE for TBW in Friesian cows was 224·68 $\pm$ 5·84 L by using plasma samples decreased to 221·58 $\pm$ 5·27 L by using milk samples because milk was higher than plasma corrected activity by 6·5%. When the values of TBW were expressed as L/100 kg LBW, the averages were 74·78 $\pm$ 0·61 and 73·77 $\pm$ 0·27 by plasma and milk samples, respectively and the difference was found to be insignificant. The averages of WTR as L/day were 36·16 $\pm$ 0·26 and 35·30 $\pm$ 0·83 that estimated by using plasma and milk samples, respectively, (Table 3).

The results that mentioned above were associated with those revealed by Aschbacker *et al.* (1965) who found that TBW estimated from tritium content of plasma, urine and milk was 71·9, 73, respectively, when they were expressed as percent of live body weight.

The "t" test of significance indicated that milk samples in Friesian cows has insignificant effect on WTR determination when they were compared with plasma samples. This was due to the fact that tritiated water disappearance rate constant (- K x 10^{-3}) (Table 4) and biological half life time of time of tritiated water that obtained from plasma and milk had same values.

TABLE 2. Activities, quenching and corrected sample activity of plasma and milk after dosing the tritiated water in lactating cows.

Hrs after dosing	N	Plasma net activity cpm/1 ml	Milk net activity cpm/1 ml	Diffe-rence %	Plasma quenching	Milk quenching	Diff-rence %	Plasma corrected cmp/1 ml	Milk corrected cpm/1 ml	Diffrence. %
24	8	1441±127	1747±167	21.23	2.29±0.35	1.78±0.24	22.27	3222±710	3408±854	5.77
48	8	1194±228	1548±367	29.64	2.23±0.23	1.66±0.21	25.56	2542±768	2807±1030	10.42
72	8	860±305	1241±495	44.30	1.95±0.20	1.58±0.22	18.97	2046±778	2129±1012	4.05
96	8	665±285	999±404	50.22	2.04±0.25	1.60±0.23	21.56	1659±742	1753±831	5.66
120	8	692±245	847±432	22.39	2.25±0.64	1.56±0.17	30.66	1328±670	1437±869	8.20
X-		970	1276	33.55	2.15	1.63	23.80	2159	2307	6.82
SE		±150	167		0.06	0.03		±333	±357	
"T"		7.70*			9.47			3.37		

* Significant at (P 0.01).

TABLE 3: Total body water, water turnover rate and biological half life time of tritiated water by using plasma compared with milk samples after injection tritiated water in lactating cows.

	Plasma			Milk		
	X-	SE	C.V.	X-	SE	C.V.
Total body water, ...	224.68	5.84	10.40	221.58	5.27	9.51
1. "b"			0.861			
Total body water, ...	74.78	0.61	3.28	73.77	0.27	1.46
1/100 kg., LBW . . . . . "t"			0.261			
Water turnover rate, . .	36.16	0.26	2.90	35.30	0.83	9.46
1/day . . . . . "t"			0.488			
Half life time, ...	4.23	0.16	10.63	4.27	0.18	11.94
days. . . . . "t"			0.107			

TABLE 4. Tritiated water disappearance rate constant ($-K \times 10^{-3}$) in plasma and milk after intravenous injection in Friesian cows.

Cow's No.	Plasma	Milk
1	7.53	7.21
2	5.92	5.77
3	7.21	7.53
X-	6.88	6.83
E.S.	0.42	0.46
"t"	0.26	

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تقدير الميزان المائى فى ابقار الفريزيان

محمد جمال الدين قمر ، تيمور حسين كمال ، حسن جبر وكمال المصرى

كليتى الزراعة بجامعتى القاهرة والزقازيق وهيئة الطاقة الذرية ، مصر

كانت متوسطات كمية ماء الشرب الكلى للأبقار الحامل والحلابة هى ٢٧ر٢٥ ± ١ر٨٨ لتر/يوم على التوالى – بينما متوسطات معدل استبدال ماء الجسم كانت ٢٧ر٧٨ ± ٤٩ر ، ٣٥ر٩٦ ± ١ر٢٩ لتر/يوم على التوالى – ولقد وجد أن الفرق الحقيقى بين المتوسطات ٠

قدر الماء المفقود عن طريق الجهاز التنفسى وسطح الجلد معا وذلك بالطريقة العادية فى كل من الأبقار الحامل والحلابة ووجد أن المتوسطات على التوالى كانت ١١ر٤٧ ± ١ ، ١٦ر٣٧ ± ٢ر٠٨

أما بطريقة تخفيف الماء المرقم بالترثيوم فان المتوسطات كانت ١٢ر٤٠ ± ٩٨ر ، ١٨ر١٢ ± ١ر٥٨ لتر/يوم على التوالى واتضح أن الفرق بين متوسطات الطريقتين فرق حقيقى وأن متوسط طريقة تخفيف الماء المرقم بالترثيوم كان أعلى من متوسط الطريقة العادية بحوالى ٨ – ١٠٪ ٠

وعند تقدير كمية الماء المفقودة على درجة ١٨°م عن طريق الجلد بالطريقة العادية وطريقة تخفيف الماء المرقم بالترثيوم كانت متوسطاتها ٣٦ر ± ٠٥ر ، ٣٧ر ± ٠٥ر لتر/يوم على التوالى ٠ وعلى درجة ٣٨°م – كانت كمية الماء المفقودة عن طريق الجلد ٦١ر ± ٠٧ر لتر/يوم بالطريقة العادية مقارنة مع ٦٥ ± ٠٧ر لتر/يوم بطريقة تخفيف الماء المرقم بالترثيوم ٠

كان متوسط عدد النشاط الاشعاعى فى البلازما هو ٩٧٠ ± ١٥٠ عدة فى الدقيقة لكل ميلليلتر ازدادت زيادة حقيقية فى اللبن الى ١٢٦٧ ± ١٦٧ عدة فى الدقيقة لكل ميلليلتر ٠

كان متوسطات معامل تصحيح النشاط الاشعاعى ٢ر١٥ ± ٦ر ، ١ر٦٣ ± ٠٣ر ، وذلك لعينات البلازما واللبن على التوالى – واتضح أن هناك فروق حقيقية بين متوسطات معامل التصحيح للسوائل المختلفة وأن اختلاف قيمةم هذا المعامل تعود الى اختلاف التركيب الكيمائى واللبن والخواص الفيزيقية بين كل من البلازما واللبن ٠

وعندما صحح نشاط العينات باستخدام معامل التصحيح تغير العد الاشعاعى الى ٢١٥٩ ± ٣٣٣ ، ٢٣٠٧ ± ٣٥٧ عدة فى الدقيقة فى عينات البلازما واللبن على التوالى ٠

متوسطات ماء الجسم الكلى لكل ١٠٠ كجم من وزن الجسم كانت ٧٤ر٧٨ ± ٦١ر ، ٧٣ر٧٧ ± ٢٧ر ، وذلك باستخدام عينات البلازما واللبن على التوالى، ومتوسط معدل استبدال ماء الجسم كان ٣٦ر١٦ ± ٢٦ر لتر/يوم وذلك باستخدام عينات البلازما انخفضت الى ٣٥ر٣ ± ٨٣ باستخدام عينات اللبن٠