THE IN VIVO DETERMINATION OF BODY WATER SPACE IN CATTLE USING THE TRITIUM DILUTION TECHNIQUE

A. B. CARNEGIE*† and N. M. TULLOH*

Summary

Tritiated water (TOH) space was determined in 26 steers (13 Angus, 13 Jersey) without prior starvation. The animals were killed and desiccated to determine total body water (TBW). The regression equation relating TOH space (Y) and TBW (X) was as follows:

\[ Y = 1.150 X \quad (SE_b \pm 0.009) \]

The regressions of TOH space and TBW on body weight were computed for both breeds. Both sets of equations showed that, at the same body weight, Angus had a higher body water content than Jersey steers.

I. INTRODUCTION

An accurate in vivo method for determining body composition would be useful in many physiological and nutritional investigations. Panaretto and Till (1963) with goats and Panaretto (1963) with sheep and goats have reported good relations between body water space estimated by tritiated water (TOH) and total body water (TBW) estimated by desiccation. However, their animals were deprived of food and water for 48 h before determining TOH space. There is a need for an examination of these relations in animals which have been fed until the start of the TOH space determination. This paper reports an investigation of this type in two breeds of cattle.

II. MATERIALS AND METHODS

(a) Animals and Their Management

The animals used were 13 Angus and 13 Jersey steers. These cattle were aged one to three weeks when bought and were fed milk substitutes up to a weight of 50 kg, after which they were kept in a small paddock and fed pellets and lucerne hay ad libitum.

Pairs of animals, one member from each breed, were killed at the same body weights. Killing weights were distributed at approximately 20% increments within the body weight range 62-355 kg.

(b) Injection with Tritiated Water

A blood sample was taken from each animal immediately before injection. The dose of TOH was prepared on the basis of 1 mc for each 40 kg of body weight and was injected into the jugular vein using the technique of Panaretto and Till (1963). Further blood samples were taken at 6 h and 8 h after injection. Animals

‡ 50kg-100 kg: Barastoc calf starter pellets 18% Crude Protein
100 kg -140 kg: Barastoc calf grower pellets 15% Crude Protein
140 kg+: Barastoc cattle cubes 14% Crude Protein
Supplied by Barastoc Products, 143 Queen Street, Melbourne.

†Present address: Marcus Oldham Agricultural College, Geelong, Victoria.
were not fasted before the injection, but after injection each animal was kept in a sheltered yard without food and water until the 8 h blood sample had been collected.

(c) Assay of Tritium

The TOH was recovered quantitatively from the blood by vacuum sublimation. The specific activity of the TOH was measured in a Packard liquid scintillation counter using three 0.2 ml aliquots of radioactive water in 10 ml of a scintillation liquid containing 3.6 g PPO (2, 5-diphenyloxazole), 0.09 g POPOP (1, 4-bis-(5-phenyloxazolyl) benzene) in 400 ml ethanol and 500 ml toluene. The TOH space was calculated as:

$$\text{TOH space} = \frac{\text{Dose TOH (mc) injected}}{\text{Specific activity of serum water (mc/l) post injection}}$$

(d) Analysis for Total Body Water

The animals were slaughtered as described by Seebeck (1967). Blood was collected, weighed, and a sample was taken to determine water content by desiccation. After slaughter, the digesta was removed from the alimentary tract and the percentage of water in the digesta was obtained by desiccation. Each carcass was sawn down the medial line and quartered between the 10th and 11th ribs. It was assumed that each half of the carcass had the same water content.

The following parts of the animal were used for chemical analysis: head, hide, feet, digestive tract, viscera, left forequarter and left hindquarter. With the exception of the hide which was sampled before freezing, each part was frozen, and minced in a Jeffco cutter-grinder using a die plate with 3/8 in. (9.5 mm) holes. The mince from each part of the animal was mixed by hand and three samples of 300 g were taken for analysis. Losses of weight during mincing were assumed to be water and results were corrected for this loss. TBW was calculated from the sum of water in the carcass, plus the water content of the other parts, plus the water content of the digesta and blood, plus evaporative losses.

The body weight of each slaughtered animal was adjusted to its earlier body weight measured 8 h after injection, assuming that any increase or decrease in weight was due to gain or loss of weight of digesta.

(e) Statistical Analysis

The results were analysed by one-way analysis of covariance (Snedecor 1956). In the regression of TOH space on TBW, the original data were used. However, before computing the relations between TOH space and body weight and TBW and body weight, the data were transformed to logarithms. This enabled Huxley’s (1932) allometric growth equation to be used in the logarithmic form as the basis for these analyses.

III. RESULTS

(a) TOH Space and TBW

The relation between TOH space and TBW is shown in Figure 1. The difference between breeds was not statistically significant. The data were therefore, pooled, and the common regression was obtained. As the intercept on the Y-axis
was not significantly different from zero, it was assumed that the regression should pass through the origin. It was computed in this form, the relation being as follows:

\[ Y = 1.150X (\text{SE}_b \pm 0.009) \]

where \( Y \) = TOH space and \( X \) = TBW. The 95% confidence limits for a casual observation of TOH space were calculated as:

\[ Y_o = bX_o \pm t \left( \frac{1 + X_o^2}{\sum X^2} \right)^{1/2} \text{S.E.} \]

Where \( X_o \) = TBW at casual observation \( Y_o \) of TOH space. At the mean and at the extremes of TBW obtained in the experiment, these limits were as follows:

(i) TBW 46.1 l, \( Y = 1.15X \pm 11.81 \) l.
(ii) TBW 111.4 l, \( Y = 1.15X \pm 12.01 \) l.
(iii) TBW 214.5 l, \( Y = 1.15X \pm 12.51 \) l.

(b) Relations Between Body Water and Body Weight

Table 1 shows the analyses of covariance calculated for TOH space and TBW using body weight as the independent variate.

There was no significant difference either between breeds- or between techniques in the slopes (values of b) of the regression equations.
Table 1

Constants in the regression equations, adjusted means and F-values in the analyses of covariance of TOH space and total body water (TBW) using log body weight as the independent variate (X) in the equation: \( Y = a + bX \)

<table>
<thead>
<tr>
<th>Dependent Variate (Y)</th>
<th>Constants</th>
<th></th>
<th>Adjusted Means* (Anti-log Y, l)</th>
<th></th>
<th>F-Values†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Angus</td>
<td>Jersey</td>
<td>Common</td>
<td>Angus</td>
<td>Jersey</td>
</tr>
<tr>
<td></td>
<td>( a )</td>
<td>( b \pm \text{S.E.} )</td>
<td>( a )</td>
<td>( b \pm \text{S.E.} )</td>
<td>( a )</td>
</tr>
<tr>
<td>Log TOH</td>
<td>0.117</td>
<td>0.903 ± 0.042</td>
<td>0.186</td>
<td>0.858 ± 0.031</td>
<td>0.152</td>
</tr>
<tr>
<td>Log TBW</td>
<td>0.110</td>
<td>0.875 ± 0.016</td>
<td>0.092</td>
<td>0.876 ± 0.016</td>
<td>0.101</td>
</tr>
</tbody>
</table>

†Adjusted to geometric mean body weight (150.7 kg) along the slope of the common regression line.

*With 1,22 degrees of freedom for between slopes and 1,23 for between adjusted means

**P < 0.025    ***P < 0.01.
When compared at the same body weight, the difference between breeds in weight of body water was statistically significant for both techniques, there being more water in Angus than in Jersey steers.

IV. DISCUSSION

(a) TON Space and TBW

If successive determinations in vivo of body composition are required in individual animals, it is desirable that they be obtained with minimal disturbance to the animal and hence to its growth pattern. Therefore, in this investigation food and water were withheld from the steers for only 8 h, i.e. from the time of TOH injection until the 8 h post injection blood sample had been collected. The specific activity of the 6 h samples were not significantly different from that at 8 h, but body weights were not always measured at 6 h and only the 8 h data have been used in the statistical analyses. The specific activity of rumen samples was also measured in two pairs of identical Jersey twin steers being grown under the same conditions as the steers in this experiment. The specific activity of the 8 h post injection blood samples was significantly greater than that of rumen samples collected at the same time (difference: 2.6 ± S.E. 0.5 %, n = 8, P < 0.025). The time post injection, at which the specific activity of rumen and blood samples equilibrated, was not determined.

During the 8 h post injection period, there were losses of TOH in urine, in faeces and in evaporative losses from the skin and respiratory passages. Measurements were carried out over a period of 13 months and environmental temperatures ranged from 10 to 38°C. The percentage difference between evaporative losses at these two extremes of temperature can be assumed to be similar to those observed by Till and Downes (1962), namely 0.450.80% over the 8 h period. A further loss of TOH from the body water would have resulted from exchange with labile hydrogen atoms in various compounds in body tissues. The effect of these losses would have been to lower the equilibrium activity of TOH in the 8 h post injection blood sample, hence leading to an overestimate of total body water by TOH space. Without correction for any losses of TOH, our mean overestimate of TBW was 15 %. Panaretto and Till (1963) and Panaretto (1963) overestimated TBW by 0.8 and 0.4 %, respectively, when values were calculated as percentages of body weight. However, their estimates were made after deducting 3 % to allow for hydrogen exchange and evaporative losses and their animals were without food and water for 48 h before injection and a further 6 h before the equilibrium blood samples were taken. These differences in technique may partly explain the differences between their results and ours.

The apparent accuracy of the technique of Panaretto and Till (1963) and Panaretto (1963) was increased because they used TOH space as their independent variate when computing the relation between TOH space and TBW. We have used TBW as the independent variate on the assumption that it is an accurate estimate of the body water content of animals. It appears that, when animals are not deprived of food and water before estimating TOH space, a substantial overestimate of TBW will be obtained.
(b) Relations between Body Water and Body Weight

The slopes of the common regression equations in Table 1 indicate that the relative growth ratio for body water (for both techniques) was 0.88; that is, as body weight increased body water increased but, as a percentage of body weight, it decreased. The exponential equation used in these analyses provides a convenient way of expressing change in the water content of animals as body weight increases.

Because the slopes of the regression equations were all similar, the difference between Angus and Jersey steers in body water content can be expressed as a constant percentage difference over the body weight range of the experiment. At the geometric mean of body weight, the breed differences (Table 1) for the two techniques were as follows:

<table>
<thead>
<tr>
<th></th>
<th>TOH space</th>
<th>Angus &gt; Jersey</th>
<th>6.8%</th>
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<tbody>
<tr>
<td></td>
<td>TBW</td>
<td>Angus &gt; Jersey</td>
<td>3.5%</td>
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The levels of statistical significance in Table 1 indicate that, although a smaller percentage difference was obtained with TBW, it was more reliable than the difference obtained with TOH space. This breed difference in body water content is an indication of a difference in body composition. The body water content of these cattle will be related to other components of body composition in a later publication.

In spite of the overestimate of TBW given by TOH space, the determination of TOH space can be used as a reliable field technique to study relative changes in growing cattle and relative differences between groups of cattle, in body water content.

V. ACKNOWLEDGMENTS

We wish to thank Mr. H. Moog and Mr. D. Whitfield for technical assistance. This work was financed by a grant from the Australian Meat Research Committee.

VI. REFERENCES


