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UREA SPACE VERSUS TRITIATED WATER SPACE AS AN IN VIVO PREDICTOR OF BODY WATER AND BODY FAT

H.H. Meissner
Animal and Dairy Science Research Institute, Irene

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OPSOMMING: UREUMRUIMTE VERSUS TRITIUMRUIMTE AS 'N *IN VIVO* BERAMER VAN LIGGAAMSVOG EN -VET

Die ureumverdunningstegniek vir die bepaling van liggaamsvog en -vet *in vivo* is vergelyk met die titriumverdunningstegniek in terme van akkuraatheid, gemak van uitvoering, koste en toepassingsmoontlikhede. Altesaam 19 skape is in die studie gebruik waarvan sommige op 'n ruvoerrantsoen gevoer was en ander op 'n kragvoerrantsoen. Ongeveer 100–250 mg/kg ureum en 0,4 mCi tritium in 0,9% NaCl is intraveneus of geïnfuseer of ingespuit. Na bloedmonstering vir ureum- en tritiumanalise is van die skape geslag en opgemaal vir direkte bepaling van liggaamsamestelling. Die resultate dui op die volgende: 1) Liggaamsvog en -vet kon net so betroubaar vanaf ureumruimte beraam word indien monsters geneem is tussen 6 en 10 minute na toediening as met monstering by ekwilibrium; soortgelyk kon liggaamsvog en -vet vanaf tritiumruimte net so betroubaar beraam word indien monstering tussen 14 en 20 minute na toediening geskied het as by ekwilibrium. 2) Liggaamsvog en -vet is akkurater deur die titriummetode as die ureummetode beraam. 3) Die tydsduur geneem tot ekwilibrium van die ureum of titriummolekuul is nie beïnvloed deur die grootte van die spysverteringskanaal nie. 4) Sowel ureum- as titriumruimte bereken by ekwilibrium, het totale vog en nie slegs liggaamsvog (vog in spysverteringskanaal uitgesluit) beraam nie. 5) Die ureummetode is nie so maklik om uit te voer as die titriummetode nie, aangesien ureum in groot hoeveelhede per kateter geïnfuseer moet word (inspuiting was onsuksesvol) terwyl tritium in klein hoeveelhede ingespuit kan word. 6) Die ureummetode is ongeveer 40% goedkoper as die tritiummetode. Die gevolgtrekking is gemaak dat die ureummetode nie voldoende akkuraat is vir kritiese proefwerk nie, maar dat dit toepassingsmoontlikhede het vir liggaamsamestellingberamings by prestasietoetsskemas in die lig van die akkuraatheid wat vereis word, die kostefaktor en die feit dat afgekeurde diere onmiddellik geslag en verbruik kan word, wat nie die geval is met tritium nie weens die wet op radio-aktiewe materiale.

SUMMARY:

The urea dilution technique for the determination of body water and body fat *in vivo* was compared with the tritiated water dilution technique with respect to accuracy, ease of determination, costs, and application possibilities. Nineteen sheep were used in the study, some of which had access to a roughage diet and the other to a concentrate diet. About 100–250 mg/kg urea and 0,4 mCi tritiated water in 0,9% NaCl solution was either infused or injected intravenously. Following blood sampling for urea and titrium analyses some of the sheep were slaughtered and minced for direct determination of body water and body fat. The results showed the following: 1) Body water and body fat could be predicted with similar accuracy from urea space measurement at 6 to 10 minutes post-administration of urea than at equilibrium; similarly could body water and body fat be predicted with the same accuracy from tritiated water space at 15 to 20 minutes following administration than at equilibrium. 2) Body water and body fat could be predicted more accurately with the tritiated water method than with the urea method. 3) The time period necessary for the titrium or urea molecule to equilibrate completely was independent of amount of digesta in the digestive tract. 4) Both urea and tritiated water space at equilibrium measured total water and not merely body water (water exclusive of digestive tract). 5) The urea method is not as easy as the tritiated water method due to the fact that urea has to be infused in large amounts through a catheter (injection was unsuccessful) whereas tritium can be injected fairly easily in small amounts. 6) The urea method is approximately 40% cheaper than the tritiated water method. It was concluded that the urea method is not accurate enough in critical experiments but may be applicated for body composition determinations in performance testing due to its sufficient accuracy, the costs involved, and the fact that the carcases of culled animals can be utilised immediately, which is not the case with tritiated water because of the law on radio-active materials.

The value of the tritium dilution technique as a convenient and probably the most accurate method (Robelin, 1973) of estimating body composition in live animals has been amply demonstrated. This has been shown in rabbits (Reid, Balch & Glascock, 1958), pig-tailed monkeys (Kodama, 1970), rats (Gordon, Topps & Begg, 1971), rats, cats, rabbits, guinea pigs and man (Foy & Schnieden, 1960), man (Done & Payne, 1957), cattle (Aschbacher, Kamal & Cragle, 1965; Carnegie & Tullch, 1968), sheep (Panaretto, 1968; Searle, 1970; Hofmeyr, Olivier, Kroon & van Rensburg, 1971; Meissner & Bieler, 1975), and the pig (Kay, Jones & Smart, 1966; Kemm, 1974). However, the application of the tritium method for practical use with farm animals (for example, performance testing) is less convenient – the main reasons being: 1) the tritium molecule equilibrates relatively slowly in the fluid medium of the body, taking approximately 5 to 8 hours in sheep (Searle, 1970; Meissner, unpublished) and cattle (Carnegie & Tulloh, 1968), 2) standard laboratories have to be specially adapt-

ed to cope with radio-active materials, 3) the carcass of the animal cannot be utilised immediately after experimentation due to legislation on radio-active materials.

Urea may be an acceptable alternative if the measurement of urea space proves to be as accurate and easy as the measurement of tritiated water space as a means of predicting body composition. The urea molecule appears to equilibrate within 1 hour in the dog (Painter, 1940), 15–40 minutes in man (Donovan & Brenner, 1930; San Pietro & Rittenberg, 1953), within 30 minutes in goats (Gärtner, 1961) and only 12 minutes in cattle (Preston & Kock, 1973). Furthermore, urea can be measured accurately and easily in either whole blood or plasma, it has no toxic manifestation, is not foreign to the body and causes no physiological disturbances when administered in sufficiently small amounts. Also, urea is not selectively stored, secreted or metabolized (San Pietro & Rittenberg, 1953) and is excreted sufficiently slowly to allow time for uniform distribution in the body (Painter, 1940). Painter (1940) using dessication, confirmed that the water available for solution of urea is equal to the total water content of the dog body. This was also shown for man by San Pietro and Rittenberg (1953), using deuterium oxide and urea. However, Gärtner (1961) and Preston and Kock (1973) consider urea space in the ruminant to be a measure of empty body water (total body water less the water in the digestive tract) rather than total body water.

The study reported in this paper evaluates the pros and cons of urea space measurement as compared to tritiated water space measurement in terms of accuracy, ease of determination, costs involved, and application possibilities.

Procedure

Animals and treatment procedures

A total of 19 sheep comprising 6 Karakuls (K), 8 South African Mutton Merinos (SAM), 2 Merino types (M) and 3 Mutton x Walrich Merino crosses (W) ranging in body mass between 13 and 80 kg were used. The sheep were given either a pelleted concentrate diet or a roughage diet and were not fasted before administration of the urea and tritium solutions. However, feed and water were taken away during distribution of the test substances through the body. The animals were weighed immediately before administration and after the last blood sample was taken. The mean of the two measurements was taken as representative. Nine animals were slaughtered after blood sampling.

Infusion and blood sampling

Urea and tritium were either both injected intravenously over a 2 minute period using a 20 ml sterilized syringe or infused by means of an indwelling catheter through a 12 gauge needle. The infusion solution contained 50% urea and approximately 2 g tritiated water dissolved in 0,9% NaCl solution. The volume injected was accurately weighed; the quantity of urea injected approximated 100–250 mg/kg body mass whereas the activity of the tritium solution averaged 0,2 m Ci/g. Jugular blood samples were taken prior to and at varying times after administration and stored at 4°C when immediate analysis was possible or at −20°C (plasma) when this was not convenient.

Sample analysis

Blood samples obtained for urea analysis were centrifuged and the plasma analysed for urea –N according to the automated method of Marsh, Fingerhut and Miller (1965) (Technicon Auto Analyzer, Model 1). Blood samples obtained for tritium analysis were counted by means of liquid scintillation, adopting the procedure as described by Meissner and Bieler (1975). The slaughter procedure of the animals and analysis for body water and body fat are described by Hofmeyr, Kroon, van Rensburg & van der Merwe (1972).

Calculation and statistical treatment

The apparent urea or tritiated water space was calculated from the ratio of urea or tritium injected to the concentration at equilibrium, or at various other stages prior to equilibrium, after correction for the pre-injection values. No correction was applied for losses of urea or tritium that might have occurred during blood sampling. The accuracy of the methods was determined using standard linear regression analysis.

Results

Equilibration period

Figure 1 illustrates a typical time-equilibration curve for urea and tritium in the fluid medium of the

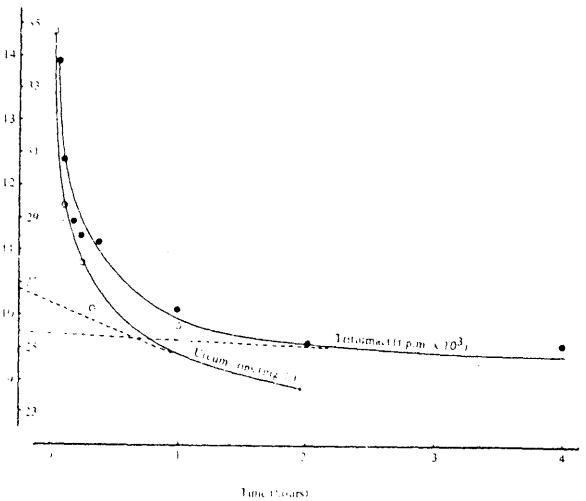

Fig. 1. – *A typical time-equilibration curve for tritium and urea in the fluid medium of the sheep body*

Table 1

Urea and tritiated water space as measured after 20 minutes or 6 hours following administration, in comparison with empty body water and total body water

No. of sheep	Urea space (kg)	TOH space (kg)	EBW (kg)	TBW (kg)
SAM 1	39,41	40,49	27,84	39,61
SAM 6	14,65	14,54	11,27	14,88
K 2	29,95	30,32	21,59	30,25
SAM 7	15,90	18,27	13,58	17,53
SAM 3	31,61	33,99	25,81	33,79
SAM 8	11,67	11,64	8,18	11,57
K 4	31,04	36,02	25,00	34,84
K 5	21,83	30,13	19,57	29,60
SAM 9	17,16	17,19	13,28	16,20

sheep body. By extrapolating the straight portion of the time-equilibration curve back to zero-time as in Figure 1, the concentration of urea or the activity of tritium if complete equilibrium was achieved immediately following administration, can be estimated. The concentration and/or activity thus arrived at corresponds with the concentration and/or activity with blood sampling at about 14 to 20 minutes followed urea administration or at approximately 4 to 6 hours following tritium administration as is shown in Figure 1. Presumably, this corresponds with the point of complete equilibrium. Urea or tritiated water space (TOH) calculated at the point of equilibrium, estimates total body water (TBW) and not merely empty body water (EBW) as is shown in Table 1.

The stage when urea distribution reached complete equilibrium varied however, among animals. For most, equilibration was complete between 14 and 20 minutes following administration, with 20 minutes giving the most accurate relationship between urea space and TBW. Yet, in the case of sheep K5 equilibrium was only achieved after about 1 hour following administration. This variation in the distribution did not occur with tritium, (at 6 hours tritium is completely distributed) which probably explains why TOH space estimated TBW more accurately than urea space as is illustrated in Table 2.

Table 2

Prediction equations and the accuracy in terms of r^2 and Sy.x of the relationship between TBW (Y) and Urea space (X) or TOH space (X) in kg

X	Prediction equation	r^2	Sy. x
Urea space	Y = 1,04X + 0,75	0,933	2,86
TOH space	Y = 0,98X + 0,04	0,998	0,49

Table 3

Urea space (Y) measured at 15 min. and TOH space (X) measured at 6 hours post-administration, together with the regression equation

No. of sheep	TOH space (kg)	Urea space (kg)	Regression equation r^2, and Sy. x
W1	10,10	9,31	
W2	15,54	13,00	
SAM 6	16,29	15,78	
M9	15,89	14,74	Y = 0,97X - 1,19
M8	22,48	21,03	r^2 = 0,992
W4	23,39	19,53	Sy.x = 1,15 kg
K12	32,13	29,74	
K11	35,81	32,99	
K13	41,73	38,58	
SAM 14	46,16	45,24	

The time for complete equilibration does not seem to be influenced by the amount of digesta present in the digestive tract. The data depicted in Tables 1 and 2 are from sheep fed on a roughage diet, thus having large digestive tract contents. None of the sheep fed on a concentrate diet (small digestive tract contents) were slaughtered, but the relationship between TOH space and urea space measured at equilibrium should theoretically be nearly the same as those illustrated in Table 2, since both estimate TBW. The relationship between TOH space measured at 6 hours following administration and urea space measured at 15 minutes following administration was the closest corresponding in terms of having a slope close to 1,0 and accuracy of fit. The time taken for complete distribution thus appear to be of the same order. TOH space values at 6 hours and urea space values at 15 minutes for the sheep on the concentrate diet together with the regression equation are illustrated in Table 3.

Estimation of EBW

Estimation of EBW from urea space or TOH space was most accurate in terms of deviation from regression (Sy.x) when X and Y were expressed in percentage units. However, a tendency for the estimation of EBW from TOH space to be more accurate when X and Y were in kilogram than the estimation of EBW from urea space and *vice versa*, was observed. Thus, regression equations between EBW and urea space or TOH space were compiled using both percentage and kilogram. The most accurate of these prediction equations and the time when blood was sampled following administration of urea and tritium are shown in Table 4.

If the prediction equations of X and Y in kilograms are considered together with those in percentage units, it is evident from the Sy. x column in Table 4 that TOH space and urea space estimated EBW with similar accuracy. The most interesting aspect in Table 4,

Table 4

The prediction equations and their accuracy of estimating EBW (Y) from TOH space (X) or urea space (X) at variou. stages post-administration

X	Time (min.)	Prediction equation	r^2	Sy.x (kg)
TOH Space	360	Y(kg) = 0,67X (kg) + 1,17	0,98	1,08
	360	Y(%) = 0,77X (%) − 3,17	0,67	1,23
	14	Y(kg) = 1,06X (kg) − 1,41	0,97	1,25
	14	Y(%) = 0,51X (%) + 21,26	0,83	0,88
	20	Y(kg) = 1,07X (kg) − 2,44	0,99	0,89
	20	Y (%) = 0,44X (%) + 24,16	0,74	1,10
Urea space	20	Y (kg) = 0,72X (kg) + 1,34	0,95	1,68
	20	Y (%) = 0,48X (%) + 17,44	0,61	1,35
	6	Y(kg) = 1,02X(kg) + 0,70	0,93	1,97
	6	Y (%) = 0,57X (%) + 20,75	0,86	0,79
	10	Y (kg) = 0,85X (kg) + 1,11	0,94	1,79
	10	Y (%) = 0,54X (%) + 17,88	0,83	0,88

*The Sy.x in kilogram for regression equations with X and Y in percentage units is the approximate value at a mean body mass of 41,96 kg

however, is that within TOH space prediction equations and within urea space prediction equations, EBW could be predicted with similar accuracy from TOH space measurement at 14 to 20 minutes post-administration and from urea space measurement at 6 to 10 minutes following administration than from measurement at equilibrium, i.e. at 360 minutes for tritium and 20 minutes for urea. Urea space measured at 6 minutes post-administration differed with a mean value of 16,4 ± 4,6% from urea space measured at 10 minutes following administration, whereas the corresponding differences between 14 and 20 minutes for TOH space measurement was only 4,3 ± 4,1% . Thus by measuring urea space at any stage between 6 and 10 minutes a larger error in EBW estimation would be expected than by chance measurement of TOH space between 14 and 20 minutes following administration.

Estimation of body fat

Urea space and TOH space, as a percentage of body mass, gave the most accurate regression equations with body fat as a percentage of body mass, the kilogram equivalent being less accurate. The sheep listed in Table 1 were also used here. Table 5 illustrates the regression equations and accuracy of estimation from TOH space measurement at 14,20 and 360 minutes post-administration and from urea space measurement at 6,10 and 20 minutes following administration.

All three prediction equations with TOH space as illustrated in Table 5, are evidently more accurate than the three equations with urea space as X. Within TOH space equations and within urea space equations, as was noticed with EBW, TOH space measurement at 14 or 20 minutes post-administration proved to be as accurate as TOH space measurement at equilibrium (360 minutes). The same applied to urea space measur-

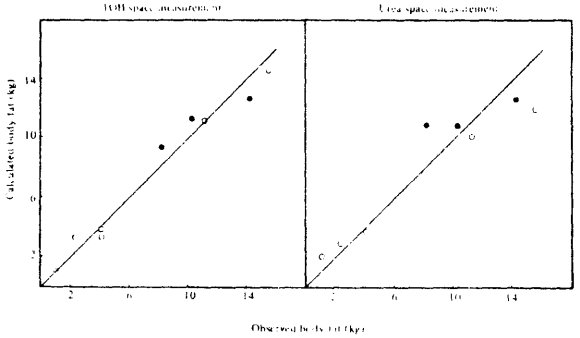

Fig. 2 – *The relationship between observed and calculated body fat from TOH space and urea space measurement respectively*
S.A. Mutton Merino : ○
Karakul : ●

Table 5

The prediction equations and their accuracy of estimating body fat (Y) from TOH space (X) or urea space (X) at various stages post-administration

X	Time (min.)	Prediction equation	r^2	Sy.x (%)
TOH space (%)	360	Y = 82,96 – 1,05X	0,79	2,90
	14	Y = 44,48 – 0,58X	0,69	3,53
	20	Y = 44,77 – 0,57X	0,81	2,80
Urea space (%)	20	Y = 47,45 – 0,52X	0,46	4,67
	6	Y = 40,14 – 0,53X	0,49	4,55
	10	Y = 44,65 – 0,55X	0,54	4,32

ed at 6 or 10 minutes after administration in comparison to measurement at equilibrium (20 minutes). A more realistic picture of the size of prediction error involved than by merely evaluating the Sy.x as in Table 5, is achieved by plotting the observed body fat values against the calculated body fat values. For this purpose the 20 minute prediction equation of TOH space and the 10 minute prediction equation of urea space were used. The results are illustrated in Figure 2.

Discussion

Method of administration

The most reliable results with urea space measurement were achieved when urea was infused through a catheter. By injecting the substance, the method proved to be less successful, mainly because it was not always possible to prevent the animal's head from moving during the period of approximately 2 minutes which was necessary to inject about 10 to 30 g into the jugular vein. Movement of the head could result in the injection needle being forced out of the jugular vein and the consequent failure of the substance to be quantitatively introduced into the bloodstream.

Preston & Kock (1973) used a 20% urea in 0,9% NaCl infusion solution, whereas a 50% solution was used in the present study which enabled the infusion or injection of only 40% of the volume they had to introduce when similar amounts of urea were administered. This would conceivably aid to the success of injection because it can shorten the period of administration. The actual amount administered varied between 100 and 250 mg per kg body mass. More reliable results were obtained with about 200–250 mg per kg than with 100 mg per kg because it raised the blood urea concentration to a great extent. Urea space is calculated by the ratio between the concentration of urea injected to the difference between pre- and post-administration concentrations. The larger this difference, the smaller the error between samples will be and thus the more reliable the calculation according to the theoretical example presented in Table 6.

Administration of urea by injection was tried in view of the fact that less labour is involved and because catheterization for the purpose of infusion is sometimes inconvenient when body composition determinations are frequently needed, say at weekly intervals.

In comparison, the administration of tritium presents no problem since it can be injected with neg-

Table 6

Reliability of calculation of urea space from duplicate blood samples which differ 1% from each other if 100 mg urea/kg body mass was administered or when 250 mg urea/kg body mass was administered

Amount administered	Pre-administered urea concentration	Post-administered urea concentration	Difference between pre- and post-admin.	Percentage influence in calculation
100 mg/kg	20 mg %	25 mg %	5 mg %	4%
	20 mg %	25,2 mg %	5,2 mb %	
200 mg/kg	20 mg %	35 mg %	15 mg %	2%
	20 mg %	35,3 mg %	15,3 mg %	

ligible error. The amount of tritiated water to be injected in the sheep can usually be limited to about 2 g which can be introduced fairly rapidly.

Costs of determination

Following blood sampling, the methods for determining TOH space and urea space are similar with respect to time taken, labour involved, and accuracy of method. However, the cost of determination differs. The urea method is about 40% cheaper than the tritiated water method. In this laboratory the cost of the urea method is approximately R0,80 per urea space measurement in comparison to the R1,10 per TOH space measurement.

Equilibration period

At equilibrium, both urea space and TOH space measured TBW and not EBW as was suggested for urea space by Gärtner (1961) and Preston and Kock (1973). Admittedly, the difference between TBW and EBW is less pronounced on a concentrate diet, which they probably used in their experiments, than on a roughage diet. However, by suggesting urea space to be a measure of EBW at equilibrium it is implied that the urea molecule is distributed selectively between the fluids of the body and the gut water. The only other explanation for the conclusion these workers came to is that complete equilibrium was not achieved.

One of the major shortcomings of the tritiated water method is its exceptionally long equilibration period. Blood sampling for TOH space measurement between 14 and 20 minutes post-administration in this study, however, proved to be no less accurate in predicting EBW as illustrated in Table 4, or body fat as illustrated in Table 5, than TOH space measurement at equilibrium. Thus blood sampling at 14 to 20 minutes post-administration of tritium would seem to be appropriate for routine body composition studies with sheep.

Although equilibration seemed to be complete between 14 and 20 minutes post-administration of urea, the actual point of equilibrium varied considerably among animals as illustrated in Table 1 (compare K5). Thus blood sampling at this stage for urea space determination is about as uncertain as blood sampling at any other stage before 14 minutes post-administration. In view of this observation and the fact that body composition could be estimated with similar accuracy when blood was sampled between 6 and 10 minutes post-administration (Tables 4 and 5), urea space measurement at this stage may be considered for future studies.

Reliability of method

EBW and body fat could be estimated more accurately using the tritiated water method than when using the urea method. This should necessarily also apply to total body composition. Since body fat is the most variable of all the components of body composition (Meissner, de la Rey, Gerhard & van der Westhuizen, 1976), the accuracy with which it can be estimated usually determines the success or failure of the *in vivo* method employed. The estimation of body fat from a single TOH space measurement may comprise an error of about 3,1% of body mass (Sy.x column, Table 5), whereas the same estimation from a single urea space measurement may induce an error of about 4,5%, which implicates the urea method to be approximately 45% less accurate than the tritiated water method for this purpose. Yet, at a large body fat content this difference in reliability of method is less significant, since the error of determination declines with increasing body fat content as is shown by the theoretical example in Table 7.

Apparently the only comparable work in the literature which related urea space with body fat, is that of Preston and Kock (1973). Unfortunately they only gave the correlation coefficient of the equation between urea space as a percentage of body mass and body fat percentage and not the error of estimate. However, by re-analysis of their data the Sy.x value appeared to be approximately 2,2% of body mass when the data of the two steers omitted due to "greater gastro-intestinal fill", were not included, and about 3,8% when these were included. Both estimations were evidently less than the error found in this study (Table 5). Greater gut fill would be expected in this study since the animals were fed on a high roughage diet and were not fasted beforehand as in the case of the abovemen-

Table 7

The influence of increasing body fat content on the magnitude of the error involved when predicting body fat from TOH space and urea space measurement

Body mass (kg)	Predicted fat (kg)	Error (kg) from:		Error as % of fat	
		TOH space	urea space	TOH space	urea space
20	2,0	0,62	0,90	31	45
40	6,5	1,24	1,80	19	28
70	17,0	2,17	3,15	13	19

tioned steers. Therefore it would seem that much of the variation which contributes to the error of estimation is related to variation in gut fill.

This conclusion is supported by observations with TOH space measurements. Meissner and Bieler (1975) found an error of estimate of body fat from TOH space of 1,8 to 2% of body mass. Their sheep were fed on a concentrate diet and in addition were fasted beforehand, which probably explains the smaller error than in this study. In another study (Meissner, unpublished) an increase in error of estimate of body fat from TOH space of nearly 70% was found from measurements on a roughage diet in comparison to measurements on a concentrate diet.

It can therefore be concluded that both methods for estimating body composition *in vivo* considered here, would be more reliable when the animals are fed on a concentrate diet than when fed on a roughage diet.

Implementation of method

Since the urea method is less accurate than the tritiated water method, it cannot substitute the latter in critical body composition studies. The urea method would also be less convenient in studies involving frequent determinations of body composition due to the fact that urea must be infused.

However, bearing in mind that: 1) the urea method would probably be more reliable on a concentrate diet; 2) at larger body fat content the urea method is more accurate than at less body fat and 3) due to the cost factor, the urea method might be a proposition in performance testing, especially also since the carcasses of culled animals can be utilised immediately after the test period.

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