EVALUATION OF TECHNIQUES FOR ESTIMATING MILK PRODUCTION BY SOWS
1. DEUTERIUM OXIDE DILUTION METHOD FOR ESTIMATING MILK INTAKE BY PIGLETS

S. Prawirodigdo[1], R.H. King[2], A.C. Dunkin and H. Dove[3]

School of Agriculture and Forestry, Melbourne University
Parkville, Vic. 3052, Australia

Summary

Two experiments were conducted to determine the validity of the deuterium oxide (D_2O) dilution method to estimate milk intake by pigs. A total of 39 piglets weaned from their dams 24 – 36 hours after birth and trained to drink from artificial nipples, were used for two experiments. Estimates of milk replacer consumption of individual piglets over 1, 3, 5 and 7 days were made by the D_2O dilution method and by dissappearance. Milk intakes estimated by this D_2O dilution method were significantly correlated (R^2 = 0.98-0.99) with milk intake measured by disappearance. The average difference between estimates by disappearance and estimates by the D_2O dilution method were –1%, +0.5%, +0.5% and –2.1% for measurement period of 1, 3, 5 and 7 days respectively.
(Key Words: Deuterium Oxide, Milk Intake, Piglets)

Introduction

The accurate determination of the amount of milk produced by lactating sows is very useful in investigations into their nutrition and physiology. The traditional method for estimating milk consumption by piglets, the weigh-suckle-weigh (WSW) technique, has been widely accepted (Lewis et al., 1978). However, this technique has been reported to have several disadvantages. Weighing the litter before and after suckling is labour intensive (Hartman et al., 1962; Mahan et al., 1972), possibly inaccurate because of piglet weight losses due to defaecation, urination (Van Spaendonck and Vanschoubroek, 1964; Yang et al., 1980) and also as a results of water evaporation and metabolism during the nursing bout (Klaver et al., 1981; Noblet and Etienne, 1986). It may also disrupt the normal maternal-offspring relationship (Pettigrew et al., 1985); agitation in the sow may prohibit normal milk ejection, and the vigor with which the piglets suckle may also be affected.

An alternative method for estimating milk consumption is the deuterium oxide (D_2O) dilution method. Deuterium oxide becomes uniformly distributed in total body water and can be used to estimate total body water in piglets (Rudolph et al., 1988). Milk intake can be calculated knowing the proportion of water derived from milk and the extent to which body water is diluted after milk intake provided that the only water consumed is that in milk.

The purpose of this study was to determine the validity of the D_2O dilution method to estimate milk intake by pigs by comparing the estimates derived from the D_2O dilution method with accurate physical measurement of the milk intake of artificial-reared pigs.

Materials and Methods

Animals

Twenty-four and 15 Large White piglets were used in experiment 1 and 2 respectively. In these experiments piglets were removed from their dams 24 – 36 hours after birth and were reared artificially on a liquid milk replacer. Neomycin

[1] Address reprint requests to S. Prawirodigdo, Research Institute for Animal Production, P.O. Box 123 Bogor, Indonesia.

[2] Animal Research Institute, Werribee, Vic. 3030, Australia.

[3] Division of Plant Industry CSRIO, P.O. Box 1600, Canberra, Act. 2601, Australia.

Received December 22, 1989.

Accepted April 3, 1990.

(Neobiotic, Upjohn) was added to the milk of animals exhibiting clinical diarrhoea, for a period of 2 – 3 days as a precaution againts the possible development of bacterial infection.

The piglets were individually housed in wire mesh cages (48cm x 58cm x 53cm high) which were raised approximately 45 cm above floor level in a heated room. The ambient temperature was maintained at 30°C for the first two weeks after which the temperature was reduced to 25°C as suggested by Campbell and Dunkin (1983a). In addition, 175-watt lamps were placed above the corner areas of the cages to provide extra warmth for the piglets.

Design and Treatments Feeding and Measurement Periods

Experiment 1

After a pre-experimental period of seven days, piglets were allocated at random to six treatments and fed the semisynthetic liquid diet as shown in table 1. Piglets were offered either 0.90, 1.15, 1.40, 1.65, 1.90 or 2.15 MJ gross energy (GE)/$W^{0.75}$/d for the next 13 days. Piglets were weighed weekly to determine feed allowances for the following week. Thereafter 12 piglets were removed from the experiment and the remaining 12 were reallocated to three levels (1.15, 1.65 or 2.15 MJGE/$W^{0.75}$/d) for the following nine days. Individual milk intakes were recorded daily. Milk intake was estimated by the disappearance of liquid milk replacer and by the D_2O dilution method over periods of 1, 11, 1 and 8 days respectively.

TABLE 1. COMPOSITION OF LIQUID MILK REPLACER

Ingredients	Amount (g)
Whey protein concentrate[1]	1263
Dextrose	705
Butter fat	837
Soya bean oil	210
Minerals solution	450
Methionine	6
Choline	6
Formalin	12
Water	11511
Chemical analysis (g/kg air dry)	
Dry matter	190.9
Crude protein	63.8
Fat	71.7
Lactose	56.8

[1] "Alacen 312" whey protein concentrate containing 76.5% crude protein and supplied by N.Z. Dairy Enterprise Ltd. P.B. 25 Mount Waverly, Victoria, 3149.

Experiment 2

The piglets were allocated at random to three levels of feeding, either 1.15, 1.65 and 2.15 MJGE/$W^{0.75}$/d after a pre-experimental period of seven days. They remained on the respective treatments for the following three weeks. Estimates of individual milk intakes by the disappearance of liquid milk replacer and by the D_2O dilution method were conducted over periods of 1, 3, 5 and 7 days in both the first and third weeks of the feeding treatments.

Procedure of D_2O dilution method

The principal component for determining the milk consumption of piglets by the D_2O dilution method included injection of D_2O into the body of the piglets. Collection of blood samples, separation of blood water, determination of D_2O concentration, calculation of water turnover and calculation of milk intake. The steps of these procedure are presented below.

Piglets were fasted for 45 minutes before and 2 hours after D_2O injection. The purpose of the first fasting was to standardizise gut fill and the second to obtain an equilibrium of D_2O with the total body water (Pettigrew et al., 1987). Deuterium oxide (99.7% D_2O) was administered by intra muscular injection into the ham of the piglets using the syringe fitted with a 23 gauge needle. The syringe, together with the needle, was weighed to nearest 0.1 mg before and after injection to calculate the amount of D_2O injected. Deuterium oxide was administered at the beginning and at the end of each measurement period to estimate total body water space. The amount of D_2O administered depended on the length of measurement period and ranged from 0.8 to 1.8 g/kg body weight to ensure that the concentration of the D_2O in the body water was at least 500mg/kg at the end of the measurement period.

Blood samples were taken from vena cava

(Love, 1985) using a 10 ml vacutainer containing lithium heparin and attached to a 20 gauge needle. An outline of the procedure involving injection of D_2O and collection of blood samples is shown in figure 1.

Suckle
Separate (fast) 45 minutes
First injection of D_2O (1.75 g/kg L.W.)
Fast 2 hours

First blood sample (initial D_2O concentration = C_1)
Measurement period
Separate (fast) 45 minutes

Second blood sample (D_2O concentration = C_2)
Second injection of D_2O (0.50 g/kg L.W.)
Fast 2 hours

Third blood sample (Final D_2O concentration = C_3)

Figure 1. Procedure of D_2O injection and collection of blooc samples.

The concentration of D_2O in the body water samples was determined using an infra red analyser as described by Byers (1979). In experiment 1, water was extracted from blood samples by vacuum sublimation using Thurnberg tubes. However, this procedure often failed to satisfactorily remove all the water. Consequently, in experiment 2, water was extracted from samples placed in long-necked flasks connected to condensation fingers. The extracted water was then assayed for D_2O by infrared spectroscopy. Total water turnover was converted to an estimate of milk intake using the determined water content of milk and estimate of the metabolic water derived from the ingested milk solids.

The calculation of milk consumption involved the determination of water turnover which was then converted to milk consumption by the methods described below.

The water turnover during each measurement period was calculated by the following formula (Dove and Freer, 1979):

$$WTO = [(V_2 - V_1)/t] \times [\ln(C_1/C_2)/\ln(V_2/V_1)]$$

in which WTO is water turnover (g/d), V_1 and V_2 are the volumes of body water pool (ml) at the beginning and end, respectively, of the measurement period, while C_1 and C_2 are the concentrations of D_2O (mg/kg) at the beginning and the end of the measurement period and C_3 is the concentration of D_2O (mg/kg) after equilibration after the second injection (dose$_2$) of D_2O. The length of the measurement period is expressed as t (days). In any cases where volume of body water pool was not determined directly at the end of the measurement period, pool size was estimated from the bodyweight and the proportion of body water determined directly at a previous measurement. V_1 and V_2 were calculated as follows:

$$V_1 = \frac{\text{dose}_1\ D_2O \times 10^6}{C_1}$$

and

$$V_2 = \frac{\text{dose}_2\ D_2O \times 10^6}{(C_3 - C_2)}$$

The water turnover estimate was then used to estimate milk consumption using the various correction described below. Not only does the water content of milk contribute to the water turnover estimate but the water turnover estimate also includes water that is liberated by oxidation of the protein, fat and lactose in milk. Thus the amount of total water obtained from milk as percent of milk, is calculated as follows:

$$\text{Water (\%)} = (100 - \%DM) + (\%CP \times 0.41) + (\%fat \times 1.07) + (\%lactose \times 0.60)$$

where DM is dry matter and CP is crude protein (Mitchell, 1962). Not all potential metabolic water from milk is liberated, as some remains bound in protein and fat deposited in the piglet's body.

The amount not liberated depends on the amount of protein and fat deposited in the piglet's body, which was estimated from the growth rate piglets in the following manner. Regression equations relating the amounts of fat and protein deposited to growth rate of piglets were established from data reported by Campbell and Dunkin (1983b); the respective equation being:

$$F = 0.238\ GR - 19.58 \text{ and}$$
$$P = 0.131\ GR + 9.339$$

where F = amount of fat deposited (g/d), P = amount of protein deposited (g/d) and GR = growth rate of piglets (g/d).

The amount of potential metabolic water deposited as protein and fat and thus not liberated, was calculated by the following equation:

$$PMWD = (P \times 0.41) + (F \times 1.07)$$

where PMWD = potential metabolic water deposited (g/d).

Thus milk consumed can be calculated by the following equation:

Estimated milk intake (g/d) =

$$\frac{WTO + PMWD}{Water\ (\%)} \times 100$$

Statistical analysis

The actual milk intakes and those estimated by the D_2O dilution method were compared using the paired t-test and by regression analysis (Steel and Torrie, 1981).

Results

The amount of liquid milk replacer consumed, estimated by disappearance and by the D_2O dilution method is shown in table 2. The milk intakes which were determined by the D_2O dilution method were significantly lower ($p < 0.001$) than actual milk intakes in experiment 1. However, in experiment 2 the estimates of milk intakes using D_2O did not differ significantly ($p > 0.05$) from actual milk consumption (table 2).

Regression analysis of the results revealed similar differences between the results of experiment 1 and 2. The coefficients of determination of actual milk intake with intake estimated by D_2O dilution over the measurement periods of 1, 11, 1 and 8 days were 0.58, 0.42, 0.86 and 0.95 respectively. In experiment 2, milk intakes estimated by the D_2O dilution method were highly correlated with actual intakes for all periods (R^2 = 0.98 – 0.99). Furthermore there was no significant difference between the regression equations for periods of 1, 3, 5 and 7 days (figure

TABLE 2. THE AMOUNT OF LIQUID MILK REPLACER CONSUMPTION AS ESTIMATED BY DISAPPEARANCE AND BY THE D_2O DILUTION METHOD

	Measurement Period(days)	Number of Measurements	Mean (g/d)	Range (g/d)
Experiment 1				
Actual milk intake	1	20	583	303 to 925
	11	20	758[a]	357 to 1451
	1	23	996[b]	414 to 1903
	8	11	750[c]	490 to 1179
D_2O dilution	1	20	515	170 to 886
	11	20	525[a]	248 to 1223
	1	23	445[b]	172 to 807
	8	11	403[c]	288 to 594
Experiment 2				
Actual milk intake	1	30	632	168 to 1193
	3	30	664	259 to 1315
	5	30	663	255 to 1315
	7	45	628	259 to 1278
D_2O dilution	1	30	630	269 to 1221
	3	30	674	244 to 1359
	5	30	677	245 to 1376
	7	45	616	250 to 1179

In experiment 1 a, b and c in rows of actual milk intake differ significantly ($p < 0.01$) from a, b and c respectively in rows of intake estimated by D_2O dilution.

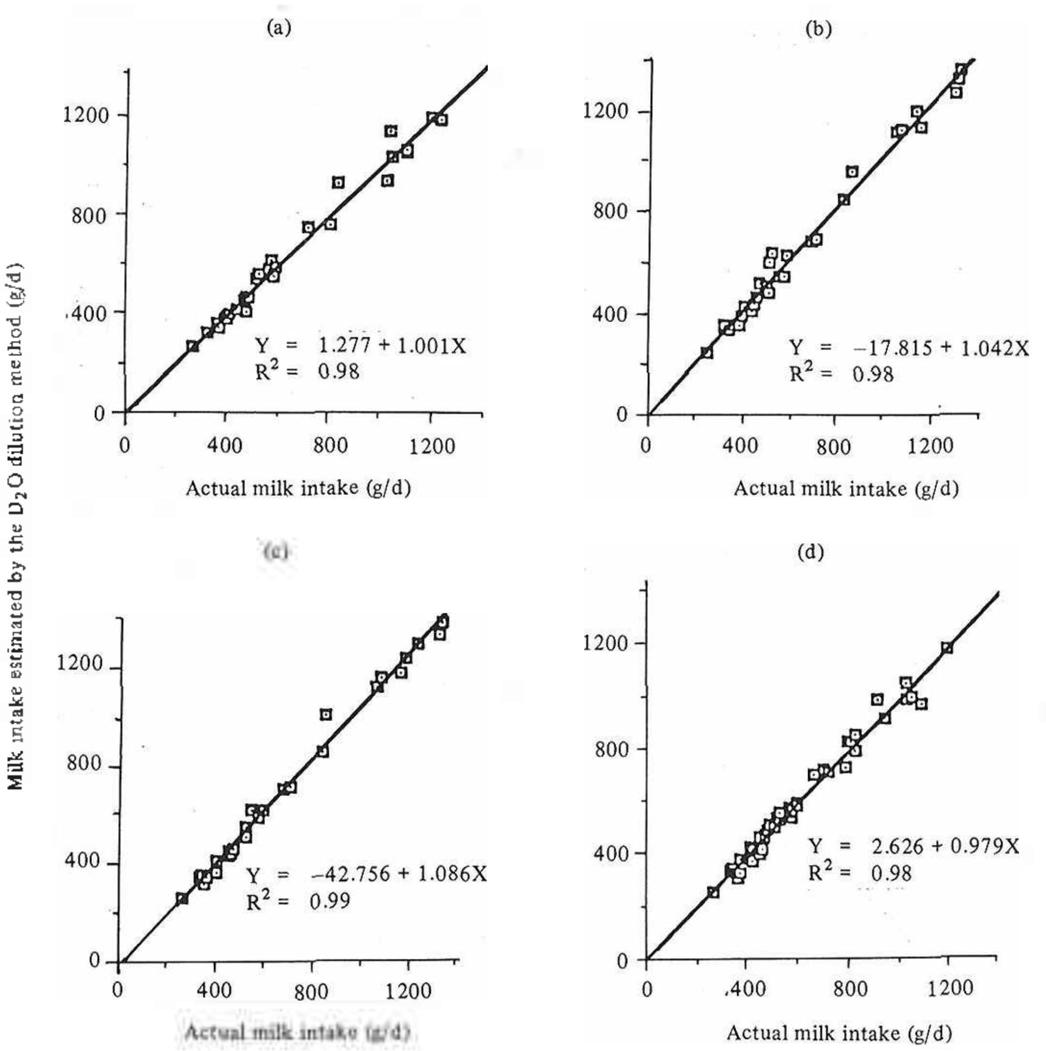

Figure 2. Correlation between actual milk intake and milk intake estimated by the D_2O dilution method over the measurement periods of 1(a), 3(b), 5(c) and 7(d) days (experiment 2).

2) and the regression equation of the combined data is presented (figure 3).

Differences within piglets between actual and estimated intakes were taken as a measure of the accuracy of the D_2O dilution method and revealed that the isotope dilution method was inaccurate in experiment 1 with the mean deviation of the D_2O dilution estimates from actual milk intakes ranging from 8.5 to 55.3% (table 3). However, the isotope dilution method proved much more accurate in experiment 2 where the mean deviations of the D_2O dilution estimates from the measurements of disappearance were only −0.1, +0.5, +0.5 and −2.1% among 1, 3, 5 and 7 day measurements.

Discussion

The results of the present study suggest that the D_2O dilution method is an accurate method for estimating the milk consumption of piglets for periods of 1 – 7 days. However, several problems were encountered in experiment 1. Firstly, blood samples were often not completely dried under vacuum sublimation using Thurnberg tubes. It is possible that D_2O, being more dense than water,

TABLE 3. THE DIFFERENCE BETWEEN ACTUAL MILK INTAKE AND MILK INTAKE ESTIMATED FROM WATER TURNOVER MEASURED BY D_2O DILUTION

	Measurement period (days)	Number of measurement	Difference between actual and estimated (D_2O) milk consumption (%)		
			Mean	SE	Range
Experiment 1					
	1	20	8.46	5.86	43.2 to 52.0
	11	20	29.94	4.73	−12.1 to 77.3
	1	23	55.32	1.43	40.0 to 72.8
	8	11	44.02	1.65	38.1 to 50.4
Experiment 2					
	1	30	−0.1	1.02	15.7 to 11.7
	3	30	+0.5	1.25	−13.3 to 15.6
	5	30	+0.5	1.18	−15.3 to 16.0
	7	45	−2.1	0.84	−15.7 to 7.2

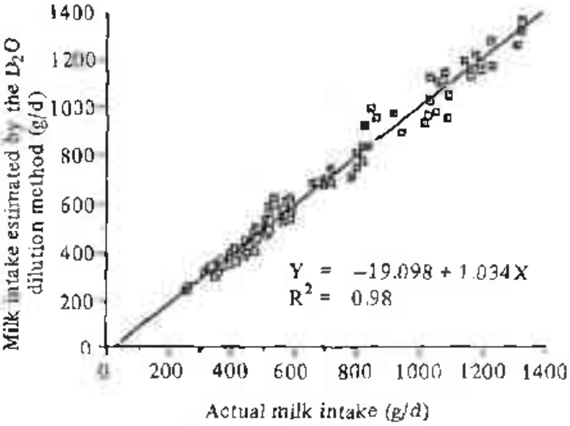

Figure 3. Correlation between actual milk intake and milk intake estimated by the D_2O dilution method (combined data) over the measurement periods of 1, 3, 5 and 7 days (experiment 2).

may be deferentially removed from blood under vacuum sublimation. Consequently the concentration of D_2O in extracted water would be less in samples which had not been completely dried. This may account for the lower values of milk intake estimated by the D_2O method which were recorded in experiment 1. Secondly, the amount of spilt milk and possible losses of water from the liquid milk replacer through evaporation were not recorded in experiment 1. These errors would contribute to an overestimate of actual milk intake. Finally, the D_2O concentration at the end of the 11-day measurement period was often very low and could not be detected accurately by infrared spectroscopy. The validity of an estimate of milk intake for the 11-day measurement period and the subsequent 1-day measurement period would be affected by this possibly inaccurate estimate of D_2O concentration.

The results of experiment 1 were included in this paper to highlight some of the inaccuracies that may be encountered when using the D_2O dilution method to estimate the milk consumption of piglets. The possible errors discussed above were eliminated in experiment 2 and in the subsequent experiments reported in later papers.

The results of experiment 2 showed that the isotope dilution technique for measuring milk consumption of piglets was acceptably accurate and precise. Pettigrew et al. (1987), who compared estimates of milk replacer consumption of individual piglets over periods of 1 or 3 days, also found close agreement between estimates derived from the D_2O dilution method and from actual measurement by disappearance. Rudolph et al. (1984) concluded that milk intake may be accurately predicted by measurement of water turnover via D_2O dilution in pigs. However, although there was a close correlation between actual milk intake and D_2O-estimated water inflow, water inflow using D_2O was between 4% and 10% lower than known milk intake (Rudolph, 1985).

Literature Cited

Byers, F. M. 1979. Extraction and measurement of deuterium oxide at tracer level in biological fluids. Anal. Biochemist. 98:208-213.

Campbell, R. G. and A. C. Dunkin. 1983a. The influence of nutrition in early life on growth and development of the pig. 1. Effect of protein nutrition prior and subsequent to 6.5 kg on growth and development to 45 kg. *Anim. Prod.* 36:415-423.

Campbell, R.G and A.C. Dunkin. 1983b. The effects of energy intake and dietary protein on nitrogen retention, growth performance, body composition and some aspects of energy metabolism of baby pigs. Br. J. Nutr. 49:221-230.

Dove, H. and M. Freer. 1979. The accuracy of tritiated water turnover rate as an estimate of milk intake in lambs. Aust. J. Agric. Res. 30:725-739.

Hartman, D. A., T. M. Ludwick and R. F. Wilson. 1962. Certain aspects of lactation performance in sows. J. Anim. Sci. 21:883-886.

Klaver, J., G. J. M. Van Kempen, P. G. B. De Lange, M. W. A. Verstegen and H. Boer. 1981. Milk composition and daily yield of different milk components as affected by sow condition and lactation/feeding regimen. J. Anim. Sci. 52:1091-1097.

Lewis, A.J., V.C. Speer and D.G. Haught. 1978. Relationship between yield and composition of sow's milk and weight gains of nursing pigs. J. Anim. Sci. 47:634-638.

Love, R. J. 1985. The T. G. Hungerford Vade mecum. Series for domestic animals. No. 6. Diseases of pigs. The University of Sydney Post-Graduate Foundation in Veterinary Science, Sydney.

Mahan, D. C., D. E. Becker, H. W. Norton and A. H. Jensen. 1971. Milk production in lactating sows and time lengths used in evaluating milk production estimates. J. Anim. Sci. 33:35-37.

Mitchell, H. H. 1962. Comparative nutrition of man and domestic animals. Academic press, New York.

Noblet, J. and M. Etienne. 1985. Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. J. Anim. Sci. 63:1888-1896.

Pettigrew, J. E., S. G. Cornelius, R. L. Moser and A. F. Sower. 1987. A refinement and evaluation of the isotope dilution method for estimating milk intake by piglets. Livest. Prod. Sci. 16:163-174.

Pettigrew, J. E., A. F. Sower, S. G. Cornelius and R. L. Moser. 1985. A comparison of isotope dilution and weigh-suckle-weigh methods for estimating milk intake by pigs. Can. J. Anim. Sci. 65:989-992.

Rudolph, B. C. 1985. Use of deuterium oxide as an estimator of milk intake and body composition of nursing pigs and the effect of oxytocin administration to the dam on early lactational performance. In Dissertation Abstracts International, B(Sciences and Engineering) 46:10.

Rudolph, B. C., T. S. Stahly and G. L. Cromwell. 1984. Accuracy of milk intake estimates in pigs by water turnover (via D20 dilution) and weigh-suckle-weigh methods. J. Anim. Sci. (Suppl 1) 59:101-102.

Rudolph, B. C., T. S. Stahly and G. L. Cromwell. 1988. Estimation of body composition of neonatal pigs via deuterium oxide dilution: validation of technique. J. Anim. Sci. 66:53-61.

Steel, R.G.D. and J. Torrie. 1981. Principles and procedures of statistics. A biometrical approach. (2nd. Ed). McGraw-Hill International Book Company, Tokyo.

Van Spaendonck, R. L. and F. X. Vanschoubroek. 1964. Determination of the milk yield of sows and correction for loss of weight due to metabolic processes of piglets during suckling. Anim. Prod. 6:119-123.

Yang, T. S., D. Howard and M. W. Macfarlane. 1980. A note on milk intake of piglets measured by tritium dilution. Anim. Prod. 31:201-203.