Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 16, Numbers 1 and 2, January and April, 1941.

> Printed in the Union of South Africa by the Government Printer, Pretoria.

The Endogenous Nitrogen Metabolism of Pigs with Special Reference to the Maintenance Protein Requirement.

By B. A. DU TOIT and D. B. SMUTS, Section of Nutrition, Onderstepoort.

THE constitution of the animal body is such that it necessitates a certain minimum quantity of organic and inorganic nutrients for the maintenance of those vital physiological functions associated with the mere existence of life.

Thus the continual catabolism of nitrogenous material in the protoplasm which forms an inevitable but nevertheless integral part of the daily metabolism and existence of the animal body must, to avoid ultimate disintegration and destruction of the cellular mass, be replaced by an equivalent amount of nitrogenous material of a 100 per cent. biological value. Hence it is clear that this quantity of nitrogen is the bare minimum to which the exogenous supply can be reduced without impairing the general health, vigour and vitality of the animal. Recently Burroughs and Mitchell have shown that there exists a distinct difference in the nature and composition of the nitrogen requirements for maintenance. Apparently 30 to 50 per cent. of the endogenous nitrogen can be replaced by almost any type of nitrogen. Thus urea and the different ammonium salts may satisfactorily replace this portion of the nitrogen losses, arising from the idling activities of the protoplasm There appears to be no doubt on the other hand that the remaining portion can only be satisfied by a selective combination of indispensable amino acids. A deficiency in any one of these amino acids impairs the total nitrogen utilization. It remains therefore to be shown in how far the latter portion influences the utilizability of the former, whether there exists any relationship between them or whether they can be satisfied independently from two different sources. If no relationship between these two fractions should exist, so that the former portion can for instance be satisfactorily replaced by non-amino acid nitrogen, irrespective of whether the requirements of the latter portion have been fulfilled, then it would mean, that the actual protein requirements as measured by the endogenous metabolism, are considerably reduced. In fact it would mean that such requirements can best be expressed in terms of total nitrogen, with a clear discrimination as to what portion of the total can be replaced by non-amino nitrogen and what fraction requires a complete assortment of indispensable amino acids in the form of crude protein. The practicability of such a differentiation will be extremely difficult due to the fact that no ENDOGENOUS NITROGEN METABOLISM OF PIGS.

protein can satisfy the amino acid requirements of the second fraction with a 100 per cent. efficiency. Consequently those amino acids dispensable for maintaining the integrity of the protoplasm will be utilized for the replenishment of the nitrogen losses associated with the first fraction. Hence the requirements of non-amino acid nitrogen will automatically be reduced proportionally, making the situation very complex indeed. Furthermore, apart from the fact that very little economical benefit can be derived from the partial replacement of the endogenous losses by ammonium compounds, it is still to be shown that the continual feeding of such compounds over a period of time does not effect the general health, vitality and vigour of the animals. It appears, therefore, that the endogenous nitrogen expressed in the conventional way as protein remains the safest and most practical way of calculating the maintenance requirement of nitrogen. In fact the requirements based on this principle will allow for a certain amount of safety, since it can be expected that the indispensable amino acid portion, which is in all probability the most essential fraction, will be satisfied first before any excess amino acids will be converted to satisfy the nitrogen needs of the former. For this reason a study of the endogenous nitrogen metabolism in view of calculating maintenance protein requirements was undertaken.

EXPERIMENTAL.

Nine large white pigs ranging in weight from 24 to 34 Kgm. were put on a standard protein ration for 10 days and then on a nitrogen free ration, the composition of which is given in Table 1. Daily collection of urine and faeces was executed during the period of nitrogen free feeding. The metabolism crates were the same as used in our sheep work. The faeces were carefully collected, the screens were washed with distilled water into the urine collecting bottle. Urine was collected in acid medium. The daily collection of faeces and urine. plus the washings were aliquoted and the latter analyzed for total nitrogen, creatine plus creatinine and urea. The urea was determined according to the method of Von Slyke and Cullen, creatine and creatinine by the method of Folin (1914) and total nitrogen by the usual Kjeldahl method. Pigs were fed twice daily and supplied with plenty of distilled water.

EXPERIMENTAL RESULTS.

In graphs 1-5 are given in graphic form the results of the daily nitrogen, creatine plus creatinine and urea excretion of 5 pigs from the first day that they were put on the nitrogen low-ration. As will be seen from these graphs there is a very sharp drop in total nitrogen excretion the first day. From then onwards the daily nitrogen excretion decreases at a slow rate, and reaches a constant output with all pigs at approximately the 6th day. Hence it can be assumed that the endogenous level with pigs of this age and receiving previously a 16 per cent. protein ration is reached at the 6th day of nitrogen free feeding. The creatine plus creatinine excretion remains absolutely constant as can be seen from the graphs, showing that no tissue catabolism has taken place. The urea excretion on the other hand behaves more or less like the nitrogen excretion. After dropping slowly the first 4 days it stays almost constant for the rest of the period. This shows that although urea is chiefly an end-product of exogenous protein metabolism, a constant portion ir nevertheless, derived from endogenous protoplasmic reactions.

In Table 2 are given the results of the endogenous nitrogen metabolism on the nine pigs together with the results of 5 pigs previously measured by Smuts (1935). The weights of these pigs vary from 24 to 79 Kgm. In column 4 the endogenous nitrogen has been converted into the conventional protein. In the following column the data for the calculation of K in the equation $P = KW^{.734}$ previously used by us for sheep, are given, With the exception of pig 5, for which a very low value namely 0.67 was obtained, the rest of the values agree fairly well. The average value obtained for the 14 pigs is 0.81, giving therefore an equation of $P = 0.81W^{.734}$ for the estimation of the maintenance protein requirements of pigs. By utilizing this equation and the weights in Kgm. of the pigs, the protein requirements were estimated. These values are given in the second last column. The percentage deviation between these values and that originally measured are given in the last column. The value obtained with pig 5 naturally deviates considerably from the measured value and influences to a certain degree the average percentage deviation of the group. If this value is omitted it is seen that in no case does the percentage deviation exceed the 10 per cent mark. However, since exceptional cases like that of pig 5 are very often encountered, it must be assumed that certain animals have a distinctly higher or lower endogenous metabolism than the average and cannot therefore be excluded from a set of data. The average percentage deviation between the measured endogenous protein and that estimated from the above equation is ± 4.9 . This deviation is extremely small and shows that the determined equation can be utilized successfully in practice for the estimation of the maintenance protein requirements of pigs.

In Table 3 all the existing data on the endogenous nitrogen metabolism of pigs as summarized by Mitchell and Hamilton (1929), and to which our own have been added, are tabulated. The idea was to ascertain in how far the constant, and hence the equation, determined from our own data, will apply to the existing data including wide variations in weight. As will be seen from Table 3. the average weight of these pigs range from 14.3 Kgm. to 79 Kgm. Thus the constant arrived at from these values ought to be very representative. Except for the three values namely, 0.67, 0.63 and 0.69 obtained by Smuts, Morgen and Pfeiffer respectively, the constants as a group do not vary to a great extent. The average value of 0.79 is in close agreement with that of 0.81 obtained by us on 14 pigs, of less variable weights. Even with the inclusion of the above three values, which definitely fall far below the average value, it is nevertheless interesting that the average percentage deviation between the measured and the estimated protein requirements is only ± 6.7 . These figures seem to indicate that the endogenous nitrogen is closely related to the power function of weight or to the surface area of an animal. Another important fact seems to be the close relationship between the endogenous nitrogen of

ENDOGENOUS NITROGEN METABOLISM OF PIGS.

different species per square meter or $\frac{3}{4}$ function of the weight. Thus we have found previously that the constant for sheep in the same equation as that of pigs is 0.74, a value which is very near to 0.79 as found for pigs. It is quite possible that if large enough numbers of animals are employed for the determination of the endogenous nitrogen, that similar equations may be obtained. It is clear from these data that the equation $P=0.81 \text{ W}^{.734}$ can be utilized successfully for the estimate of the maintenance requirements of pigs. It is quite obvious that the equation $P=0.79 \cdot W^{.734}$ will probably give a truer representation of the actual endogenous nitrogen, since it represents a more distributed set of data. However, for practical purposes K=0.81 will be safer since it allows for a certain amount of safety.

SUMMARY AND CONCLUSIONS.

The endogenous nitrogen metabolism of pigs have been measured and an equation evolved for the estimation of the maintenance protein requirements of pigs. The equation obtained is $P=0.81 \text{ W}^{.734}$, where P= protein in grams, and W weight in Kgm.

REFERENCES.

- BURROUGHS, E., WISE, H., BURROUGHS, S., AND MITCHELL, H. (1940). The independence of the endogenous and the exogenous metabolism of nitrogen. J. Ntr., Vol. 19, pp. 271-283.
- BURROUGHS, E., WISE, H., BURROUGHS, S., AND MITCHELL, H. (1940). The animo aids required for the complete replacement of endogenous losses in the adult rat. J. Nutr., Vol. 19, pp. 363-383.
- BURROUGHS, E., WISE, H., BURROUGHS, S., AND MITCHELL, H. (1940). The interdependence among amino acids in their utilization in the endogenous metabolism. J. Nutr., Vol. 19, pp. 385-391.
- FOLIN, O. (1914). On the determination of creatinine and creatine in urine. J.B.C., Vol. 17, p. 469.
- MITCHELL, H. H., AND HAMILTON, T. S. (1929). The Biochemistry of the amino acids. Americ. Monograph Series, New York.
- SMUTS, D. B. (1935). The relationship between the basal metabolism and the endogenous nitrogen metabolism with particular reference to the estimation of the maintenance protein requirements. J. Nutr., Vol. 9, p. 403.
- VAN SLYKE, D. D., AND CULLEN, G. E. (1914). A permanent preparation of urease and its use in the determination of urea. J.B.C., Vol. 19, p. 211.

TABLE 1.

Percentage Composition of N-low Ration.

Starch	88.0
Agar	3.0
Boneash	$2 \cdot 0$
Salt	1.0
Yeast	$5 \cdot 0$
Codliveroil	$1 \cdot 0$
and the set of the second set of	
TOTAL	$100 \cdot 0$

TABLE 2.

The Endogenous Nitrogen of Pigs of Various Weights.

Percentage Deviation,	1+1++++++++ 12000 12000 12000 12000 12000 12000 14++++++++++++++++++++++++++++++++++	士 4.9
P= .81W. ⁷³⁴	$\begin{array}{c} 17.93\\ 17.72\\ 20.01\\ 17.72\\ 20.01\\ 18.51\\ 18.51\\ 8.35\\ 8.35\\ 8.35\\ 8.35\\ 8.35\\ 8.35\\ 10.27\\ 10.81\\ 10.71\\ 10.81\\ 10.55\\ 10.54\end{array}$	1
$\begin{array}{c} Value of \\ K in \\ P = KW^{*33} \end{array}$	$\begin{array}{c} 0.87\\ 0.79\\ 0.87\\ 0.77\\ 0.78\\ 0.78\\ 0.78\\ 0.78\\ 0.78\\ 0.78\\ 0.78\\ 0.83\\ 0.78\\ 0.81\\ 0.81\\ 0.91\\ 0.91\end{array}$	0.81
Log W. 734	1:3451 1:3451 1:3303 1:3728 1:3763 1:3763 1:3763 1:3763 1:3763 1:0131 1:0261 1:0131 1:0261 1:0131 1:0261 1:0261 1:1241 1:1144	1
Log W.	1.8325 1.8326 1.8326 1.8751 1.8751 1.8751 1.8751 1.8751 1.8802 1.3802 1.3802 1.3814 1.3816 1.5315 1.5315 1.5315 1.5315 1.5315	- 1
Log P.	$\begin{array}{c} 1\cdot 2844\\ 1\cdot 2844\\ 1\cdot 2367\\ 1\cdot 2312\\ 1\cdot 2524\\ 1\cdot 1869\\ 0\cdot 9166\\ 0\cdot 9166\\ 0\cdot 9166\\ 0\cdot 9165\\ 0\cdot 9165\\ 0\cdot 9165\\ 0\cdot 9165\\ 1\cdot 0339\\ 1\cdot 0724\\ 1\cdot 0724\end{array}$	1
Findogenous Protein. Gm.	19-25 17-25 17-25 17-25 17-38 17-38 8-06 8-31 8-38 8-31 8-38 8-31 8-38 8-31 8-38 8-31 8-38 8-31 8-38 8-38	I
Endogenous Nitrogen. Gm.	$\begin{array}{c} 3.08\\ 3.45\\ 2.46\\ 2.46\\ 1.29\\ 1.33\\ 1.33\\ 1.64\\ 1.33\\ 1.64\\ 1.81\\$	
Weight. Kgm.	688 992 924 925 925 925 925 925 925 925 925 925 925	1
Animal No.	1 2 5 5 6 6 6 6 6 6 6 6 7 7 6 6 7 7 6 6 7 7 7 6 6 7 7 7 6 6 6 7 7 7 7 7 6 6 6 7	AVEBAGE

B. A. DU TOIT AND D. B. SMUTS.

173

3	
H	
E	
V	
E	
·	

The Endogenous Nitrogen of Pigs of Different Weights as Determined by Various Workers.

Percent	Deviation.	1.6 -	- 2.0	- 4.3	+ 5.1	+17.4	+ 5.7	+15.1	- 5.4	+25.2	- 2.9	- 0.3	1 8.6	- 2.7	-11.6	• 0.2	- 1.8	-12.2	- 1.9	- 5.3	+20.7	0.0	6.0 +	- 1.3	+ 1.0	1.1 + .	+ 2.6	- 4.4	+11.4	- 7.2	+ 6.7	
P =	wer MAL.	17.49	16.90	19.52	18.79	.18.05	17.50	18.77	13.19	12.06	11.84	11.52	11.43	11.43	11.23	10.52	10.52	10.28	10.06	8.88	8.98	8.39	8.39	8.14	8.14	8.39	6.99	6.51	6.27	5.57	The second	
Value of K in	P=KW. ⁷³⁴	0.89	0.81	0.81	0.75	19.0	0.75	0.69	0.84	0.63	0.81	0.80	0.86	0.81	11.0	0.85	0.81	06.0	0.81	0.83	69.0	0.78	0.78	0.80	0.78	0.78	12.0	0.83	0.71	0.85	61.0	
.Log W • 784	D	1.3451	1.3303	1.3928	1.3763	1.3589	1.3455	1.3759	1.2226	1.1838	1.7759	1.1638	1.1604	1.1604	1.1527	1.1241	1.1241	1.1146	1.1047	1.0506	1.0423	1.0261	1.0261	1.0131	1.0131	1.0261	0.9469	0.9160	0.8994	0.8480	1	
Log W.	0	1.8325	1.8261	1.8976	1.8751	1.8513	1.8331	1.8745	1.6656	1.6128	1.6021	1.5855	1.5809	1.5809	1.5705	1.5315	1.5315	1.5185	1.5051	1.4314	1.4200	1.3979	1.3979	1.3802	1.3802	1.3979	1.2900	1.2480	1.2253	1.1553	1	
Log P.)	1.2844	1.2367	1.3312	1.2524	1.1869	1.2191	1.2125	1.1443	0.9836	.1.0859	1.0630	1.0969	1.0697	1.0025	1.0535	1.0298	1.0686	1.0107	0.9722	0.8716	0.9232	0.9196	0.9165	0.9063	0.9165	0.8357	0.8357	0.7505	0.7782		
Endogenous Protein.	Gm.	19.25	17.25	21.44	17.88	15.38	16.56	16.31	13.94	9.63	12.19	11.56	12.50	11.75	10.06	11.31	10.71	11.71	10.25	9.38	. 7.44	8.38	8.31	8.25	8.06	8.25	6.81	6.81	5.63	6.00	-	
Endogenous Nitrogen.	Gm.	3.08	2:76	3.48	2.86	2.46	2.65	2.61	2.23	1.54	1.95	1.85	2.00	1.88	1.61	1.81	1.73	1.89	1.64	1.50	1.19	1.34	1.33	1.32	1.29	1.32	1.09	1.09	06.0	96.0		
Weights.	Agm.	68.0	67.0	0.67	75.0	71.0	0.89	74.9	46.3	41.0	40.0	38.5	38.1	38.1	37.2	34.0	34.0	33.0	32.0	27.0	26.3	25.0	25.0	24.0	24.0	25.0	19.5	17.7	16.8	14.3	Proved	
Investigator.	p .	Smuts					McCollum & Steenbock		McCollum & Hoogland	Morgen et al	Mitchell & Kick	McCollum & Steenbock	Mitchell & Kick	****** 66 66	McCollum & Steenbock	Du Toit & Smuts	95			· · · · · · · · · · · · · · · · · · ·	Pfeiffer	Du Toit & Smuts	56 66	39 99 *****	59 59 54	Pfeiffer	McCollum & Steenbock	McCollum & Hoogland	McCollum & Steenbock	McCollum & Hoogland	AVERAGE	

ENDOGENOUS NITROGEN METABOLISM OF PIGS.





.



PIG No. 2.



PIG No. 3.

ENDOGENOUS NITROGEN METABOLISM OF PIGS.



PIG No. 4.



Pig No. 5.