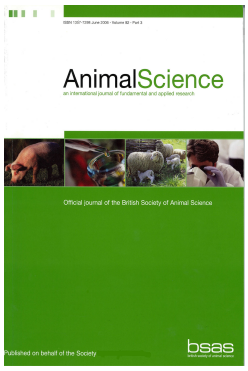

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Allometric association between *in vivo* estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs

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Abstract

The objective of this study was to develop accurate mathematical-statistical functions to estimate body composition of live pigs between 20 and 140 kg weight from total body water (TBWA) determined by the deuterium dilution technique. Chemical body compositions during the growth period are essential input parameters for biological pig growth models, which are used to estimated the nutrient requirements, improve the entire production system, determine optimal slaughter weight, optimize selection for food intake, etc. In the present study, 48 pigs (17 female and 31 castrated males) were used in an experimental station to obtain protein, lipid, ash and water content at 20, 30, 60, 90, 120 and 140 kg live weight. At each target weight, body water of the animals was determined by the deuterium dilution technique. Eight pigs of each live-weight group were slaughtered and chemically analysed. Water content of the empty body decreased from 74 to 53%, whereas lipid content rose from 7 to 30%. Between 20 and 30 kg body weight, protein content increased from 16 to 17% and thereafter decreased to 16%. Ash content was constant at 3%. To estimate body composition of the remaining animals from TBWA (%) determined by deuterium dilution technique, two sets of exponential prediction functions were used to describe the relationship between chemically analysed body components and TBWA (%). The first set of prediction functions fitted one intercept for the entire growth period and the second set of prediction functions fitted a different intercept for each weight class. Correlation coefficients between estimated and chemically determined empty body water, lipid, protein and ash for the first set of functions were 0·93, 0·86, 0·83 and 0·65, respectively. The second set of prediction functions showed higher accuracy (2 to 10%), but had the disadvantage of non-continuous estimates over the entire growth period. In contrast, by using the first set of prediction functions, a continuous accurate estimation of body composition of live pigs was obtained over a large range of growth (20 to 140 kg) based on deuterium dilution space.

Keywords: *body composition, deuterium oxide, growth, pigs.*

Introduction

Pig growth models have been substantially used to improve the efficiency of pig productions (e.g. De Lange *et al.*, 2003; Knap *et al.*, 2003; Van Milgen and Noblet *et al.*, 2003; Moughan, 2003; Pomar *et al.*, 2003). These pig growth models are also of increasing interest in animal breeding. For instance, De Vries and Kanis (1992) developed a biological pig growth model to optimize selection for food intake capacity. For this biological growth model, accurate input parameters of maximum protein deposition rate and minimum lipid to protein deposition ratio have to be estimated. These input parameters have to be obtained at different stages of growth in order to optimize the feeding strategy for growing and finishing pigs. Additionally, the accretion of carcass lean and fat tissue is of primary interest because the quantity and ratio of these two components determine the economic value of the animal (Akridge *et al.*, 1992). Different methods can be used to measure protein and lipid deposition rate in live animals. One method for determining body composition is to measure the total body water content and then estimate the body composition on the basis of the relative consistency of the composition of the content of fat free substance (FFS (%)) and on the basis of empty body weight. Thereby, FFS (%) for the whole

body is defined as percentage of non-fat mass on the empty body weight. Deuterium oxide (D_2O) is an ideal tracer for the total body water (TBWA) content because the *in vivo* kinetics and metabolism of D_2O are nearly identical to those of water (Pinson, 1952). In this study, as reference method, chemical analysis of the whole body in a serial slaughter trial is used to test its association with estimated body composition determined by D_2O method. To estimate body composition over the entire growth period in relation to total body water determined by D_2O dilution technique, exponential functions similar to the allometric function described by Huxley (1932) were used. However, in this study, exponential functions were used to estimate the percentages of body compositions and not their weights. The objective of this study was to obtain the appropriate prediction functions to estimate body composition over the entire growth period from TBWA (%) determined by D_2O dilution space. Additionally, exponential prediction functions were fitted with a different intercept for each weight group and compared with those fitting only one intercept over the entire growth period. Furthermore, the chemical body composition in the entire body and in different fractions of the body is examined.

Material and methods

Animals

Data were obtained in a three generation full-sib design that will be used finally to identify the genomic regulation of protein and lipid deposition rate. The base generation (F_0) consisted of seven unrelated Piétrain boars, heterozygous at the ryanodine receptor locus (stress susceptibility gene), of a sire line and 14 unrelated sows (Large White × Landrace × Leicoma) of a dam line which were mated to produce the F_1 generation. Animals not used to build up the F_2 generation were tested on station to obtain protein, lipid and ash deposition.

The present analysis is based on the measurements of 48 serial slaughtered pigs (17 females and 31 castrates) belonging to the F_1 generation. On average, there were seven progeny per sire and three pigs per litter. The pigs were housed in identical straw bedded pens with an electronic feeding station of type ACEMA 48 (Acemo) at the performance test station in Achterwehr. The pigs were allowed *ad libitum* access to pelleted diets which provided adequate nutrients supply (Gesellschaft für Ernährungsphysiologie, 1987) for expression of maximum protein accretion for the different body weight ranges (Table 1). Amino acid analysis (lysine, threonine) was performed by ion exchange chromatography following acid hydrolysis with 6 mol/l HCl (method 994·12; Association of Official Analytical Chemists, 1999). Methionine and cysteine were determined by oxidation with performic acid, yielding the acid stable forms methionine sulphone and cysteic acid. Tryptophan was determined following alkaline hydrolysis with barium hydroxide according to Fontaine *et al.* (1998).

Slaughter procedure and chemical analysis

In this experiment a serial slaughter trial was carried out. Eight animals were slaughtered at 20, 30, 60, 90, 120 and 140 kg live weight in a nearby slaughter house. After stunning, the blood was collected, carcass was scalded and viscera was removed. The hot carcass was split and chilled (4°C) for 24 h. From gastro-intestinal tract the contents were removed. Then, the weight of blood, separate organs (liver, lung, kidney, spleen, heart and trachea), empty intestinal tract and leaf fat was measured and accumulated to the fraction viscera. Thereafter, these viscera (excluding blood) were pooled and minced with a cutter (Rohwer, Type N45S-1) for 3 min and mixed until homogenized material was achieved. During mincing, the material was cooled with dry ice. Two samples were taken for the chemical analysis and stored at −20°C in individual polyethylene boxes to prevent moisture loss. One day after slaughter, the standard performance traits recorded on test stations such as carcass length, pH value of ham and loin, conductivity, loin eye area, fat area, backfat, side fat, belly fat, belly fat area and a fat degree B were measured at the cold left carcass side according to the principles of test stations (Zentralverband der Deutschen Schweineproduktion, 1992). At the same day the cold left carcass side was dissected into ham, shoulder, loin, neck, belly, and head (similar to DLG carcass cuts; Scheper and Scholz (1985)). After weighing these carcass cuts a further dissection took place, in which each carcass cut was dissected into bones, rind and a boneless carcass part. From all these carcass cuts, bones were pooled to the fraction 'bones' and the non-bone tissue to the fraction 'soft tissue' (lean/fat tissue). Both fractions were minced like the viscera and two 1·0-kg samples from each fraction were taken and frozen at −20°C until the chemical analysis.

The three fractions (viscera of the whole body as well as soft tissue and bones of the left side of the carcass) were analysed separately with respect to their chemical

Table 1 *The composition and chemical analysis of diets used in different phases of growth*

	Diet			
	1 20 to 30 kg	2 30 to 60 kg	3 60 to 90 kg	4 90 to 140 kg
ME† (MJ/kg)	13·8	13·8	13·8	13·4
Lysine (g/kg)	13·0	12·0	11·0	10·0
Lysine : ME (g : MJ)	0·94	0·87	0·82	0·75
Lysine : (methionine + cysteine)		1 : 0·58 – 0·60		
Lysine : threonine		1 : 0·65 – 0·70		
Lysine : tryptophan		1 : 0·18 – 0·20		

† Metabolizable energy (MJ/kg) calculated according to Gesellschaft für Ernährungsphysiologie (1987).

components. In order to obtain homogenous samples, they were autoclaved at 120°C and 2·3 bar for 1 h and thereafter mixed with a blender. The samples were analysed using the methods of the VDLUFA (Naumann *et al*., 1997). After mixing the sample with sea sand, dry matter content was determined using a drying oven at 103°C for 12 h. Ash content was determined using a muffle oven for 3 h at 550°C. Protein content was determined using the Kjeldahl procedure. Lipid content was determined according to method B pretreatment with HCl as described by Naumann *et al.* (1997).

The blood was not analysed with respect to its chemical composition. Variability of blood composition is small, therefore blood composition values was used as described in the literature (Gütte *et al*., 1978) and then added to the chemical body composition of viscera.

Based on the composition and weight of bones, soft tissue, viscera and blood, the total body composition was calculated. For the calculation of the body composition of the entire body it was considered that soft tissue and bones were collected from the left side of the carcass only, while viscera and blood were components of the whole body. Furthermore, it has to be considered that the total body composition excludes skin and hair lost during scalding.

Application and analysis by deuterium dilution technique
All pigs were measured using the deuterium dilution technique at the target live weights of 20, 30, 60, 90, 120 and 140 kg. Animals were given no food and water 20 and 3 h, respectively, before D_2O application and this status was kept until the last blood sample was taken 6 h after D_2O application. Deuterium oxide was applied with a small amount of food, which was 100, 150, 200, 400, 600 and 800 g for the different body weights, respectively. Before D_2O application a veterinarian took a blood sample from the vena jugularis to identify the basal level of deuterium. After this, the animals were given 0·7 g D_2O (isotopic purity of 80%) per kg body weight. In a pre-trial of five animals, equilibration of D_2O in the body was reached at about 5 h after application, as indicated by a constant concentration of D_2O in blood samples until at least 7 h after application. Duplicate blood samples were taken at 5 and 6 h after application of D_2O. Samples were stored (−20°C) until D_2O concentration was analysed. Extraction of D_2O from the blood was achieved by the modified method from Byers (1979) and Tissier *et al.* (1978). In the modified method, the blood samples were vacuum sublimated and the water was collected in cold finger condensers. The deuterium oxide content of this cleaned H_2O-D_2O mixture was analysed with an infrared spectrometer (Perkin Elmer, 16 PC FT-IR) with a wavelength from 2400 cm^{-1} to 2700 cm^{-1} against water. Over these wavelengths, 32 scans per sample were carried out from which the concentration of D_2O was calculated. The samples were prepared and measured as follows. Two standards (0 p.p.m. and 960 p.p.m. D_2O in H_2O) and 13 samples were placed in an ultrasonic bath with 1 l water (20°C) for 10 min to reduce small bubbles. For the 490 measurements of each used standard, i.e. 0 p.p.m. and 960 p.p.m. standard, standard deviations of 3·1 and 3·4 p.p.m. were obtained, respectively. The series of measurements started with the 0 p.p.m. standard, succeeds with each of the 13 samples, and finished with 960 p.p.m. standard. Before each measurement, the temperature-controlled cell (35°C) was rinsed thoroughly twice with the probe.

Deuterium dilution technique is based on the marker dispersed in the body and replaced like normal body water. Based on applied D_2O and D_2O concentration in the blood water as well as body weight (BW), the total body water (TBWA) was calculated using the following equation:

$$\mathrm{TBWA}(\%) = \frac{D_2O_{fed}(g)}{D_2O_{blood}(ppm)} \times \frac{100\,000}{BW(kg)} \quad (1)$$

where D_2O_{fed} is the dose of deuterium given by food and D_2O_{blood} is the concentration of deuterium in blood water at equilibrium.

Statistical analysis and calculation of body composition
The statistical analysis was performed using SAS-procedure GLM (Statistical Analysis Systems Institute, 1992). To estimate the percentage of body composition (Y) based on the percentage of TBWA (X) determined by deuterium dilution technique, exponential functions similar to the following allometric equation $Y = aX^b$ (Huxley, 1932) was used, where Y is the content (%) of the chemical component, X is the content (%) of reference components, b is the development of content of chemical components during growth and a is the intercept. The exponential function was fitted by linearizing the function as $\log_{10} Y = \log_{10} a + b\log_{10} X$. Two different sets of functions were derived to estimate body composition.

Firstly, prediction functions were estimated using the same intercept over all weight groups. Secondly, prediction functions were obtained using a different intercept for each weight group. Both sets of functions based on TBWA determined by deuterium dilution space using equation (1).

The empty body was defined as the difference between body weight and gut content (including urine content of the bladder). Empty body water (EBWA) was estimated from TBWA and body weight and accounts for the water content in stomach, intestine and bladder. EBWA was used to estimate FFS (%). FFS (%) composed of the water, protein and ash content of the empty body excluding the chemical component lipid only. Therefore, lipid content is simply the difference of FFS (%) from 100%. Protein and ash content were estimated from FFS (%), based on the knowledge that the composition of FFS is almost constant (Moulton, 1923; Robelin, 1973 and 1977). The described calculation of body composition from data using deuterium dilution technique has been recommended by Hörnicke (1959) and Susenbeth (1984).

For the second set of prediction functions, in which a different intercept was estimated for each weight group, body weight was not significant ($P > 0{\cdot}05$) for estimation of EBWA from TBWA and thus not considered. Then the same procedure was used as described for the first set of

functions but additionally fitting a different intercept for each weight group.

The following parameters were used to evaluate the precision of estimation according to Gu *et al.* (1992).

1. Correlation (r) between the predicted values ($\hat{Y}_i$) and observed values (Y_i) for each component.
2. The residual standard deviation was calculated as follows:

$$RSD = \left(\sum_{i=1}^{n} (e_i)^2 / n - p\right)^{\frac{1}{2}}$$

where e_i is the residual value for the ith observation, $n =$ number of observation and $p =$ degrees of freedom in the model.

Results

Means, standard deviations and coefficients of variation of the empty body composition analysed by chemical analysis and the change during the growth period are presented in Table 2. The protein and ash content were relatively constant with values in the range from 15·9 to 17·3% and 2·9 to 3·0%, respectively. The water content decreased from 74 to 53% during growth from 20 to 140 kg. This was due to an increase in lipid content from 7 to 30%.

The chemical composition of the three fractions (soft tissue, bones, and viscera) is given in Table 3. The change in chemical composition of the soft tissue fraction was similar as for the whole body composition. For this fraction, lipid showed highest coefficient of variation among animals. Protein content of bones was relatively constant (16·4 to 18·7%), whereas water content decreases from 66·6 to 48·0%. In contrast to water content, lipid and ash content rose from 6·7 to 16·9% and 9·7 to 16·5%, respectively. The viscera fraction showed highest water content and lowest protein content of all three fractions. Magnitude and change in lipid and ash content of the viscera fraction was similar to those of the soft tissue fraction.

Based on the difference between the mean chemical body composition between weight groups, the average deposition rates of protein, lipid, ash and water were calculated (Table 4). This assumes that each group was a random sample of the population. An increase in protein deposition rate was obtained between 60 and 90 kg live weight and in the last growth period (120 to 140 kg). Also for lipid, the deposition rate increased until the weight range of 60 to 90 kg, stayed almost on equal level during growth from 90 to 120 kg, and increased dramatically during the last period. Ash and water deposition rate increased up to 120 kg and then decreased substantially.

The results of the chemical analysis were used to obtain the prediction functions for estimation of body composition based on TBWA determined by deuterium dilution space. When fitting one intercept over all weight groups (first set of functions described in the following equations 2 to 5), the estimation of EBWA (%) analysed by chemical analysis showed the following prediction function on TBWA (%) and body weight (BW), where n is the number of animals used for this analysis, r is the correlation between predicted and chemically analysed components and RSD is the residual standard deviation:

$$EBWA(\%) = 17{\cdot}0957 \times TBWA(\%)^{0{\cdot}4131} \times BW^{-0.1141}$$

$$n = 48;\ r = 0.93;\ RSD = 2.91\%. \quad (2)$$

The actual FFS content measured by chemical analysis, composed of water, protein and ash content of the empty body, resulted in the following prediction function fitting EBWA content estimated in equation (2):

$$FFS(\%) = 3{\cdot}3270 \times EBWA(\%)^{0{\cdot}7730}$$

$$n = 48; \quad r = 0{\cdot}86; \quad RSD = 4{\cdot}37\%. \quad (3)$$

The protein and ash content were estimated based on the FFS (%) of the empty body estimated in equation (3). The actual percentage of protein of the FFS (XP_{FFS}) and actual percentage of ash of the FFS (XA_{FFS}) determined by chemical analysis showed the following prediction functions fitting FFS (%):

$$XP_{FFS}(\%) = 972{\cdot}81 \times FFS(\%)^{-0{\cdot}8804}$$

$$n = 48;\ r = 0{\cdot}83;\ RSD = 1{\cdot}05\% \quad (4)$$

$$XA_{FFS}(\%) = 255{\cdot}77 \times FFS(\%)^{-0{\cdot}9619}$$

$$n = 48;\ r = 0{\cdot}65;\ RSD = 0{\cdot}37\%. \quad (5)$$

The correlation between actual water content measured by chemical analysis and water content determined by

Table 2 *Body weight (BW), empty body weight (EBWT) and chemical composition of the empty body in different weight groups during growth using chemical analysis (with eight animals in each weight group)*

	Weight class (kg)																	
	20			30			60			90			120			140		
	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV
Chemical component																		
BW (kg)	20·2	2·25	0·11	32·8	2·14	0·07	62·6	2·60	0·04	92·8	3·46	0·04	116·5	1·91	0·02	142·4	4·02	0·03
EBWT (kg)	19·0	2·20	0·12	30·6	2·35	0·08	60·1	2·69	0·04	90·8	3·24	0·04	114·7	1·80	0·02	139·2	4·09	0·03
Water (%)	74·07	1·39	0·02	71·33	1·63	0·02	62·35	1·99	0·03	58·90	2·70	0·05	60·70	5·83	0·10	52·87	4·47	0·08
Lipid (%)	7·04	1·11	0·16	8·39	1·49	0·18	17·40	2·26	0·13	21·49	2·84	0·13	22·86	5·35	0·23	29·75	4·61	0·15
Protein (%)	15·91	0·81	0·05	17·32	0·70	0·04	17·15	0·85	0·05	16·58	0·30	0·02	16·07	1·08	0·07	15·88	1·16	0·07
Ash (%)	3·01	0·25	0·08	2·99	0·39	0·13	3·13	0·22	0·07	3·06	0·22	0·07	3·14	0·22	0·07	2·91	0·34	0·12

Table 3 *Chemical composition of the soft tissue and bones of the left carcass side as well as viscera fraction of the different weight groups during growth (with eight animals in each weight group) using chemical analysis*

	Weight class (kg)																	
	20			30			60			90			120			140		
Fraction	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV	Mean	s.d.	CV
Soft tissue																		
Weight (kg)	4·5	0·84	0·19	8·1	0·54	0·07	16·8	1·25	0·07	27·9	0·94	0·03	37·6	0·76	0·02	46·0	1·90	0·04
Water (%)	74·88	2·04	0·03	70·53	2·39	0·03	60·54	2·64	0·04	57·68	3·64	0·06	57·48	5·02	0·09	49·98	4·51	0·09
Lipid (%)	8·10	1·76	0·22	10·33	2·19	0·21	20·94	3·16	0·15	24·64	3·84	0·16	25·36	6·05	0·24	33·29	5·65	0·17
Protein (%)	16·05	1·20	0·07	18·16	1·04	0·06	17·66	0·97	0·05	16·87	0·36	0·02	16·35	1·07	0·07	16·01	1·50	0·09
Ash (%)	0·97	0·03	0·03	0·99	0·03	0·03	0·85	0·03	0·04	0·81	0·04	0·05	0·81	0·06	0·07	0·73	0·06	0·08
Bones																		
Weight (kg)	1·9	0·23	0·12	2·8	0·18	0·06	4·6	0·32	0·07	6·4	0·37	0·06	8·0	0·59	0·07	8·8	0·47	0·05
Water (%)	66·55	1·74	0·03	63·46	1·37	0·02	55·80	2·08	0·04	52·95	2·48	0·05	51·33	3·90	0·08	47·96	2·38	0·05
Lipid (%)	6·65	1·16	0·17	7·54	0·93	0·12	12·46	0·51	0·04	13·90	1·88	0·14	15·98	2·07	0·13	16·85	1·27	0·08
Protein (%)	17·11	0·68	0·04	18·09	0·47	0·03	17·98	1·04	0·06	18·00	0·70	0·04	16·36	3·39	0·21	18·65	1·11	0·06
Ash (%)	9·69	1·07	0·11	10·91	1·48	0·14	13·76	1·28	0·12	15·15	1·30	0·09	16·34	1·12	0·07	16·54	1·85	0·11
Viscera																		
Weight (kg)	2·8	0·44	0·16	4·6	0·36	0·08	7·4	0·56	0·08	10·2	0·70	0·07	12·0	1·23	0·10	14·1	1·40	0·10
Water (%)	81·74	1·04	0·01	81·09	1·51	0·02	73·89	1·71	0·02	68·42	3·31	0·05	64·37	6·41	0·10	57·00	4·61	0·08
Lipid (%)	4·19	0·78	0·19	5·09	0·92	0·18	12·14	1·84	0·15	18·29	3·89	0·21	23·52	7·67	0·33	31·78	5·79	0·18
Protein (%)	13·14	0·59	0·05	12·89	0·87	0·07	13·11	0·85	0·06	12·47	0·64	0·05	11·24	1·58	0·14	10·55	1·17	0·11
Ash (%)	0·93	0·05	0·05	0·93	0·06	0·06	0·86	0·05	0·06	0·82	0·07	0·09	0·88	0·27	0·31	0·67	0·06	0·09

deuterium dilution space was high with $r = 0{\cdot}93$. Also, FFS (%) measured by chemical analysis showed high association ($r = 0{\cdot}86$) with EBWA (%) estimated from deuterium dilution space. The accuracy for the estimation protein content of FFS was only slightly lower than the accuracy of estimation of FFS (%). Lowest correlation ($r = 0{\cdot}65$) was obtained for the estimation of ash (%) of the FFS. The RSD of estimation of FFS (%) was higher than those for the other body components.

The second set of functions, described in the following equations 6 to 9, was created to estimate body composition by the deuterium dilution technique with a different intercept for each weight group. The *b* value of the allometric function was the same over all weight classes only the intercept changed. For the determination of TBWA the same equation (1) described for the first set of functions was used. Then, the chemically analysed body components showed the following functional associations with the deuterium dilution space estimated components:

$$\text{EBWA}(\%) = a \times \text{TBWA}(\%)^{0{\cdot}3276}$$

$$n = 48;\ r = 0{\cdot}95;\ \text{RSD} = 2{\cdot}62\% \qquad (6)$$

Table 4 *Protein, lipid, ash and water deposition rates using chemical analysis*

	Weight range (kg)				
	20–30	30–60	60–90	90–120	120–140
Chemical component					
Protein (g/day)	110	109	127	117	147
s.e.	8·2	7·2	9·6	14·8	30·9
Lipid (g/day)	61	173	243	220	636
s.e.	10·4	12·2	66·6	70·7	137·7
Ash (g/day)	17	21	24	27	17
s.e.	2·4	1·6	3·4	3·6	7·9
Water (g/day)	386	340	430	443	187
s.e.	37·9	19·9	55·6	59·0	110·5

where *a* is 17·61, 17·04, 15·51, 14·72, 14·73 and 13·44 for the 20, 30, 60, 90, 120 and 140 kg weight class, respectively.

$$\text{FFS}(\%) = a \times \text{EBWA}(\%)^{0{\cdot}1350}$$

$$n = 48;\ r = 0{\cdot}93;\ \text{RSD} = 3{\cdot}14\% \qquad (7)$$

where *a* is 52·13, 51·48, 47·27, 45·26, 44·24 and 41·06 for the above described weight classes, respectively.

$$\text{XP}_{\text{FFS}}(\%) = a \times \text{FFS}(\%)^{0{\cdot}0860}$$

$$n = 48;\ r = 0{\cdot}92;\ \text{RSD} = 0{\cdot}77\% \qquad (8)$$

where *a* is 11·57, 12·82, 14·19, 14·52, 14·34 and 15·67 for the above described weight classes, respectively.

$$\text{XA}_{\text{FFS}}(\%) = a \times \text{FFS}(\%)^{2{\cdot}7687}$$

$$n = 48;\ r = 0{\cdot}75;\ \text{RSD} = 0{\cdot}32 \qquad (9)$$

where *a* is $1{\cdot}15 \times 10^{-5}$, $1{\cdot}20 \times 10^{-5}$, $1{\cdot}86 \times 10^{-5}$, $2{\cdot}21 \times 10^{-5}$, $2{\cdot}43 \times 10^{-5}$ and $3{\cdot}19 \times 10^{-5}$ for the above described weight classes, respectively.

The correlation between analysed and predicted body components increased by 8, 11 and 15% for FFS content of the empty body, protein content of the FFS, and ash content of the FFS, respectively, using a different intercept for the weight classes in comparison to using one intercept over the entire growth period.

Figure 1 shows the chemical analysed EBWA and the estimated EBWA by the deuterium dilution technique. The differences between estimated EBWA using deuterium dilution technique and chemically analysed EBWA was highest at low empty body water. Lowest EBWA was obtained at the end of the growth period and was associated with an extreme increase in lipid.

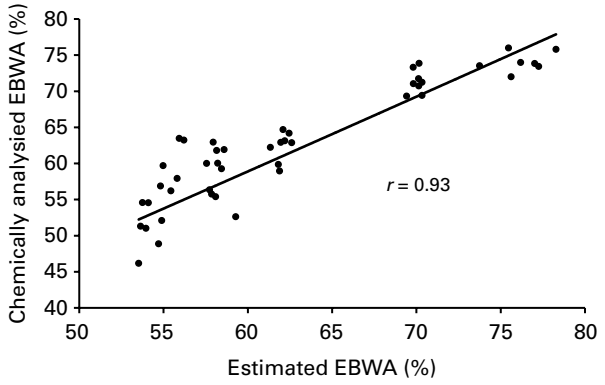

Figure 1 The relationship between chemical analysed empty body water (EBWA) and estimated EBWA determined by deuterium dilution technique fitting an exponential function with one intercept for the entire growth period.

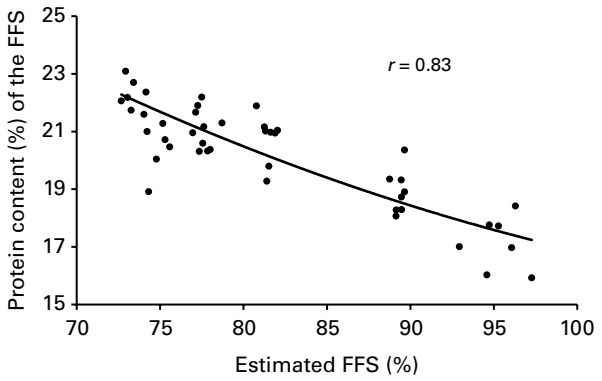

Figure 3 The relationship between chemical analysed protein content of the fat free substance (FFS) and estimated FFS determined by the deuterium dilution technique fitting an exponential function with one intercept for the entire growth period.

Figure 2 shows the increase in FFS (%) content with increasing estimated EBWA (%), which was determined by deuterium dilution technique. At high body weight, a low EBWA content was reached ranging from 52 to 60%. This was due to the high lipid content of animals. As a consequence of high variation of lipid at low EBWA (%), the variation of FFS (%) was also high. Additionally, at low EBWA (%), the highest difference between estimated FFS (%) and chemical analysed FFS (%) from the serial slaughter trail was obtained.

The protein content of FFS analysed by chemical analysis decreased with increasing estimated FFS (%) determined by deuterium dilution technique (Figure 3). The differences between chemically analysed and estimated protein content of the FFS were almost randomly distributed over the entire range of FFS (%). Also, the ash content of FFS analysed by chemical analysis decreased with increased estimated FFS (%) determined by deuterium dilution technique (Figure 4). However, the correlation between chemically analysed and from FFS (%) estimated ash content was much lower than for protein content.

Discussion

The results of composition of empty body are in accordance with Shields *et al.* (1983), Wagner *et al.* (1999) and Möhn and De Lange (1998). Especially the development of protein content was similar. In the first growing phase of our experimental pigs, protein content of the empty body increased from 16·0 to 17·3% between 20 and 30 kg body weight. Shields *et al.* (1983) found an increase from 15·2 to 16·3% between 6 and 36 kg body weight. Wagner *et al.* (1999) reported an increase from 13·8 to 17·5% between 26 and 63 kg body weight and Möhn and De Lange (1998) from 16·5 to 17·4% between 25 and 71 kg body weight. The increase in protein content of the empty body in the first growing phase (20 to 30 kg) and its decrease thereafter can be explained by the development of this component in the FFS. In the FFS, the percentage of protein increased continuously with 17% at 20 kg to 22% at 140 kg (Figure 3) while water content decreased. In the first growing phase (20 to 30 kg) this increase in protein content was higher than the increase in lipid deposition. After 30 kg body weight, there was a substantial increase in lipid deposition,

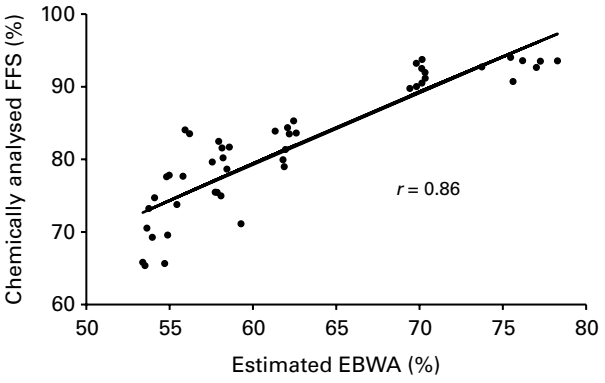

Figure 2 The relationship between chemical analysed fat free substance (FFS) and estimated empty body water (EBWA) determined by deuterium dilution technique fitting an exponential function with one intercept for the entire growth period.

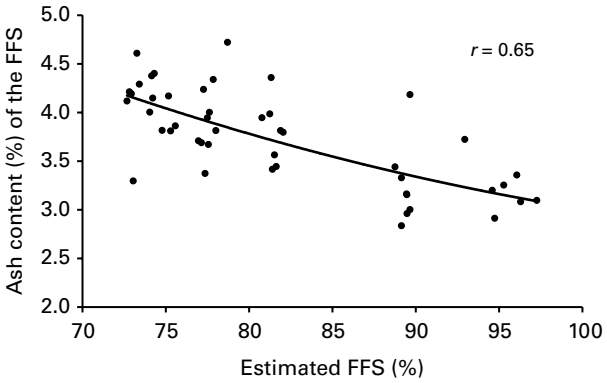

Figure 4 The relationship between chemical analysed ash content of the fat free substance (FFS) and estimated FFS determined by the deuterium dilution technique fitting an exponential function with one intercept for the entire growth period.

which over-compensates the increase in protein content of the FFS so that the protein content relative to the empty body decreased (Table 2). In our study the increasing protein content of the empty body results primarily from the increasing in protein content of the soft tissue fraction from 16 to 18%. During a test period between 15 and 115 kg, Susenbeth (1984) found an increase in lipid content of the empty body from 8 to 28%, a decrease in water and protein content of the empty body from 71 to 53% and 17 to 15%, respectively. This high percentage of lipid content was in the present study obtained at a much higher weight of 140 kg body weight, probably due to selection for low fatness at around 100 kg body weight, which seems to have postponed lipid accretion to a later stage of development. Puberty of the female animals may have partly contributed to this rapid increase in lipid accretion. However, the relative amount among body components seems to be consistent to those of Susenbeth (1984).

With increasing body weight, the composition from soft tissue, as the largest fraction of total body, resulted in the same trend as the composition of the total empty body. Lipid content showed highest coefficient of variation in comparison with corresponding values of water, protein and ash content. Individual differences between animals with regard to lipid content resulted in high coefficients of variation. In a stress-free environment with an adequate accommodation with nutrient and considering an marginal minimal lipid to protein deposition ratio depending on the breed of interest, the protein deposition depends on energy intake above the maintenance and the genetically determined protein deposition (PD_{max}) (Möhn and De Lange, 1998). The protein deposition increases with rising energy intake up to a genetically determined PD_{max} (Whittemore and Fawcett, 1976; Moughan and Verstegen, 1988). If PD_{max} is reached, ME is mostly used for lipid deposition. Different genetic potential for protein deposition and food intake capacity can be the reason for the variation of lipid content between animals in higher ages. As expected, high magnitude of ash content was found in bones (10 to 17%). In this fraction, protein and lipid content reached almost similar percentages as the ash content starting from 17 and 7%, respectively. These high contents of protein and lipid can be explained by the low age of the animals. Because these pigs were far from maturity, collagen content of bones is expected to be high. The extreme rise in lipid content in the viscera fraction resulted mainly from the abdominal fat. High protein and lipid deposition rates were obtained between 60 and 90 kg of body weight. After a slight reduction between 90 to 120 kg of body weight, deposition rates substantially increased again until 140 kg. The high protein deposition rate at 60 to 90 kg corresponds well with estimates using enhanced breeds tested from 25 to 125 kg (Eissen, 2000). However, the increase in protein deposition rate after 120 kg in the present study is interesting. The slight reduction in lipid deposition in the range from 90 to 120 kg body weight was also found by Eissen (2000). This may be due to sexual maturity, physiological changes etc. resulting in a reduced food intake. In the present trial, the extreme increase in lipid deposition during 120 to 140 kg indicates that crosses with Piétrain (used as a lean sire line) still result in high lipid accretion when optimal slaughter weight was passed. In addition, this high lipid accretion was associated with high protein deposition. The first set of functions to estimate chemical body composition over the entire growth period showed slightly lower correlations than the second set of functions for which a different intercept was estimated for each weight group. Generally, EBWA and FFS were more accurately estimated than protein content and in particular ash content. Although the accuracy of the second set of functions was slightly higher, the first set of functions has two main advantages. The first set of equations resulted in continuous estimates of body composition over the entire growth period. In contrast, estimates using the second set of functions showed high differences in body composition between slightly different body weights belonging to two different weight classes. For example, using the second set of functions, at 38·5 kg body weight, 10% higher lipid content was estimated than for 38 kg body weight. These steps in body composition between weight groups are of high disadvantage for an analysis over an entire growth period. Also the variance in body composition among animals is highly reduced by using the second set of functions, in which equations are available at every weight class. Therefore, the first set of functions may be the more general choice for estimation of body composition. The second set of equations may only be used when the weights are similar to those of the pigs in our experiment.

The validity of the D_2O dilution technique rests upon the assumption that defined relationships between chemical body components exist. The principle is based on a two-compartment model, which divides the entire empty body into lipid and FFS. The FFS showed an almost consistent composition. Therefore, for this analysis the best method is expected to be based on the principle provided by Hörnicke (1959) and Susenbeth (1984). First, the EBWA (%) was determined, which then was used to estimate FFS (%) of the empty body weight. Based on the FFS (%), the protein and ash content of the FFS can be accurately estimated. Lipid content is simply the difference FFS (%) from 100%. EBWA (%) estimated as allometric function of TBWA (%) (determined by D_2O space) and body weight resulted in a high accuracy. The study of Rozeboom *et al.* (1994) estimated EBWA (kg) based on linear regression on body weight and D_2O space and resulted in similar accuracy ($r = 0{\cdot}94$ and 0·99 depending on breed) than in the present study. Again, using linear regression, Rozeboom *et al.* (1994) achieved accuracies for prediction of lipid mass based on the predictors body weight and D_2O space of $r = 0{\cdot}98$ and 0·97 for the analysed breeds. The same model was selected by Knudson (1986 and 1990) and Ferrell and Cornelius (1984). Our model, obtaining lipid content as the difference of FFS (%) from 100%, showed a smaller correlation ($r = 0{\cdot}86$) than those reported by Rozeboom *et al.* (1994). In the present study, the estimation of protein and ash content showed a lower accuracy than the estimation of EBWA (%) and FFS (%). Rozeboom *et al.* (1994) reported higher correlations for the estimation of protein mass of the empty body with $r = 0{\cdot}93$ and 0·99 and for ash mass with $r = 0{\cdot}63$ and 0·92. However, the prediction equations of the present study were derived on percentages of body composition in order to avoid the high autocorrelation between body weight and chemical body compositions in units of

weights. This autocorrelation resulted in high accuracy of estimation of chemical body composition (kg) even when body weight (kg) was the only predictor in the linear regression model of Rozeboom *et al.* (1994). Therefore, the correlations in the present study have to be interpreted as highly accurate given that the relative body composition was estimated. Also, it should be mentioned that a comparison of accuracies of prediction obtained in different studies has to be interpreted carefully, because different populations, different weight ranges, different models, etc. are used.

In the present study, the second set of functions resulted in a slightly higher accuracy than the first set of functions. This indicates the influence of body weight on the estimation, which was fitted as a different intercept for each weight group in the second set of functions. Figures 1 to 4 show estimates of the first set of functions in relation to the variables from which they are determined. Overall, the curves showed a good fit to the values of the chemical analysis, as also indicated by the accuracy ($r = 0{\cdot}65$ to $0{\cdot}93$). Therefore, these functions are very valuable to estimate body composition of pigs, when D_2O dilution space is recorded. In further analyses, these functions will be used to determine the protein, lipid, water and ash deposition of live pigs in order to use these estimates in a biological growth model. This can be used to optimize selection for food intake capacity, estimation of nutrient requirements, optimal slaughter weight, improvement of the production system, etc.

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