The use of imaging to learn on piglet level of development

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Abstract

Piglet maturity at birth is defined as the level of full development, which is a major determinant of postnatal survival. Our objective was to identify biometric indicators of the level of piglet maturity using external body length measurements. Dead piglets were fixed on a board to be photographed in a standardised way. Images were subsequently analysed using ImageJ software (Abramoff et al., 2004). Measurements included body length, body width, body surface, head surface, skull length, head length and head width. In addition, it was possible to measure humerus, femur, tibia and foot lengths because these bones were clearly visible through the skin. Body measurements enabled us to compare allometric development of 312 progeny from contrasting breeds for maturity at birth: the Meishan (MS) and the Large White (LW). A crossbreeding design with use of mixed semen from the two breeds enabled us to study differences in development between purebreds (PB) and crossbreds (CB) developing in a LW or a MS uterine environment. Similar allometric slopes (>0.70) for body width and femoral length relative to body length were found at 90d dvp in LW PB and MS PB, but null slopes observed at 110d dvp in MS PB indicated a slowing of development. Head development did not differ between genetic types. In conclusion, body development was slower in MS PB than in other genetic types in late gestation, indicating that full body development was reached in MS PB only.

Key-words: Image analysis, biometrics, allometry, piglet development, crossbreeding effects

Introduction

Pig breeders constantly express concern about the high level of piglet losses that occur in the neonatal period. The heritability of piglet survival traits is too low to expect a direct genetic improvement. Selection for lean growth rate and litter size has had a negative impact on the physiological status of piglets at birth (Canario

et al., 2007). Genetics have a strong impact on the so-called level of maturity at birth, as shown by Herpin et al. (1993) in a comparison of Meishan (MS) piglets with White breeds of piglet. Prenatal development is a predisposing factor to perinatal losses, which have increased in recent decades in parallel with selective breeding. Due to heterosis effects, crossbred (CB) piglets have higher vigour than purebred (PB) piglets (Sellier 1970). In order to decipher the genetic roots underlying piglet maturity, the use of imaging was proposed to facilitate biometric analysis of 312 piglet foetuses obtained from crossbreeding of two contrasting breeds for piglet vitality at birth. The comparison was therefore based on PB and CB developing in a LW or a MS dam uterine environment.

Materials and methods

Data

The experiment was carried out in the INRA experimental herd at Le Magneraud (INRA GENESI, Charentes-Maritimes, France). Sows were in their second gestation. Caesarean sections were performed at 90 d (+1d) and 110 d (+/- 1d) of gestation. Foetuses were euthanised at birth by IV injection of 5 ml saturated potassium chloride solution, and the inert body was moved to a different location for macroscopic examination. Dead piglets were fixed on a board to be photographed in a standardised way. The body weight (BW) of each foetus was then measured.
The reason for using two types of semen mixed in equal proportions to inseminate each sow was to obtain ideally 50% purebreds and 50% crossbreds within a litter. The population, including 312 foetuses from 9 LW sows and 10 MS sows, is described in Table 1.

Table 1 – Mean characteristics (SD) for second parity Large White (LW) and Meishan (MS) sows producing both purebreds and crossbreds within the same litter

Sow breed	LW	LW	MS	MS
Gestation stage (j)	90	110	90	110
N	6	3	7	3
Litter size at caesarean	16,5	19,2	15,1	13,6
N purebred piglets	78	49	31	16
N crossbred piglets	23	10	77	28

Image analysis

Images were subsequently analysed using ImageJ software (Abramoff et al., 2004). Image capture was standardised, as a) all foetuses were placed in the same position on the work area, b) a reference measuring tape was used at a consistent position from the head, and c) the camera was fixed at a standardised distance from the work area to minimise measurement inaccuracies. Measurements included body length, body width defined as the distance between a tangent line from the top of the back to the rear of the front leg (Figure 1), body surface, head surface, skull length, head length (= skull length + snout length) and head width defined as the distance between a tangent line from the top of the head to the throat. It was also possible to measure humerus, femur, tibia and foot lengths because these bones were clearly visible through the skin. In the event of uncertainty due to a non-optimal position of the foetus on the board, the measurement was disregarded.

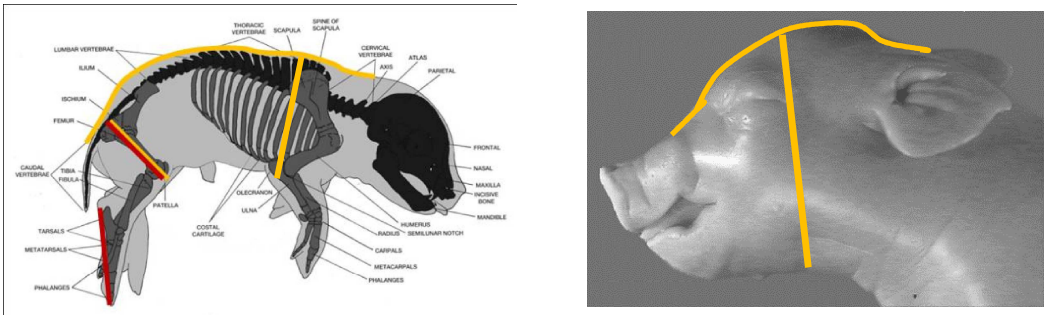

Figure 1. Left: Illustration of foetal body and bone length measurements from image analysis; Right: Measurement for head width to head length ratio.

Biometric analysis

All the measurements collected were tested as biometric indicators of maturity at birth.

Statistical analysis

The statistical methodology was inspired by Vallet and Freking (2006). To compare phenotypic differences between the four genetic types, raw data were analysed after natural log transformation. The model for analysis under the PROC MIXED procedure of SAS (SAS Institute, 2006) included the fixed effect of sex, the interaction between genetic type and age of development (dvp), the percent of PB in the litter and litter size as covariates, and the sow random effect.

Bivariate allometry relating body or skeletal length (y) to body length (x) in each line was analysed by Huxley's (1932) allometric equation transformed to natural logarithms $ln\, y = ln\, a + k\, ln\, x$ where $ln\, a$ is the intercept and k is the regression coefficient. The slope that is the coefficient of allometry defines a simple constant of proportionality, and the intercept represents a scaling coefficient (White and Gould, 1965). The model for analysis of allometric growth relative to body shape included the fixed effect of gender, the interaction between genetic type and age of dvp, the linear effect of the log of foetal BL, the genetic type x age x linear effect of foetal BL interaction, the percent of PB in the litter and litter size as covariates, and the sow random effect.

Results and discussion

Table 2 – Estimates for biometric measurements of purebred and crossbred foetuses which developed in the same maternal Large White (LW) or Meishan (MS) environment

	Age (d)	**LWxLW**	**MSxLW**	**LWxMS**	**MSxMS**		**LWxLW vs MSxLW**	**MSxMS vs LWxMS**
		lsmean	lsmean	lsmean	lsmean	RSD	Prob(D=0)	Prob(D=0)
BW (g)	90	524.95 e	602.15 e	579.58 e	500.55 e	177		0.10
	110	1166.78	1218.04	1065.07	903.16			
Body length (cm)	90	18.53 e	18.62 e	18.58 e	16.77 e	1.66		<.0001
	110	23.91	23.08	22.91	21.36			0.11
Body width (cm)	90	3.50 e	3.94 c	4.03 b	3.90	0.49	0.001	
	110	4.78	4.93	4.66	4.53			
Body surface (cm²)	90	92.56 e	104.74 c	104.63 b	95.34 a	21.33		
	110	148.06	150.51	136.67	123.26			
Head length (cm)	90	10.86 e	11.24 e	11.19 e	10.89 e	0.63		
	110	13.33	13.56	13.14	12.96			
Head width (cm)	90	6.02 e	6.21 d	6.47 e	6.34 e	0.36		
	110	6.80	6.85	7.24	7.16			
Humerus length (cm)	90	3.96 e	4.19 e	4.27 e	3.99 d	0.44		0.04
	110	5.36	5.34	5.19	4.79			
Femur length (cm)	90	6.17 e	6.67 d	6.42 e	6.19 d	0.63	0.02	
	110	7.91	8.23	7.74	7.47			
Foot length (cm)	90	4.08 e	4.29 e	4.43 e	4.11 e	0.45		0.01
	110	5.56	5.52	5.51	5.11			

Level of significance for changes from 90d to 110d dvp: a: p<0.10; b: p<0.05; c: p<0.01; d: p<0.001; e: p<0.0001. RSD: residual standard deviation.

The comparison focused on 1) within-genetic type changes from 90d to 100d dvp and 2) differences between PB and CB genetic types developed in a given dam breed environment.
In LW sows, PB and CB did not differ in terms of BW and BL (Table 2). In MS sows, CB foetuses tended to be heavier than PB at 90d dvp and to be longer than PB at 90d and 110d dvp. In LW sows, the thorax was 11% thinner in PB than in CB and the difference between the two genetic types was not significant at 110d dvp. With regard to the ponderal index (BW/BL^3), we observed in MS PB only that BW increased at the expense of BL at 110d dvp, so that BL reached a plateau before birth in that genetic group. LW PB had a shorter femoral bone (7.5%) at 90d dvp than their CB counterparts but this contrast was offset at 110d dvp. A large increase in head dimensions from 90d to 110d dvp was found in the four piglet genetic types, with increases of 20% in head length and 12% in head width. We proceeded with the comparison of body development in proportion to BL or head length.

Table 3 - Linear slopes ± SE for the relationships between the log of body (head) size and the log of body (head) length in purebred and crossbred foetuses developed within the same maternal Large White (LW) or Meishan (MS) environment

	Age (d)	**LWxLW**	**MSxLW**	**LWxMS**	**MSxMS**	**LWxLW vs MSxLW**	**MSxMS vs LWxMS**
		lsmean	lsmean	lsmean	lsmean	Prob(D=0)	Prob(D=0)
Head width	90	0.35 ± 0.10 b	0.09 ± 0.27 b	0.35 ± 0.11	0.05 ± 0.18		
	110	0.65 ± 0.10	1.32 ± 0.51	0.48 ± 0.19	0.38 ± 0.36		
Body Width	90	0.83 ± 0.13 a	0.76 ± 0.40	0.85 ± 0.16	0.73 ± 0.22 a		
	110	1.17 ± 0.14	1.33 ± 0.54	1.08 ± 0.23	0.08 ± 0.32		0.008
Femur length	90	1.00 ± 0.08	0.55 ± 0.25 a	0.92 ± 0.10	0.83 ± 0.14 b	0.08	
	110	1.10 ± 0.08	1.25 ± 0.33	1.09 ± 0.15	0.29 ± 0.19		0.0006

Level of significance for changes from 90d to 110d dvp: a: p<0.10; b: p<0.05; c: p<0.01; d: p<0.001; e: p<0.0001.

Allometric analyses are shown in Table 3. Proportional changes in foetal bone size with changes in foetal size (i.e. isometric changes) are reflected by a slope of 1. A slope greater than 1 is indicative of a positive allometry, i.e. foetal tissue dvp is enhanced compared to foetal whole body dvp. Conversely, a slope of less than 1 refers to a negative allometry and is indicative of a sparing effect, i.e. foetal tissue dvp is slower than foetal whole body dvp.
A negative allometry on head dvp was observed in the four foetal genotypes at 90d dvp. The acceleration of head widening relative to head lengthening was high in LW CB and moderate in LW PB. The analysis of body width relative to BL indicated that the dvp of LW PB was accelerated in late gestation ($p<0.05$). At 110d dvp, an abrupt change in the allometric slope towards zero was observed in MS PB. This slowing of dvp suggests full dvp in MS PB. The PB and CB dvp was more heterogeneous in MS sows ($p=0.008$) than in LW sows. The trend for delayed femoral bone dvp relative to BL in LW CB as compared to LW PB detected at 90d dvp ($P=0.08$) changed to isometry at 110d. In MS sows, a difference between PB and CB in femur lengthening relative to body lengthening was found at 110d dvp. Maximum femur length relative to breed standards at birth was likely to be reached in MS PB.

Conclusions

Our study validated the suitability of imaging to analyse piglet developmental level. Rapid development occurred at the end of gestation with some acceleration perceived in LW CB. Only MS PB foetuses achieved full body size development before birth. Several measurements were validated as biometric indicators of maturity at birth.
In this study, we identified new criteria that could be included in the breeding goal of maternal lines, as a means of limiting delayed maturity at birth. We are now confident about the validity of several new traits for routine recording on the farm. The genetic determinism of these new phenotypes is under study. To save time and facilitate data acquisition by farmers, the next step will be to develop a tool which can be used to take pictures or measurements automatically. Different technologies are suitable, including sensors coupled with ISO electronic devices which enable individual piglet identification. Development of algorithms to extract the relevant information will also be necessary. The outcome will be provide a reliable tool system for individual recording of maturity data in young animals which could be used on a large scale in breeding herds for the acquisition of high-throughput phenotypes.

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References

Abramoff M.D., Magelhaes P.J., and Ram S.J. 2004. "Image Processing with ImageJ". *Biophotonics International* **11**(7) 36-42.

Canario L., Père M.C., Tribout T., Thomas F., David C., Gogué J., Herpin P., Bidanel J.P., and Le Dividich J. 2007. Estimation of genetic trends from 1977 to 1998 of body composition and physiological state of large white pigs at birth. *Animal.* **1**(10) 1409-1413.

Herpin P, Le Dividich J, and Amaral N. 1993. Effect of selection for lean tissue growth on body composition and physiological state of the pig at birth. *Journal of Animal Science* **71**(10) 2645-53.

Huxley, J. S. 1932. *Problems of relative growth.* Methuen: London. Reprinted 1972, Dover Publications: New York.

Sellier P. 1970. Hétérosis et croisement chez le porc. *Annales de Génétique et Sélection Animale.* **2** 145-207.

Vallet, J.L., and Freking, B.A. 2006. Changes in fetal organ weights during gestation after selection for ovulation rate and uterine capacity in swine. *Journal of Animal Science* **84** 2338-2345.

White J.F., and Gould S.J. 1965. Interpretation of the coefficient in the allometric equation. *American Naturalist* **99** 5-18.