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RESEARCH ARTICLE

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Colostrum production of Alentejano and Large-White × Landrace sows: consumption, passive immunity and mortality of piglets

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Abstract

Colostrum production and composition of 45 Alentejano (AL) and 45 crossed (Large–White × Landrace, LL) sows together with consumption and passive immunization of piglets, were evaluated. Piglets were weighed at birth, 24h, and 21 and 28 days of age. Colostrum was collected, chemically analysed and immunoglobulin G (IgG) determined. On piglets, serum IgG concentrations were determined at 2d and 28d of age. Alentejano piglets consumed 19% less colostrum than LL piglets (267 *vs* 328 g; $p<0.001$), but when adjusted for birth weight, the difference between genotypes was not significant ($p=0.891$). Alentejano sows produced less colostrum (1985±139 g *vs* 3761±139 g, $p<0.001$) than LL sows, even after adjustment for litter birth weight. IgG concentrations were higher in AL colostrum at 12 h and 36 h after the onset of farrowing. They were higher in AL than in LL piglets ($p=0.025$) and higher at 2d than at 28d of age ($p<0.001$). Mortality rate of piglets until d21 post–farrowing was higher in AL genotype (27% *vs* 16.1%, $p<0.001$). In both genotypes main factors influencing piglet mortality were birth weight and colostrum intake (g/kg birth weight). Higher mortality in AL than in LL genotype was attributable to the higher proportion (28.7% *vs* 11.6%, $p<0.001$) of light piglets. It is concluded that for similar birth weight, both AL and LL piglets have a similar ability to ingest colostrum and that AL sows are less able to produce colostrum than LL sows. IgG concentrations of colostrum and of piglets serum were higher in AL than in LL genotype.

Additional key words: neonatal pig; birth weight; birth order; colostrum composition; immunoglobulin G; survival.

Abbreviations used: AL (Alentejano swine breed); AUC (Area under curve); BW0 (piglet birth weight); BWa (piglet weight at second weighing); CI (colostrum intake); COLP (colostrum production); CP (crude protein); CV (coefficient of variation); DE (digestible energy); DM (dry matter); E (experimental); IgG (immunoglobulin G); IgG2 (serum IgG at 2 days of age); IgG28 (serum IgG at 28 days of age); LIP (total lipids); LL (Large–White × Landrace genotype); LS (litter size); LWB (litter weight at birth); RBO (relative birth order); SE (standard error); SEM (standard error of mean).

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Introduction

The Alentejano pig belongs to the Iberian breed raised all over the Southwest region of the Iberian Peninsula. The breed is raised mainly in an extensive system under oak canopy (green and cork) in the Alentejo region (Portugal). Compared to commercial crosses, the Iberian sow is less prolific (Vázquez *et al*., 1994; Marques, 2001) while the pre–weaning mortality of piglets is high, ranging from 20 to 28% (Marques *et al*., 1996; Robledo *et al*., 2008). Further, the Iberian sow has a shorter gestation length (≈ 4d, Charneca *et al*., 2012) which, however, does not affect the energy stores and the physiological maturity of the newborn (Charneca *et al*., 2010).

Because of the important commercial value of the Iberian pig (for a review, see Lopez–Bote *et al*., 1998), most of research has been devoted to the finishing period. To our knowledge, less it is known on the development of the neonatal pig. Data on milk composi-

tion and production of Iberian sows are scarce and even more limited on colostrum composition, consumption of piglets, and production of sows (Aguinaga *et al*., 2011; Castellano *et al*., 2013). Yet, pigs are born with no immune protection (Wagstrom *et al*., 2000) and with low body energy stores (Mellor & Cockburn, 1986). Therefore, it is vital that the newborns ingest adequate amounts of colostrum to establish a robust passive immunity and to ensure sufficient supply of energy for metabolism.

The objectives of this experiment were to determine in the Alentejano pig breed, piglet consumption of colostrum, colostrum production and composition, the acquisition of colostral immunity, and the survival of piglets in comparison with a commercial cross (Large White × Landrace).

Material and methods

The experiment was carried out at the Experimental Centre of Mitra (E) of the University of Évora and at two private farms in Portugal. It was conducted according to the European Community regulations concerning the production of experimental animals (Directive 86/609/EEC; EC, 1986).

Animals and management

Forty–five purebred Alentejano (AL), 23 at E site and 22 in a private AL farm and 45 Large–White × Landrace (LL) sows, 22 at E site and 23 in private LL farm and their litters were used. Parity of sows ranged from 1 to 10.

Housing, management and feeding of the sows were previously described by Charneca *et al*. (2012). Briefly, Large–White × Landrace females were mated by Large White boar at the E site or inseminated with Piétrain semen in the LL private farm. Alentejano sows were mated by AL boars in both sites but mating dates were only controlled at E site.

All LL sows were kept in indoor facilities during the gestation period and maintained in groups of 3 to 4 sows (E site) or in individual crates on a concrete slatted floor (private farm). Alentejano sows were also maintained in groups of 3 to 4 sows in indoor facilities (E site) until gestation was confirmed. Thereafter, they were moved to an outdoor park where they had access to huts. In the private AL farm all gestation period took place in an outdoor park. Irrespective of genotype or production site, on day 105±1 of gestation all sows were moved to the farrowing house and placed in individual farrowing crates. Although at E site AL sows used to farrow in outside huts, in this study farrowing took place in indoor farrowing houses due to experimental purposes (piglets weighing, colostrum collection, piglet bleeding). All farrowing rooms had an ambient temperature between 20ºC and 23ºC. Piglets were provided with local heating (175 W infrared lamps) in the creep area. Details on gestation and lactation diets and also creep feeding of the piglets are given by Charneca *et al*. (2012). Diets were the usual at the farms and were not modified for experimental purposes. Briefly, LL sows were fed twice a day with commercial gestation (14–16% CP; 13–13.4 MJ DE) and lactation (17–18% CP; 13.8 MJ DE) diets based on cereals and soybean meal. They were not fed at farrowing day and afterwards feed supply was gradually increased until *ad libitum*. Alentejano sows at E site were fed with the same diets than LL sows when they were in indoor facilities, in outdoor conditions (second and third month of pregnancy) additionally to the commercial feed they had access to unquantifiable amount of natural feedstuffs. During autumn/winter gestations, some AL sows (7/23), had unlimited access to acorns. Alentejano sows at private farm were fed with wheat (2–2.5 kg/day) during free range gestation period and commercial lactation diet (16% CP; 13.8 MJ DE) since the last week of pregnancy. During lactation feed allowance was gradually increased until ad libitum at E site and until 3kg/day at private farm. In all facilities, water was available for sows and piglets from a low–pressure nipple–drinker.

Farrowing surveillance, and data and samples collection

Gestation lengths were only determined at the E site and at the private LL farm. Three LL sows from the private farm had abnormally long farrowing (> 9h). They were excluded from the analyses. All farrowings were attended but none was induced. Soon after birth, piglets had their umbilical cord cut at about 10–12cm from the navel after which, they were identified, roughly dried, weighed to the nearest 1g (birth weight, BW0), using an electronic balance equipped with an integration system, and returned to the sow at birth place. The birth time was recorded. These operations were performed very quickly, usually within 2–3 min of birth. All piglets were re–weighed at 24h, 21d, and 28d (the private LL farm excepted) after birth. Piglet weight and approximate time of death were also recorded. Because of complementary studies, 6 AL and 7 LL piglets were euthanized at birth. No cross–fostering was practised before the 24h weighing. Thereafter,

due to sow capacity to nurse, 40 LL piglets averaging 1246±47g in weight from litters having 9 to 20 born alive piglets, were fostered to non experimental sows. Considering the piglets losses in the first day and the cross–fostering (only in LL), at 24h there were 307 AL and 465 LL piglets with experimental sows.

Study of colostrum composition and piglet immunisation was only possible at the E site. Colostrum and milk samples (30–40 mL) were manually collected from most teats of 17 AL and 17 LL sows. The first colostrum sample was taken right after the birth of the first piglet (time 0) and then 3, 6, 12, 24 and 36h later. All samples were immediately filtrated on gauze and stored at–20ºC until analysed. Sows were intramuscularly administered 20 IU of oxytocin (Facilpart, Syva, Léon, Spain) to induce colostrum release from 3h onwards.

Blood samples (1.0–1.5 mL) were taken at 2 and 28d of age by vena cava puncture on all AL and LL piglets. Blood was allowed to clot at room temperature, centrifuged (1400 g), then the serum was removed and kept at–20°C until analysed.

Estimation of colostrum intake of piglets

Colostrum intake (CI) of piglets during the first day of life was estimated from the prediction equation of Devillers *et al*. (2004b). The equation is as follows: $CI=–217.4 + 0.217\ T + 1{,}861{,}019\ BWa/T + BW0\ (54.80–1{,}861{,}019/T)\ (0.9985–3.7\cdot10^{-4}\ t_{FS} + 6.1\cdot10^{-7}\ t_{FS}^2)$ in which CI is individual colostrum intake (g), T is the time elapsed between birth and second piglet weighing (which define "duration of colostrum intake"), BWa is the BW at second weighing (kg), BW0 is the piglet body weight at birth (kg), and t_{FS} is the interval between birth and first sucking (min). The interval from birth to the first sucking was estimated to average 15min. The 24h after birth was calculated by adding 24h to the mid duration of farrowing. Colostrum intake was not estimated for piglets dying before 17h due to inaccurate colostrum intake estimation (Devillers *et al*., 2004b). Thirteen AL and 12 LL piglets lost much weight (from 58 to 275 g) between birth and 24h of age or death if occurring after 17h, so that predicted values for colostrum intake were negative. We consider that those piglets had not consumed colostrum (CI=0). Colostrum consumption was estimated on 93.8% of AL (334/356) and 98.5% of LL (524/532) suckling piglets. The production of colostrum (COLP) of the sow was obtained by adding individual consumptions of piglets.

Mortality analyses were made considering only the period from birth to 21 days of age because in the private LL farm weaning occurred between 21 and 28 days of age. The weaning ages at E site were at 45 and 28 days respectively for AL and LL piglets, and at private AL farm weaning was made at 45 days. One LL piglet died during blood sampling at 2d of age and therefore was not considered for mortality study. Cross–fostered piglets and those killed for experimental purposes (n=53) were also excluded of mortality study.

Analytical procedures

Dry matter (DM) of colostra and milk was determined after drying at 102ºC until constant weight. Crude protein (CP) was determined according to Dumas method (CP=N·6.38) using a LECO FP–528 Nitrogen/Protein Determinator. Total lipids (LIP) content was determined according to Gerber method (AOAC, 1990). Lactose was determined using a commercial kit Boehringer Mannheim® Lactose/D–Galactose (ref. 0176303). Lactose was determined on 12 sows per genotype and not determined on 3h samples. IgG concentrations were measured on colostrum and piglets serum by ELISA test (pig IgG ELISA Quantification kit, Bethyl Laboratories, Montgomery, TX, USA) following the manufacturer's protocol. Detailed procedure is given in Devillers *et al*. (2004a).

Statistical analysis

Statistical analyses were made using the IBM SPSS Statistics software (v.21, 2012). All analyses were first made within–genotype, comparing production sites. When traits residues were not normally distributed the comparison of means was made by the Mann–Whitney *U* non–parametric test. Comparison between genotypes of performance of sows and piglets was analysed by analyses of variance (ANOVA). For piglets weight at birth and 21d analyses of covariance (ANCOVA) were performed using litter size of born alive and litter size at 21d, respectively, as covariates. To compare litters of different sizes and to determine whether birth order affected within–litter birth weight of the piglets or CI, the order of the piglets in the birth sequence was expressed as relative piglet birth order (RBO) and were calculated as follows RBO=(Birth order–1)/(Total piglets born–1), with RBO ranging from 0 to 1.

Within–litter regression analyses were used to determine the relationships between RBO, birth weight of born alive piglets, CI and serum IgG2. Analyses were based on residues obtained by ANOVA using litter as fixed effect. To evaluate the homogeneity of

slopes among genotypes, analyses of covariance (ANCOVA) were performed using the regression independent factor as covariate and evaluating the significance of the interaction between the covariate and genotype. Data on colostrum production of the sows were adjusted by covariance to litter size (LS) and to litter weight (sucking piglets, LWB) using genotype as fixed effect. To determine the effects of parity, gilts and sows were grouped as follow: group 1, parities 1 and 2 (AL=15 sows, LL=20 sows); group 2, parities 3, 4 and 5 (AL=17 sows, LL=19 sows); and group 3, parities higher than 5 (AL=13 sows, LL=6 sows). Effects of parity and acorn intake (only in E site and AL sows) were analysed by ANOVA.

For colostrum composition and serum IgG concentrations the repeated measures ANOVA procedure was used using time of collection as within–subjects factor and genotype as between–subject factor. Comparison of means was assessed by Bonferroni test. Correlation between colostrum protein and IgG was evaluated by using Pearson's coefficient. Areas under the curves (AUC) of IgG were calculated using the trapezoidal procedure and compared by ANOVA.

No attempt was made to evaluate the effect of parity on colostrum composition because of the small number of sows per parity and because parities were not equally distributed between genotype.

Comparisons of piglet mortality rate between birth and 2d and between birth and 21d was assessed using a Chi–squared test.

Birth weight (Stanton & Caroll, 1974) and colostrum intake (Quesnel *et al*., 2012) are major determinants of mortality. Within each genotype, two categories of birth weight: (≤1.0 kg, low birth weight; >1.0 kg, high birth weight); and two categories of colostrum intake: (CI/kg BW0 ≤ 180g, low colostrum intake) and (CI/kg BW0 >180g, high colostrum intake) were created. Values of 1.0 kg of BW0 and 180g CI/kg BW0 were taken as the limit between light and heavy piglets, and between low and high consumers of colostrum on the basis that piglets from modern genotype weighing 1.0 kg or less (Rydmer, 1992; Léon & Madec, 1992; Herpin *et al*., 1996), or consuming 180g CI/kg BW0 or less (Quesnel *et al*., 2012) are at increased risk of dying during the suckling period.

Main factors that could influence piglet mortality were analyzed by logistic regression. To adjust a logistic regression model it was followed the methodology recommended by Hosmer *et al.* (2013). The variables tested by the univariate model were: genotype, sex, litter size (LS, born alive), RBO, BW0 category, CI category and IgG2 concentration. Odds ratio for mortality from birth to 21d are presented jointly with 95% confidence intervals.

Unless otherwise mentioned, all presented values are mean±standard error of mean (SEM). Differences were considered significant for a p–value < 0.05.

Results

In this study, within each genotype, no significant effect ($p > 0.10$) of farm was observed on any trait of sows and litters performance. Therefore, farm was not taken into account in the analysis of the results.

General

Performance of sows and litters are shown in Table 1. Sows from the two breeds had similar average parity, being 4.0±0.3 and 3.4±0.3 (p=0.150) for AL and LL sows, respectively. AL sows had shorter gestation length (–4.2d, p<0.001) than LL sows and shorter farrowing duration (≈–1h, p=0.032). Alentejano sows had smaller litters (p<0.001) and piglets were lighter at birth (p<0.001), at 21d (p<0.001) and 28d (p=0.011) than LL piglets and differences remained significant (p<0.01) after adjustment for litter size (born alive or piglets alive at 21d). The within–litter relationship between birth weight of born alive piglets and RBO was independent (p=0.891) upon genotype. The within–litter common slope of the regression line relating birth weight to RBO was: $b_{BW0(g)}$=58.2 (±21.4, SE) RBO; R^2=0.008; p=0.007 (Fig. 1).

Colostrum intake of piglets

From birth to 24h of life, AL piglets consumed 267±8g of colostrum that was 19% (p<0.001) lower than the 328±6g recorded in LL piglets. The colostrum intake by kg of BW0 was no longer significantly different between genotypes (p=0.891), with values being 241±6g and 242±5g/kg BW0 for AL and LL piglets, respectively. The coefficient of variation (CV) of colostrum intake was high and similar in both genotypes, averaging 36.4±2.1%.

Intra–litter CI was dependent on birth weigh (p<0.001) and independent on relative birth order (p=0.966). The effects of genotype on the relationship between CI and birth weight was significant (p<0.001). The within–litter slopes of the common regression lines relating CI to BW0 was in AL genotype $b_{CI(g)}$=0.37 (±0.03, SE) BW0 (g) (R^2=0.308; p<0.001) and in LL genotype $b_{CI(g)}$=0.22 (±0.02, SE) BW0 (g) (R^2=0.187; p<0.001) (Fig. 2). The relationship between CI and RBO was not different between genotypes (p=0.462).

Table 1. Alentejano (AL) and Large-White × Landrace (LL) sows reproductive and productive traits. Each value represents mean (± SEM) for each genotype.

Traits	AL (n=45)	LL (n=45)	p-value
Gestation length (d)	110.9±0.3 (n=23) [1]	115.1±0.2	< 0.001
Duration of farrowing (min)	137±13	193±12 (n=42) [2]	0.032
Litter size			
At birth			
Total born	8.3±0.4	12.9±0.4	< 0.001
Born alive	8.0±0.4	12.0±0.4	< 0.001
Sucking piglets	7.9±0.4	11.8±0.4	< 0.001
At d21	5.8±0.3	9.2±0.4	< 0.001
Weight of piglets/litter (g)			
Born alive	1091±13 (n=362)	1338±11 (n=539)	< 0.001
Sucking piglets	1090±23 (n=356)	1345±11 (n=532)	<0.001
At 21d	4558±77 (n=260)	5722±62 (n=412)	<0.001
At weaning (28d) [3]	5783±85 (n=253)	6588±90 (n=226)	0.011

[1] not determined in AL private farm; [2] three abnormally prolonged farrowings were excluded; [3] LL only for the experimental farm.

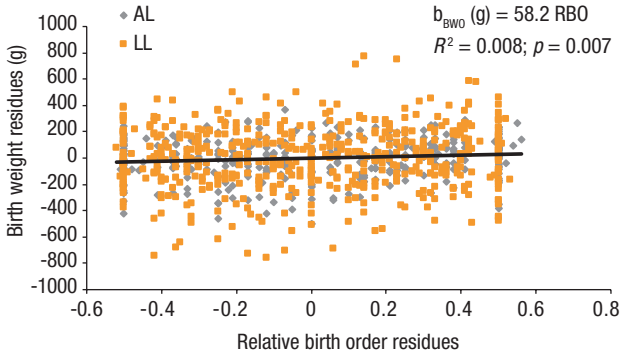

Figure 1. Intra-litter relationship between birth weight (BW0) and relative birth order (RBO) in Alentejano (AL, n=356) and crossbred (LL, n=532) piglets. Data presented are residues calculated after correction from litter effect.

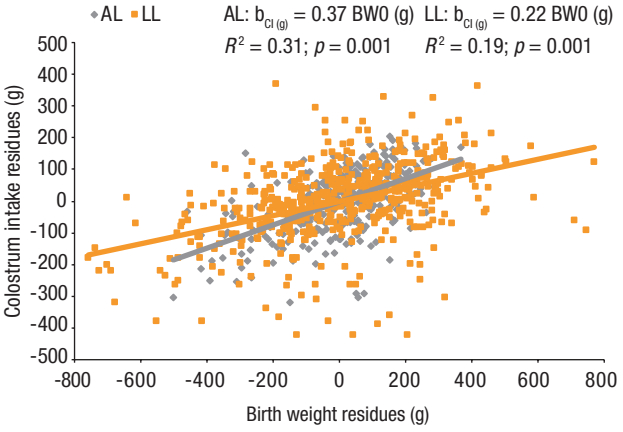

Figure 2. Intra-litter relationship between colostrum intake and birth weight in Alentejano (AL, n=356) and crossbred (LL, n=532) piglets. Regression slopes were different between genotypes (p<0.001). Data presented are residues calculated after correction from litter effect.

Colostrum production of sows

During the first 24h after the onset of farrowing, AL sows produced 1985±139g of colostrum that was 47% lower (p<0.0001) than the 3761±139g produced by LL sows. Within genotype, the CV of colostrum yield was high (32.4% AL and 30.5% LL). Within genotype, parity had no significant effect on COLP (p=0.26 and 0.34 for AL and LL sows, respectively). Within AL sows, acorns consumption had no effect on COLP (p=0.203). There was no significant (p=0.146) effect of genotype on the relationship between colostrum production and litter size or litter weight (p=0.138) The slopes of the common regressions lines relating colostrum production to litter size and litter weight were, respectively, $b_{COLP(g)}$=185(±36) LS (R^2=0.24; p<0.001) and $b_{COLP(g)}$=0.17 (±0.03) LWB(g) (R^2=0.33; p<0.001). After adjustment to litter size, AL sows produced 30% less colostrum (2369±143g *vs* 3377±143g, p<0.001) than LL sows and after adjustment to litter weight, AL sows produced 16% less (2622±150g *vs* 3124±150g; p=0.05) colostrum than LL sows.

Colostrum composition

Colostrum composition was determined on the 17 sows of each genotype at E site (Table 2). There was no significant effect of genotype on colostrum DM and lactose contents. In contrast, colostral CP and LIP contents were higher (p< 0.05) and lower (p< 0.01) in AL and LL sows, respectively.

Table 2. Dry matter (DM), crude protein (CP), total lipids (LIP), lactose, and immunoglobulin G (IgG) contents on colostrum of Alentejano (AL) and Large-White × Landrace (LL) sows (means±SEM).

	G	Time of colostrum collection (T)						Effects (*p*-value)		
		0h	3h	6h	12h	24h	36h	G	T	G × T
DM (%)	AL	27.2±0.9^{a}	27.3±0.8^{a}	26.1±0.8^{b}	21.0±0.6^{c}	20.9±0.7^{c}	21.1±0.6^{c}	0.435	$1.8 \cdot 10^{-15}$	0.096
	LL	26.1±0.9^{a}	25.6±0.8^{a}	24.4±0.8^{b}	22.0±0.6^{c}	20.3±0.7^{c}	21.2±0.7^{c}			
CP (%)	AL	18.5±0.8^{a}	17.0±0.8^{b}	16.0±0.8^{bA}	11.1±0.7^{c}	9.1±0.6^{dA}	9.0±0.6^{dA}	0.048	$3.7 \cdot 10^{-29}$	0.321
	LL	17.0±0.8^{a}	15.9±0.8^{b}	13.2±0.8^{cB}	9.9±0.7^{d}	7.3±0.6^{eB}	6.7±0.6^{eB}			
LIP (%)	AL	4.6±0.4^{a}	6.1±0.4^{bc}	6.0±0.4^{abc}	5.3±0.4^{abA}	6.3±0.6^{bcA}	7.6±0.6^{cA}	0.007	$2.1 \cdot 10^{-21}$	0.006
	LL	4.9±0.4^{a}	5.9±0.3^{b}	7.0±0.4^{bc}	7.8±0.3^{cB}	8.2±0.5^{cdB}	9.5±0.6^{dB}			
Lactose (%)	AL	2.7±0.3^{a}	–	3.2±0.3^{a}	3.4±0.2^{a}	5.8±0.4^{b}	4.5±0.2^{c}	0.877	$1.7 \cdot 10^{-12}$	0.489
	LL	3.0±0.3^{a}	–	3.3±0.3^{a}	3.6±0.2^{a}	5.5±0.4^{b}	4.1±0.2^{c}			
IgG (mg/ mL)	AL	113.8±11^{a}	82.5±7.5^{b}	65.9±6.2^{c}	49.2±5.0^{dA}	19.8±2.9^{e}	12.2±1.9^{fA}	0.050	$1.0 \cdot 10^{-13}$	0.051
	LL	103.7±11^{a}	68.2±7.5^{b}	48.0±6.2^{c}	30.8±5.0^{dB}	12.5±2.9^{e}	v5.4±1.6^{fB}			

G, genotype. Different letters in the same row represent differences among collection times. Within columns, different capital letters represent differences between genotypes. Results for 17 samples of each genotype, at each collection time.

In both genotypes, there was a significant (p<0.001) decrease in DM and CP contents during the first 12h after the birth of the first piglet. Afterwards, DM content remains practically unchanged whereas a further decrease in CP content was observed until 36h. In contrast to DM and CP, both LIP and lactose contents increased after the first piglet was born. Twelve hours after the onset of parturition, lactose content had similarly increased by 23% (p<0.01) in both genotypes. Lipid content also increased, however the increase was higher (p< 0.001) in LL (+ 60%) than in AL (+28%) during the first 12h following the onset of parturition.

Immunoglobulin G concentrations in colostrum from AL sows were always higher than those observed in LL colostrum. However, statistical difference was only observed at 12h and 36h from the onset of parturition. When estimated by the areas under curve (AUC) the total concentrations of IgG, were significantly higher in AL colostrum both from 0 to 24h (p=0.039) and from 0 to 36h (p=0.035). In both genotypes IgG concentrations were maximal in the first colostrum and subsequently decreased with time. At 3, 6, and 12h after the onset of parturition, concentrations of IgG had declined (p<0.001) similarly in both genotypes by 30, 48 and 63%, respectively. Both genotypes shown a close relationship between CP and IgG concentrations (r=0.89, p<0.001). Further, it should be noticed that the IgG concentrations were very variable with a CV determined 12h after the first piglet was born, being as high as 52% and 45% in AL and LL colostrum, respectively.

Compared to AL sows which had not access to acorns during gestation, those having access had higher (p<0.05) lipids concentrations, but concentrations of DM, CP, IgG and lactose were similar.

Passive immunity of piglets

Serum IgG were determined at E site on piglets suckled by the 17 sows per genotype with known composition of colostrum. A total of 91 AL and 131 LL piglets with a mean BW0 of 1175±22g and 1363±19g (p<0.001), respectively, were sampled at 2 and 28d of age. Alentejano piglets consumed 19% less colostrum (p<0.001) than LL piglets (269±11g *vs* 332±9g). When expressed per kg BW0, colostrum intake averaged 292±11g and 316±9g for AL and LL piglets, respectively. The difference was not significant (p=0.092).

Genotype and age at sampling had effects on piglets' serum IgG concentrations (Fig. 3). Concentrations

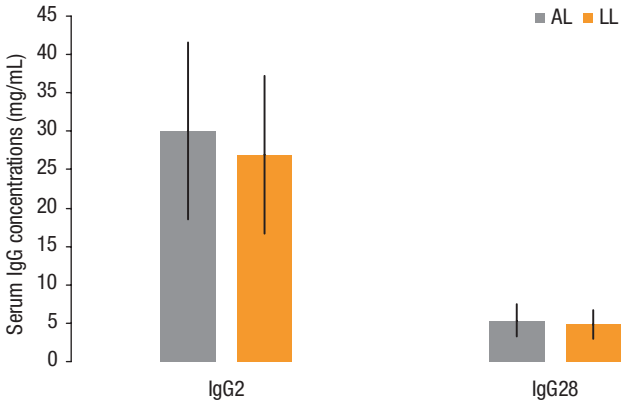

Figure 3. IgG concentrations in the serum of Alentejano (AL, n=91) and crossbred piglets (LL, n=131) at 2 days (IgG2) and 28 days (IgG28) of age (mean ± SD). IgG2 concentrations were higher than IgG28 concentrations in both genotypes (p <0.001). Within ages, AL piglets presented higher IgG concentrations in day 2 (p=0.032) and day 28 (p=0.049). Globally genotypes differences were significant (p=0.025). Bars indicate standard deviations, SD.

were higher in AL than in LL piglets (p=0.025) and in both genotypes, higher at 2d than at 28d of age (p<0.001).

Piglet mortality

Total mortality between birth and 21 d of age was dependent on genotype, being higher in AL than in LL piglets (27 *vs* 16.1%, p<0.001). Rate was the highest in the early life of piglets with 75 and 56% (p<0.01), of total mortality occurring in the first two post–natal days in AL and LL piglets, respectively.

The univariate logistic regression analysis showed that sex (p=0.576), litter size (p=0.582), relative birth order (p=0.913) and IgG2 (p=0.569) were not significant, whereas the piglet genotype, BW0 category and CI category were all highly significant (p<0.001) for mortality until 21d. The odds ratio for mortality of AL piglets was 1.9 (1.4–2.7) fold higher than for LL piglets. The odds ratio for mortality of piglets from low birth weight category were 7.1 (4.8–10.3) fold higher than for piglets in high birth weight category and the odds ratio for mortality of piglets from low colostrum intake category were 7.2 (4.9–10.5) fold higher than for piglets in high colostrum intake category.

The inclusion in the multivariate model of genotype and categories of BW0 and CI made genotype to become not significant (p=0.632), so genotype was excluded from the final model that remained with BW0 category, CI category and their interaction (p=0.006). The AUC of the final model was 0.78 which indicates a good discrimination of the model.

Considering the low birth weight category (≤1kg) the odds ratio for mortality was 18.9 (8.0–45.5) fold higher (p<0.001) for piglets with CI equal or lower than 180g/kg BW0 compared to piglets with CI higher than 180g/kgBW0. In high birth weight category, although piglets with low colostrum intake presented higher odds ratio for mortality than piglets with high colostrum intake, the difference was not so pronounced, being 4.7 fold higher (2.9–7.7; p<0.001).

Conversely, for piglets from low colostrum intake category (CI≤ 180g/kgBW0) the odds ratio for mortality was 16.7 (7.5–37.0) fold higher (p<0.001) in lighter piglets (≤1kg) than in heavier piglets (>1kg). Piglets with CI higher than 180g/kg BW0 and from low birth weight category also presented higher odds ratio for mortality than heavier piglets but in this case also in a less marked way being 4.1 (2.2–7.5; p<0.001). In other words, although CI is important to avoid death independently of BW0, for lighter piglets a good CI is more crucial to survive than for heavier piglets.

Discussion

Overall, performance of reproduction of both genotypes was similar to that reported and discussed previously (Charneca *et al*., 2012) and hence will not be re–discussed here. Briefly, AL sows had shorter gestation length, shorter duration of farrowing and are less prolific than LL sows, while AL piglets are lighter at birth and at weaning than LL piglets. Interestingly, in both genotypes, birth weight increases similarly with RBO which is consistent with previous results of Beaulieu *et al*. (2010) and Charneca *et al*. (2013). Nevertheless, it should be mentioned that RBO accounted only for approximately 0.8% of the total variation observed in piglet birth weight, which is very low. In contrast, Friend & Cunningham (1966) and Motsi *et al*. (2006) reported that late born piglets were lighter at birth than their earlier–born littermates.

Colostrum consumption of piglets and production of sows

In this study, estimation of colostrum consumption was assessed using an equation determined in a lean genotype, similar to our LL animals. To some extent, its use for AL piglets, a fatty–type genotype, could be inappropriate. However, when we compared the weight gain of piglets in the first 24h, similar results were obtained when expressed per kg birth weight. In absolute terms, AL piglets gain less (p<0.001) than LL piglets, while when expressed per kg birth weigh, gains were similar (p=0.20). Therefore we can assume that the estimation of colostrum intake determined on a lean genotype could be valid for AL piglets. However, because AL piglets have been reported to use less efficiently the sow's milk than conventional piglets (Aguinaga *et al*., 2011), there is a possibility that colostrum intake of AL was underestimated.

On average, in the first 24h, AL piglets consumed 267g of colostrum and AL sows produced 1.99 kg colostrum, values which are lower than those ranging from 300 to 380g and from 3.4 to 4.9 kg, respectively, as reported in modern crosses (this study; Devillers *et al*., 2007; Quesnel, 2011; Loisel *et al*., 2013). Particularly, the CI of AL piglets was 38% lower than the 432g estimated by Aguinaga *et al*. (2011) in Iberian piglets. However, this estimation was based on measurements made on day 2 after birth and on piglets that were markedly different with respect to litter size (6 *vs* 8.4) and birth weight (1409g *vs* 1148g), therefore making difficult the comparison with our data. The difference in colostrum consumption between AL and LL piglets can be largely attributed to difference in piglets' birth

weight. When CI per piglet was adjusted for piglets' birth weight, genotype had no significant effect on colostrum consumption of piglets suggesting that the ability to consume colostrum is similar in both genotypes. Further, birth weight was an important factor accounting for the high within–litter variability of colostrum intake. Indeed, compared with lighter littermates, piglets with high birth weight are advantaged by their better ability to compete at the udder and to extract milk from the teats (King *et al*., 1997). In the present study, data indicated that in both genotypes, CI increases with the increase in piglets' birth weight being (per 100g increase in BW0) of 37g in AL and 22g in LL piglets. Value for LL piglets is close to the 28g reported by Devillers *et al*. (2007) in similar genotype. In agreement with Devillers *et al*. (2007) colostrum intake was independent of birth order despite that, compared to the first–born, late born piglets nursed with more competition and had less time to suck. This is likely because the rate of colostrum consumption is the highest during the first few hours after birth (Castrén *et al*., 1991; Fraser & Rushen, 1992; Le Dividich *et al*., 1997). We hypothesise that, when late born piglets were born, the first–born were sated and therefore less active, allowing late–born piglets to display more teat seeking activity. Further, our data are consistent with the fact that birth weight, a major determinant of colostrum intake, is increased in late–born piglets.

The difference in colostrum production between AL and LL sows resulted, at least in part, by both the lower litter size and litter weight of AL sows which reduce the nursing demand (Auldist & King, 1995). Similarly, to what is observed for milk production (Étienne *et al*., 2000) colostrum production increased linearly with litter size. Yet, the increase was not proportional to litter size. In contrast, according to Devillers *et al*. (2007) and Quesnel (2011) colostrum yield is found to be independent on litter size. Reasons accounting for this difference are not known. However, at best, AL sows produce 16% less colostrum than LL sows, suggesting a lower capacity of AL sows to produce colostrum. According to Quesnel *et al*. (2012), the consumption of colostrum at the rate of 180g/kg BW0 would be necessary to ensure survival, growth and health of the neonatal pig. On this basis, 24 and 18% of AL and LL sows, respectively, did not produce enough colostrum, suggesting that factors controlling colostrum production, especially in AL sows, warrant future research.

Colostrum composition

The pattern of composition of colostrum of both genotypes over the first 36 hours after the onset of parturition is largely similar to those reported previously by Klobasa *et al*. (1987), Le Dividich *et al*. (2004), and Markowska–Daniel *et al*. (2010). Major results of this study indicated that concentrations of CP and IgG are higher in AL than in LL colostrum. In this respect, Duroc sows are reported to have more protein in their colostrum than Landrace sows (Farmer *et al*., 2007). Similarly, comparing Meishan and Yorshire sows, Zou *et al*. (1992) found higher protein concentrations in the Meishan colostrum but not Le Dividich *et al*. (1991). Further, Csapó *et al*. (1996) failed to find significant difference in protein concentrations in colostrum from Danish Large–White, Danish Duroc and Norwegian Landrace sows. On the other hand, according to Inoue *et al.* (1980) concentrations of IgG were less in colostrum from Hampshire, Large–White and Landrace × Large–White and high in colostrum from Landrace × Hampshire sows. These results suggest that the influence of genotype on colostral protein and immunoglobulins concentration need to be clarified.

Acorns contained high level of lipids (~6.3% DM, Rey *et al.*, 1997). Within the AL sows, those having access to acorns during gestation had also higher lipids concentrations in their colostra which agreed with the fact that colostral and milk lipids are closely related to the lipids contents of the sow diet (Jackson *et al.*, 1995).

Passive immunity of piglets

Overall and irrespective of the genotype, levels of passive immunity of piglets at 2d and 28d of age agree well with those reported by Klobasa *et al*. (2004), Le Dividich *et al*. (2005), Svendsen *et al*. (2005) and Devillers *et al.* (2011). The marked decrease in IgG levels from 2d to 28d is consistent with the observations by Le Dividich *et al*. (2004). This decrease is related to both the clearance of IgG, with a half–life of about 10 days (Curtis & Bourne, 1973), and the dilution effect associated with the increase of blood volume in that period. Present results indicate that serum IgG levels at 2d of age were higher in AL than in LL piglets. The effect of genotype can be related to the amount of ingested IgG. Indeed, Werhahn *et al*. (1981) reported a dose response relationship between the amount of porcine IgG administrated to the newborn pig and plasma IgG concentrations at 12h post–feeding. In this study, the combined effects of the higher colostral IgG concentrations in AL sows and the similar CI, adjusted to birth weight of piglets in both genotypes, can explain the higher serum IgG levels at 2d in AL piglets.

Piglet mortality

The 27% mortality from birth to 21d recorded in AL piglets was in line with the 24% to 29.3% observed by Robledo *et al*. (2008) in Iberian piglets and the 20–28% reported by Marques *et al*. (1996) for AL pigs. The 16.1% of mortality observed in LL piglets was only slightly higher than the 13.9% reported for French herds national results (IFIP–GTTT, 2013). The high mortality occurring in the first 2 days of life contributing to 75% and 56% of the total mortality of AL and LL piglets, respectively, was also observed in other studies (Marchant *et al*., 2000; Casellas *et al*., 2004; Strange *et al*., 2013).

Logistic regression analysis showed that litter size, RBO and acquisition of passive immunity did not influence the mortality of piglets. Regarding litter size our observations are contrary to those reported by Andersen *et al*. (2011) which observed a positive influence of litter size on mortality, the reason for this difference is probably the higher mean litter size in the mentioned study (higher than 13 born alive piglets) when compared to average values of 8 and 12 in the present study. The RBO of the piglets also did not influence their possibility of dying from birth to 21d in accordance with the observed marginal influence of RBO on piglets BW0 and no influence on CI, which were important explanatory factors for mortality in the present study.

The acquisition of passive immunity is of vital importance for the survival (Varley *et al*., 1987). In our study there was no influence of IgG2 on the mortality of the piglets suggesting that serum IgG concentrations at 2 d of age are a poor predictor of the piglet survival (Tyler *et al*., 1990; Rootvelt *et al*., 2012). Yet, piglets dying before 21d of age have been reported to have lower serum IgG concentrations (Blecha & Kelley, 1981; Devillers, 2004). However, these mortalities may simply be the consequence of insufficient colostrum intake rather than diseases. This is illustrated by the Devillers's study (2004) showing that piglets dying during the first four post–natal days had 36% less serum IgG concentrations than survivors but had consumed three times less colostrum (70 *vs* 233 g/kg birth weight) and hence energy. Nevertheless, in our study, the majority of losses (75% of AL; 56% of LL) occurred before blood sampling at 2 d of age and it is not known whether insufficient passive immunity was the cause of death.

Birth weight (Tyler *et al*., 1990; Casellas *et al*., 2004; Baxter *et al*., 2008) and the resulting insufficient amount of colostrum intake (Quesnel *et al*., 2012) are major factors influencing piglet mortality. Therefore, the higher mortality of AL piglets was likely related to the fact that they were lighter at birth and consumed less colostrum. The significant roles of the two above mentioned factors were also observed by the logistic regression analysis of present data. The piglets in low birth weight category present higher odds ratios for mortality than high birth weight category piglets. Also, low colostrum intake category piglets present higher odds ratios for mortality than piglets from high colostrum intake category. However these two factors interact because even if low birth weight piglets present higher odds ratios for mortality than heavier piglets, the degree of risk was colostrum intake dependent, being almost 4 fold higher when CI was lower than the 180g/kg BW0 threshold (18.9 *vs* 4.7). In other words, for lighter piglets a superior CI is more crucial to survive than for heavier piglets. Similar interaction was also observed by Ferrari *et al*. (2014) who observed that the probability of death of low birth weight piglets (1.1–1.2kg) compared to newborn heavy piglets (1.3–1.7kg) was substantially reduced when CI of low birth weight piglets reached 250g. Piglets of low birth weight have a greater surface area per unit body weight and this result in greater heat loss and hence they require more energy per unit body weight (Curtis, 1970). Additionally their energy stores at birth are lower (Mellor & Cockburn, 1986) and they are less competitive at the udder and consequently they tend to consume less colostrum (Devillers *et al*., 2007; this study) and hence, less energy. In other words, to survive light piglets must consume more colostrum than their heavier littermates. Our results suggest, therefore, that the determination of colostrum intake needed to survive and to thrive should take into account the birth weight of piglets.

These crossed influences of BW0 and CI on mortality were observed in both genotypes but to justify the differences in total mortality rate between genotypes we have to consider that the distribution (proportion) of piglets in the different BW0 categories which was not similar. Proportionally there were about 2.5 times more AL piglets in low birth weight category than LL piglets (28.7% *vs* 11.6%, $p<0.001$). Considering that low BW0 piglets also consume less colostrum, these different proportions in BW0 categories can justify, at least partially, the higher mortality rate in AL genotype.

In summary, this study provides the first estimate of colostrum intake of piglets and colostrum production of Alentejano sows. Main results show that there is no significant difference in colostrum intake expressed per kg birth weight between AL and LL piglets. Alentejano sows produce 16% less colostrum than LL sows, but AL colostrum is richer in protein and IgG. At 2d of age Alentejano piglets have 9% more serum IgG than LL piglets. In both genotypes the main factors influencing piglet mortality are birth weight and colostrum intake. The higher mortality rate of AL piglets can be explained,

at least partially, by the higher proportion of AL piglets with low birth weigh (≤ 1.0kg). These findings are expected to give new insights on the high pre–weaning mortality rate observed in AL piglets and suggest that future research on AL sows should focus on factors influencing colostrum production and piglet birth weight.

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