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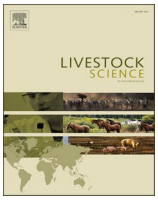

The impact of genetic groups (Alentejano and F1 Landrace x Large White pigs) and body weight (90, 120 and 160kg) on blood metabolites

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HIGHLIGHTS

- In obese pigs, high levels of feed intake determine elevated plasma triglyceride levels.
- At 90 kg live weight, fat deposition in alentejo pigs is associated with high HDL cholesterol levels.
- Elevated serum albumin levels in early finishing phase is a possible indicator of obesity in pigs.

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ABSTRACT

This research work was carried out with the goal of studying the impact of genetic groups-GG (Alentejano-AL, $n = 30$, and F1 Landrace*Large White-F1 pigs, $n = 30$) and body weight-BW (90, 120 and 160 kg) on plasma metabolites. Blood parameters were correlated with animal production traits, carcass measurements and meat quality. Individual records for feed conversion index (CI) and daily feed intake were recorded on a weekly basis, for a period of 15 weeks. Compared to the F1, AL pigs displayed ($P < 0.05$) higher average levels of glucose, total cholesterol-TC, triglycerides-TG, HDL-cholesterol (HDL), LDL-cholesterol (LDL) and total protein (TP), by about 14.0, 21.0, 42.2, 18.2, 21.2 and 5.0%, respectively. AL pigs (120–160 kg) showed higher TG levels, when compared to the values at 90 kg (2.6 and 1.6 times higher). High TG levels occurred when animals exhibited high daily feed intake (0.450 and 1.810 kg, for AL and F1 pigs). In the AL high TG levels were correlated with high fat deposition, at 120 kg ($r = 0.51$). At 90 kg, however, high fat deposition was related to HDL ($r = 0.59$), a lipoprotein associated to cholesterol transport. A progressive increase in ALB was found in the F1, as expected, but AL pigs showed higher and similar ALB means at 90, 120 and 160 kg. As for meat color, AL pigs with high cholesterol were negatively associated to L*, while high TG levels were associated to low b*. Animals with high ALB produced more tender meats (low shear force). Pigs with higher levels of lipid metabolism showed *Longissimus thoracis* muscles with decreased luminosity and yellowness (meats of a less attractive appearance). However, these meats were tenderer.

Abbreviations: AL, Alentejano; GLU, Glucose; TC, Total cholesterol; TG, Triglycerides; BUN, Urea-N; HDL, HDL-cholesterol; LDL, LDL cholesterol; ALB, Albumin; CI, Feed conversion index; DFI, Daily feed intake; ABF, Abdominal fat depots; MBL, Marbling; DB12, Dorsal backfat 12 cm; DB6, Dorsal backfat 6 cm; ALT, Area L. thoracis; L2 or L9, L* coordinate at 2 or 9 d *post mortem*; A2 or A9, a* coordinate at 2 or 9 d *post mortem*; B2 or B9, b* coordinate at 2 or 9 d*post mortem*; MOI, Moisture; ASH, Ash; PB, Crude protein; IMF, Intramuscular fat.

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1. Introduction

The Alentejano pig is an autochthonous porcine breed, included in the Iberian type. These animals have been bred in Spain and Portugal, under an extensive production system, in a Mediterranean ecosystem traditionally known as Montanheira / Dehesa (Gama et al., 2013). Their hind legs are used to produce cured dried meat, especially Iberian ham, a product with high sensory acceptability (Lopez-Bote, 1998). This trait is attributed to the high amount of intermeshed fat present in the muscle tissue, due to genetic factors and environmental conditions (Almeida et al., 2018; Bressan et al., 2016). Iberian pigs are well adapted to natural conditions – periods of scarcity (summer) followed by periods of high food availability, usually acorn and pastures, during autumn and winter (Rodríguez-Estévez et al., 2009; Tejeda et al., 2020). When compared to commercial breeds Iberian pigs present a very different set of traits, namely their food intake behavior (Martins et al., 2019; Muñoz et al., 2009; Torres-Rovira et al., 2012) and intense energy storage in the form of adipose tissue (Nieto et al., 2002), as abdominal, intramuscular and subcutaneous fat (Almeida et al., 2019; Bressan et al., 2016).

Physiological, biochemical and genetic mechanisms responsible for the differences between fatty and lean breeds are still not fully clarified (Amaral et al., 2019; Poklukar et al., 2020). These properties can be deemed responsible for increased *de novo* biosynthesis, improved adipogenesis and different levels of lipid mobilization between autochthonous and modern breeds. In general, native pigs show higher capacity for adipocyte hypertrophy and hyperplasia than commercial breeds (Brossard et al., 2019; Hausman et al., 2018) . Nevertheless, metabolites involved in supplying energy to the body are kept within a narrow range in the bloodstream (homeostatic control). Glucose, one of these nutrients, is soluble in the circulatory system and an immediate source of energy; the lipid fraction (cholesterol, triglycerides and free fatty acids), however, is insoluble in the bloodstream and must be transported in association with proteins and lipoproteins (Hegele, 2009).

Currently, studies envolving lipid mobilization metabolites and tissue development in the finishing phase are scarce and contradictory. A positive association between feed intake, cholesterol levels, lipoproteins and fat deposition was suggested by Rauw et al. (2007). In pigs with a genetic predisposition to obesity, high urea levels (Madeira et al., 2016) or high cholesterol and triglyceride levels (Nakajima et al., 2019) were associated with high fat deposition. However, in commercial breed pigs, (Muñoz et al., 2012) described low levels of correlation between blood parameters and fat deposition. In the context of meat quality, there are a few studies relating lipoprotein levels and quality characteristics based on serum indicators of lipid mobilization. Thus, our hypothesis is that serum levels associated with lipid mobilization can be used as a tool to estimate tissue development, carcass characteristics and meat quality.

The goal of our study was to analyze plasma metabolites collected at 90, 120 and 160 kg of live weight, from pigs of two genetic groups (Alentejano and F1 Landrace x Large White), as well as their relationship to animal and carcass production traits and the chemical composition and quality of meat.

2. Material and methods

2.1. Animals and treatments

A total of 60 barrows belonging to the Alentejano-AL ($n = 30$) and F1 Large White*Landrace-F1 ($n = 30$) genetic groups were used in this study. The experimental procedures employed followed the European Union legislation concerning the protection of animals used for scientific purposes (European Parliament 2010), and the animals were raised according to Portuguese and EU legislation on pig production. The AL pigs were born and raised outdoors, with grass feed, in the Montanheira (or Dehesa) until they reached 40 kg of live weight, with limited access to commercial feed when necessary. This cycle corresponds to standard procedure for AL pigs. The F1 pigs were born and raised indoors, in an intensive facility, and managed according to standard commercial procedures, until they reached 40 kg of live weight. Afterwards, pigs from both genetic groups were provided the same commercial feed until they reached an average weight of 90 kg. Given the faster growth rate of F1 pigs, their mean age was 5 mo, whereas for AL pigs of the same weight, the mean age was 11 mo. During the finishing period, which spanned fifteen weeks, on average, the AL and F1 Landrace*Large White pigs were allocated to groups of three animals in cages, with an unobstructed floor area of 2.6 m^2 per pig. At mealtime, each pig had access to an individual cage, so that daily feed intake, as well as leftovers, were measured individually. The pigs received dry food twice a day (at 9 h and 16 h), in amounts that corresponded to 4% of their live weight. The animals remained in these facilities (intensive system) until they reached the target slaughter weight (160 kg). The diet of animals used in the experiment was formulated to provide 14.7% crude protein and 13.7 Mj kg-1 DM of gross energy. There was no information on the amount of cholesterol in the feed. Yet only very small amounts would have been present, in view of the low levels of animal fat. The detailed composition of the finishing diet has been previously reported (Bressan et al., 2016). Water was provided *ad libitum*. Animals were weighed weekly. Total feed intake, daily feed intake by week and feed conversion index-CI (feed/gain ratio) were computed for each individual over the finishing period (15 weeks).

2.2. Sample collection and biochemical analysis

Blood samples for biochemical analysis were collected from pigs with body weights of approximately 90 kg, 120 kg and 160 kg, after a 12-hour fast. In general, AL pigs reared in a traditional system usually begin and end the fattening phase with live weights around 90 and 160 kg. Blood collection was performed during sanitary management procedures (screening of Porcine Reproductive and Respiratory Syndrome and planning for control and eradication of Aujeszky's disease). In all cases, blood samples were obtained by jugular venipuncture, using heparinized and plain vacutainer tubes. Samples were then chilled for at least 1 h and centrifuged at 3000 x G for 20 min. The serum obtained was then divided into several Eppendorf tubes and stored at −20 °C, pending analysis.

Concentrations of serum glucose (mmol/L); triglycerides (mmol/L); urea-N (mg/dL); total protein (g/dL); albumin (g/dL); total cholesterol (mmol/L) and high-density lipoprotein-HDL (mmol/L) were obtained using commercial kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany), following the standard procedures described by the manufacturing company. Absorbance readings were performed on a micro-flow spectrophotometer (Shimadzu Co., Kyoto, Japan). Low-density lipoprotein-LDL (mmol/l) was calculated by indirect determination (Friedewald et al., 1972). Briefly, enzymatic colorimetric methods were used in six protocols, such as: glucose (glucose oxidase and peroxidase); urea-N (with urease); triglycerides (glycerol kinase, glycerol-3-phosphate oxidase and peroxidase); total cholesterol (peroxidase, cholesterol esterase and cholesterol oxidase); and high-density lipoprotein-HDL (cholesterol oxidase, cholesterol esterase and peroxidase). Colorimetric methods were used for determining total protein (according to the Biuret method, with cupric ions) and albumin (using bromocresol green).

2.3. Slaughtering procedures, carcass information, and sample collection

Pigs weighing 160 kg (± 5 kg) were slaughtered in an experimental abattoir. During the rest period (10 h), the pigs were kept in groups of two or more per farm - avoiding contact with unknown animals - with free access to water (without food). The slaughtering process was carried out according to the standards recommended by the European Union legislation (European Council 2009). Briefly, pigs were stunned with a head-only electric stunner (250 V and 1.3 A) and slaughtered by

exsanguination. During evisceration, internal organs (heart-HEA and liver-LIV) and abdominal fat depots-ABF (omental fat + mesenteric fat + kidney fat) were removed and weighed individually. Hot carcasses were split longitudinally and weighed individually before and after cooling (at 2 ± 1 °C, for 24 h).

At the time of boning, carcasses were separated into the major joints, as described by Bressan et al. (2016), and a 100 g sample was collected from the M. *Longissimus thoracis* (LT), between the 9th and 11th vertebrae, for chemical analysis. External fat and epimysium were removed from meat samples, which were then individually minced in a commercial mixer-blender, vacuum-packaged and frozen at −18 °C, until further processing for proximate composition determination.

Each right half-carcass was cross-sectioned between the 10th and 11th thoracic vertebrae, where the area of the M. *Longissimus thoracis*-ALT was measured, by tracing the outer perimeter of the muscle on an acetate sheet, the surface of which was later measured with ImageJ Software (Schneider et al., 2012). Backfat thickness was measured with a caliper on the surface of the cut, over the midline and at 6.5 cm (DB6) and 12 cm (DB12) from the midline, as described by Bressan et al. (2016). The level of marbling-MBL was visually determined in the surface cut of the LT, according to the Pork Quality Standards (National Pork Board 2015), using a 6-point scale (where 1 = total absence of marbling, …, and 6 = high level of marbling). Each sample was evaluated for level of MBL at 9 d *post mortem* by two trained technicians, and the mean value was used for statistical analysis.

Meat samples were collected from the LT between the 12th and 16th vertebrae, to carry out physical analysis (500 g extracted from the right half-carcass). These samples were split perpendicularly to the muscle fiber into two subsamples, which were individually vacuum-packaged and refrigerated at 2 °C. One of the subsamples was randomly selected for physical analysis at 2 d *post mortem*, while the other subsample was stored at 2 ± 0.2 °C and analyzed at 9 d *post mortem*.

2.4. Chemical and physical analysis (meat)

Analyses of moisture content-MOI, intramuscular fat-IMF, crude protein-CP, and ash content-ASH were performed in duplicate, using (AOAC 2000) methods. Briefly, protein was quantified by the micro-Kjeldahl method (954.01); IMF content was determined by the Soxhlet method (920.39) using a Soxhlet extractor (Velp Scientifica SER 148/6 Solvent Extractor, Italy); MOI was determined in an oven, at a temperature of 105 °C (method 950.46); and ASH was determined by carbonization and incineration of the samples in a muffle furnace, at a temperature of 550 °C (method 920.153).

Meat pH was measured at 24 h *post mortem*, by making a scalpel incision in the LT between the 10th and 11th vertebrae, and inserting a glass electrode, model FC200 (Hanna Instruments, Leighton Buzzard, UK), attached to a portable pH meter, approximately 2.5 cm into the muscle. Three pH measurements were taken from each point sampled and the mean of these measurements was used for statistical analysis.

Meat color was evaluated at 2 and 9 d *post mortem,* using a CR-400 chroma meter (Minolta Camera Co., Ltd., Osaka, Japan), with the illuminant D65, at an observation angle of 2° and a 1 cm diameter of measurement (Mancini and Hunt, 2005). Color coordinates were obtained after 60 min of blooming at 4 °C, by averaging three readings performed in the median region of each sample, at regular distance intervals in the mid-space of 45 mm, which corresponds to the average diameter of each piece. Lightness (L*), redness (a*) and yellowness (b*) values of the LT surface were recorded, according to the CIE color space (Almeida et al., 2018).

For analysis of Warner-Bratzler Shear Force-WBSF, samples with 200 ± 25 g were boiled in water at 80.0 ± 0.2 °C, until they reached a final internal temperature of 75.0 °C, measured with a thermocouple (type T fine-gage thermocouples read with Omega RDXL4SD, Omega Engineering, Inc., Manchester, UK). After 24 h, cooked samples were cut parallel to the direction of muscle fibers (1 × 1 × 3 cm). These subsamples were sheared using a TA-XT2 texturometer (Stable Micro System, Surrey, UK), equipped with a Warner-Bratzler shearing device at a crosshead speed of 300 mm/min, and the results were expressed in kilograms. Meat samples from each animal in the experiment, aged for 2 or 9 d *post mortem*, were analyzed, and the mean of 15 to 25 measurements per sample was used for statistical analysis.

Regarding the animals in this research project, a study of the effects of genetic groups on carcass, internal organ weight and fat deposits was previously presented by Bressan et al. (2016), and the chemical and physical analysis of meat was described by Almeida et al. (2018). In the present study, data related to animal production, carcass and meat was used to analyze the relationship with blood metabolites, as well the as the relationship between the metabolites themselves.

2.5. Statistical analysis

Data was considered to have originated from a 2 × 3 factorial, with two genetic groups (Alentejano-AL and F1 Landrace*Large White-F1 pigs) and three body weights (90, 120 and 160 kg). The SAS GLM procedure (SAS Institute Inc., Cary, NC, USA) was used for analyzing biochemical parameters with a linear model, including the main effects of genetic group and body weight, as well as their interaction. The individual pig was the experimental unit for all data analysis. The analysis comprised the following metabolites: glucose (GLU), total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL), LDL-cholesterol (LDL), urea-N (BUN), total protein (TP) and albumin (ALB). Values for HDL- and LDL-cholesterol were only obtained at 90 and 160 kg of body weight. For all variables analyzed, least squares means were obtained for the main effects of genetic group, body weights and their interaction, and tests of significance were carried out using Bonferroni adjustment for multiple tests. If the interaction was not significant ($P > 0.05$), means were reported, and comparisons were only made for significant different main effects. The following experimental model was used:

$$Yijk = \mu + Oi + Cj + (OC)ij + eij$$

Where, Yijk is the dependent variable; μ is the overall mean; Oi is the effect of genetic group, where $i = 1, 2$; Cj is the effect of body weight j, where $j = 1, 2$ and 3; (OC)ij is the interaction between genetic group i and body weight j; eijk is the error term.

The daily food intake/week data were subjected to regression analysis using SPSS 22 software (SPSS Inc., Chicago, IL, USA), with a significance level of $P = 0.01$.

The Pearson correlation coefficient was determined using the PROC CORR (SAS Institute Inc., Cary, NC, USA). The correlation coefficient for serum parameters was initially determined between six specific sets of observations, namely 90 kg, 120 kg and 160 kg, for both AL and F1 pigs. Metabolite data was then correlated with animal performance data (feed conversation index-CI and daily feed intake/week), fat deposits (abdominal fat-ABF, marbling-MBL, dorsal backfat 12-DB12 and dorsal backfat 6-DB6) and organ weight (liver-LIV and heart-HEA). All these attributes were correlated with meat traits: Warner-Bratzler shear force (WBSF 2 d and WBSF 9 d), color coordinates (L*, a* and b* at 2 and 9 d *post mortem*), pH and centesimal composition (moisture-MOI, ether extract-IMF, crude protein-CP and ash-ASH).

3. Results

3.1. Analysis of variance (ANOVA)

The analysis of variance (Table 1) showed that the biochemical parameters were influenced ($P < 0.05$) in 100% by the genetic factor and in 75% by the effect of body weight. Significant interaction ($P < 0.05$) was observed for TG, BUN and ALB. The coefficients of determination (R^2) for TG and BUN were close to 0.60, and variation in results was explained in about 60% by the main factors (genetic group and body

Table 1
Least-squares means and standard error of the mean (SEM) for plasma biochemical parameters from Alentejano pigs- AL and F1 Landrace*Large White pig-F1, obtained at 90, 120 and 160 kg of body weight[1].

Variables	Genetic group - GG		Body weight - BW				*P* value[2]				
	AL (n = 30)	F1 (n = 30)	SEM	90 (n = 60)	120 (n = 60)	160 (n = 60)	SEM (gap)	GG	BW	GG*BW	RSD[3]	(R^2)[4]
GLU (mmol/L)	5.386[a]	4.632[b]	0.083	4.875[b]	4.428[a]	5.722[c]	0.100 - 0.105	<0.0001	<0.0001	0.0979	0.774	0.43
TC (mmol/L)	2.706[a]	2.135[b]	0.033	2.316[b]	2.534[a]	2.411[b]	0.039 - 0.042	<0.0001	0.0007	0.3351	0.308	0.50
TG (mmol/L)	0.827[a]	0.478[b]	0.026	0.475[a]	0.822[b]	0.659[c]	0.032 - 0.033	<0.0001	<0.0001	<0.0001	0.245	0.59
BUN (mg/dL)	28.574[a]	22.431[b]	0.408	25.240[a]	28.826[b]	22.442[c]	0.490 - 0.517	<0.0001	<0.0001	<0.0001	3.798	0.61
TP (g dL)	7.540[a]	7.169[b]	0.040	7.313[a]	7.232[a]	7.518[b]	0.049 - 0.051	<0.0001	0.0003	0.0794	0.377	0.28
HDL (mmol/L)	0.883[a]	0.723[b]	0.016	0.815	–	0.790	0.016 - 0.017	<0.0001	0.2756	0.9788	0.123	0.31
LDL (mmol/L)	1.619[a]	1.275[b]	0.031	1.406	–	1.488	0.030 - 0.032	<0.0001	0.0610	0.9171	0.233	0.37
ALB (g/dL)	3.892[b]	3.785[a]	0.022	3.728[c]	3.852[b]	3.934[a]	0.027 - 0.029	0.0013	<0.0001	0.0041	0.244	0.23

GLU = Glucose, TC = Total cholesterol, TG = Triglycerides, BUN = Urea-N, TP = Total protein.
HDL = HDL-cholesterol, LDL = LDL cholesterol, ALB = Albumin.
[1] Means with different letter in line differ significantly ($P \leq 0.05$).
[2] *P*-values for the effect of genetic group (GG), body weight (BW), and genetic groups*body weight interaction (GG*BW).
[3] RSD = residual standard deviation.
[4] R^2 = coefficients of determination (R^2).

weight) and by the interaction (genetic group-GG x body weight-BW). For ALB, with R^2 = 0.23, the main effects and the interaction explained only 23% of the variation in the results. The other variables (GLU, TC, HDL, LDL and TP) presented an R^2 between 0.28 and 0.50.

3.2. Main effects (genetics groups and body weight) and interaction effect

The means for TC, HDL and LDL showed a significant effect (P <0.05) of the genetic group variable. Compared to F1 pigs, AL pigs showed higher TC, HDL and LDL averages, by about 20.0%, 18.0%, and 21.0%, respectively. Glucose and TP averages were significantly higher (P <0.05) in AL than in F1 pigs (differences were close to 14.0% and 5.0%, respectively).

Regarding body weight, animals at 120 kg showed significantly higher values (P <0.05) of TC, TG and BUN, when compared to animals at 90 or 160 kg. For the TC parameter, these differences were around 8.5% and 4.8% for animals weighing 90 and 160 kg, respectively. On the other hand, high levels of GLU, TP and ALB were found in animals at 160 kg (P <0.05), when compared to animals at 90 and 120 kg. Those values were higher by about 15.0 and 22.60% for GLU, 2.73 and 3.80% for TP, and 5.24 and 2.10% for ALB, when compared to pigs at 90 and 120 kg, respectively. Results for TG, BUN and ALB will be presented below (interaction). Pigs at 90, 120 and 160 kg showed similar results for LDL and HDL.

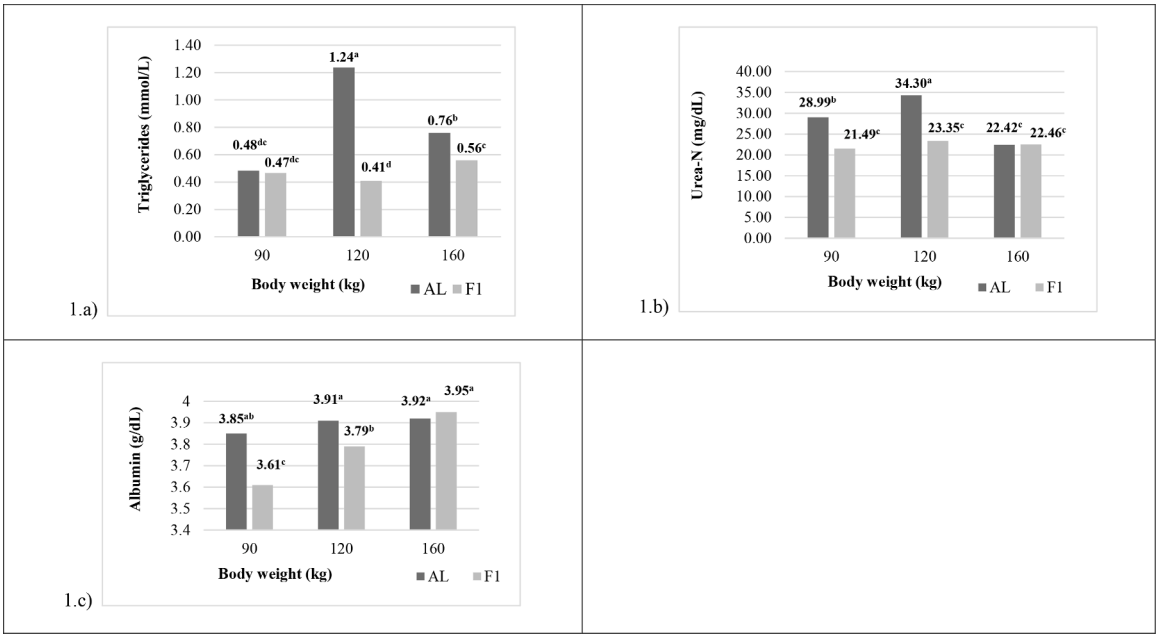

Fig. 1. Graphical representation of variables with significant interaction (P <0.05) between genetics groups (Alentejano pig-AL and F1 Landrace*Large White) and body weight (90 kg, 120 kg and 160 kg): a) triglycerides (mmol/L); b) urea-N (mg/dL); c) albumin (g/dL).

The average results of the interaction between genetic groups and body weight for TG, BUN and ALB are shown in Figure 1a, 1.b and 1.c, respectively. For the TG parameter, AL and F1 pigs had similar results, at 90 kg. However, at 120 and 160 kg, AL pigs showed TG values approximately 2.6 and 1.6 times higher than those found in F1 pigs. On the other hand, F1 pigs presented similar TG averages, at 90, 120 and 160 kg. ALB levels in F1 pigs (Fig. 1.c) increased progressively, with differences of 8.6 and 9.5%, as body weight increased from 90 to 120 kg, and from 120 to 160 kg, respectively. However, AL animals showed similar ALB means at 90, 120 and 160 kg. BUN levels found in F1 pigs (90, 120 and 160 kg) and AL pigs at 160 kg were similar. However, when compared to F1 pigs (90 and 120 kg), AL pigs (90 and 120 kg) presented high BUN averages, about 26 and 31% higher, respectively.

Throughout the finishing period (between 90 and 160 kg of live weight), the mean values for daily feed intake/week, total feed intake and feed conversion index (feed / gain ratio) were significantly influenced ($P < 0.05$) by genetic groups. The means for daily feed intake/week were 4.777 kg and 3.342 kg for AL and F1 pigs, respectively, with a standard error of 0.054 kg (data not shown). This represented a difference of about 1435 kg, or 30% higher, for AL pigs, when compared to F1 pigs. Total feed consumption was higher for AL pigs (81,102.466 kg), when compared to F1 pigs (408,501.133 kg) (data not shown), representing a 20% increase. Feed conversion index means were 5.695 and 4.720 for AL and F1 pigs, respectively, with a standard error of 0.08 (data not shown). The feed conversion index in AL pigs was higher (less efficient) than in F1 pigs, by about 0.975 (or 17%).

Data for daily feed intake/week, obtained over a period of 15 weeks, is shown in Fig. 2. The regression analysis of the variable daily food intake/week (kg), showed that, for AL pigs, the data were adjusted to a cubic model, where $y = 0.002x\hat{}3 - 0.074x\hat{}2 + 0.745x + 3.052$, where y represents the daily feed intake, with $R^2 = 93.2\%$ and significance $P = 0.000$. For F1 pigs, the regression analysis showed that the daily feed intake data were adjusted to a quadratic model, where $y = -0.074x\hat{}2 + 0.067x + 3.413$, with $R^2 = 97.2\%$ and significance $P = 0.000$. In F1 pigs, the average daily feed intake values were similar for the first 8 weeks (an average of 3.5 kg/week) and decreased thereafter, from 3.4 to 3.2 kg/week, between the 9th and 12th weeks, and from 3.0 to 2.8 kg/week, between the 13th and 15th weeks. For AL pigs, the daily feed intake/week in the first 5 weeks increased from 3.4 to 5.2 kg/week, followed by similar values between the 5th and 10th weeks (an average of 5.3 kg/week), then decreasing about 0.4 kg, to 4.8 kg/week (11th week) and stabilizing until the 15th week, at 4.4 kg/week. Between genetic groups, daily feed intake/week differences increased progressively, with values between 0.470 and 1.610 kg from the 1st to the 5th week; maximum differences between the 6th and 10th week (1.810 and 1.940 kg, respectively), and then decreasing to values between 1.340 and 1.600 kg (11th and 15th weeks, respectively).

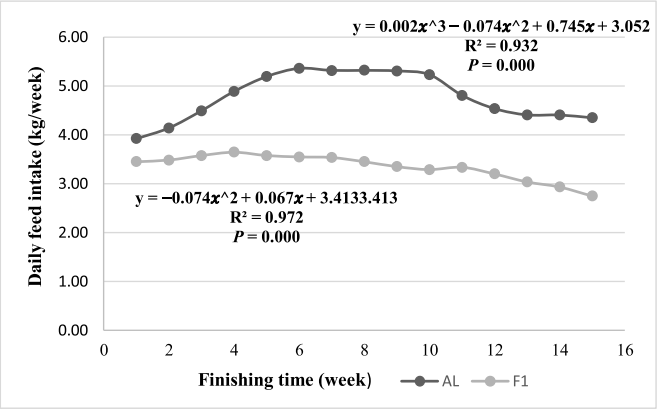

Fig. 2. Graphical representation of average daily feed intake/week (kg), regression equations, coefficients of determination (R^2) and significance for Alentejano (AL) pigs and Large White*Landrace (F1) animals.

High average daily weight gains were found ($P < 0.05$) in AL animals, when compared to F1 animals. In the period between 90 and 120 kg, the animals showed daily weight gains of 0.843 and 0.638 g/d for AL and F1, respectively (with a difference of about 24%). In the period between 120 and 160 kg, average values of 0.657 and 0.578 g/d were obtained for AL and F1 pigs, respectively (with a difference of about 12%) (Almeida et al., 2019).

3.3. Analysis of correlations

The correlation coefficients were obtained from the response variables, grouped individually according to genetic groups (AL and F1 pigs) and live weights (90, 120, and 160 kg). Correlations were studied between biochemical parameters and animal production traits, carcasses and organs (heart and liver). These results are presented in Table 2. Correlations between biochemical parameters, proximate analysis and meat characteristics are presented in Table 3. In these tables, rows and columns with no significant correlation indexes were removed.

3.3.1. Glucose (GLU)

In Alentejano pigs at 120 kg, significant correlations ($P < 0.05$) were found between GLU and ALB ($r = 0.37$) and between GLU and ALT ($r = 0.38$). Also, a negative correlation index was detected between GLU and DB6 ($r = -0.44$). In AL pigs at 160 kg, significant positive correlations were identified between GLU and ALT ($r = 0.66$), and negative correlations were observed between GLU and DB6 ($r = -0.31$) and GLU and MBL ($r = -0.56$). In F1 animals at 90 kg, significant correlations ($P < 0.05$) were found between GLU and PB ($r = 0.43$) and between GLU and pH ($r = 0.52$). In F1 pigs at 120 kg, GLU was positively correlated with daily feed intake ($r = 0.41$). These results demonstrate that in AL pigs (120 and 160 kg), high glucose levels coincided with high L. *thoracis* areas, high albumin levels (120 kg), high crude protein in meat (160 kg) and high final pH values. On the other hand, high glucose serum content was negatively related to backfat and MBL (120 and 160 kg).

3.3.2. Triglycerides (TG)

In pigs at 90 kg of body weight, TG were significantly correlated ($P < 0.05$) with ALB (0.45 and 0.44 for AL and F1 pigs, respectively) and BUN (0.54 for AL). In F1 animals, TG were correlated with ALT (0.36). At 120 kg, AL pigs show positive correlations between TG and TP (0.65), ALB (0.59), ABF (0.51), MBL (0.48), DB12 (0.47), DB6 (0.42), as well as a negative correlation with HEA (−0.39). At 160 kg, the correlation between TG and DB12 (0.42) was also significant for AL pigs. As for physical and chemical characteristics of meat, TG values were negatively associated ($P < 0.05$) with the b* coordinate (−0.36, AL at 120 kg), pH (0.44 and 0.39, in AL and F1, respectively) and ASH (0.38 in F1, at 160 kg). These results show that high TG values were associated to high levels of TP, ALB and BUN. Regarding tissue development, for AL pigs at 120 kg of live weight, high TG rates were associated with high levels of fat deposition (abdominal, subcutaneous and intramuscular); for F1 pigs (90 kg), high TG rates were associated with high areas of the L. *thoracis*.

3.3.3. Total cholesterol (TC), HDL-cholesterol (HDL) and LDL-cholesterol (LDL)

Total cholesterol was strongly correlated (r between 0.70–0.90) with HDL, LDL, BUN (AL pigs) and moderately correlated (r between 0.50–0.70) with TG, TP, ABF, MBL and DB12. At 90 kg, significant correlations ($P < 0.05$) were found between TC and HDL (0.73 and 0.69, for AL and F1, respectively), TC and LDL (0.93 and 0.92, for AL and F1), TC and BUN (0.70, for AL), TC and TP (0.52 and 0.40, for AL and F1), TC and ABF (0.42 and −0.51, for AL and F1) and TC and MBL (0.39, for AL). At 120 kg of live weight, AL pigs with high TC levels showed high TG (0.52), TP (0.39), ALB (0.43), ABF (0.49) and DB12 (0.57). However, F1 pigs with high TC showed low ABF (−0.56). In the 160 kg groups, high TC was correlated with high HDL (0.55 and 0.81, for AL and F1), LDL

Table 2
Correlations among biochemical parameters, feed conversion index (CI), daily feed intake/week (DFI), fat depots (abdominal - ABF and dorsal backfat - DB6 and DB12), marbling (MBL), area L. *thoracis* (ALT), and weight of organs (liver - LIV and heart - HEA) of the Alentejano-AL and F1 (Landrace*Large White) pigs, intensively finished between 90 and 160 kg of live weight.

Genetic group	Biochemical parameters[1, 2]	TG	HDL	LDL	BUN	TP	ALB	CI	DFI	ABF	MBL	DB12	DB6	ALT	LIV	HEA
AL pigs 90 kg	Cholesterol–TC	0.347	**0.737**	**0.939**	**0.703**	**0.519**	0.149	0.149	−0.072	**0.422**	**0.390**	0.249	0.345	−0.006	0.147	−0.127
	Triglycerides–TG	–	0.322	0.215	**0.544**	0.244	**0.454**	0.083	−0.084	0.203	0.065	0.127	0.032	0.369	0.038	−0.025
	HDL		–	**−0.468**	**0.422**	**0.455**	**0.415**	0.303	**−0.424**	**0.489**	**0.596**	**0.389**	**0.464**	0.075	0.027	−0.130
	LDL			–	**0.670**	**0.448**	−0.041	0.045	0.114	0.306	0.225	0.132	0.232	−0.080	0.179	−0.104
	Urea-N–BUN				–	**0.458**	0.154	0.238	−0.041	0.316	**0.435**	0.295	0.329	−0.033	0.050	−0.167
	Total protein–TP					–	**0.518**	**0.402**	−0.309	0.187	**0.462**	0.006	0.330	−0.354	−0.188	−0.284
F1 pigs 90 kg	Cholesterol–TC	0.227	**0.696**	**0.919**	−0.205	**0.400**	0.327	0.048	−0.143	**−0.508**	−0.115	0.066	0.104	0.311	−0.129	−0.107
	Triglycerides–TG	–	0.224	0.070	−0.187	−0.005	**0.441**	−0.130	−0.271	−0.336	−0.172	−0.177	−0.068	**0.362**	−0.002	0.196
	HDL		–	**0.369**	−0.022	0.051	0.263	0.006	−0.130	**−0.436**	−0.147	−0.269	−0.068	0.048	**−0.426**	−0.005
	LDL			–	−0.236	**0.494**	0.249	0.073	−0.090	**−0.400**	−0.055	0.244	0.180	**0.389**	−0.038	−0.115
	Urea-N–BUN				–	−0.146	−0.014	0.165	−0.140	**0.427**	0.274	0.050	**0.407**	**−0.367**	−0.161	−0.048
	Total protein–TP					–	0.121	−0.003	**−0.429**	0.038	0.053	0.110	0.286	−0.012	−0.182	0.241
AL pigs 120 kg	Glucose–GLU	0,235	–	–	0.144	0.283	**0.376**	−0.237	−0.030	0.038	−0.186	−0.253	**−0.449**	**0.383**	0.274	0.068
	Cholesterol–TC	**0.518**	–	–	0.243	**0.391**	**0.433**	−0.125	0.037	**0.494**	0.275	**0.571**	0.376	0.222	0.315	−0.184
	Triglycerides–TG	–	–	–	0.122	**0.650**	**0.597**	0.137	−0.334	**0.512**	**0.487**	**0.474**	**0.422**	0.015	0.217	**−0.398**
	Urea-N–BUN				–	0.338	**0.434**	−0.268	0.344	0.193	0.149	−0.049	0.016	−0.018	0.251	−0.290
	Total protein–TP					–	**0.733**	**0.420**	**−0.406**	**0.615**	**0.570**	0.358	**0.450**	−0.036	−0.105	−0.310
	Albumin–ALB						–	0.172	−0.250	**0.490**	0.308	0.312	0.190	**0.371**	0.177	−0.232
F1 pigs 120 kg	Glucose–GLU	0.066	–	–	0.144	0.026	0.021	0.049	**0.417**	0.051	0.101	0.079	−0.194	0.179	0.349	−0.085
	Cholesterol–TC	0.321	–	–	0.006	−0.034	0.338	−0.020	0.0087	**−0.557**	−0.263	−0.167	−0.173	0.350	0.010	0.064
	Triglycerides–TG	–	–	–	**0.370**	0.275	0.290	0.005	−0.035	−0.153	−0.293	−0.095	−0.019	0.146	−0.042	0.029
	Albumin–ALB						–	−0.298	0.326	−0.358	−0.101	−0.159	−0.362	0.222	**0.364**	0.048
AL pigs 160 kg	Glucose–GLU	0.280	0.124	0.224	0.036	−0.114	−0.020	−0.365	0.196	−0.079	**−0.559**	0.155	−0.318	**0.666**	0.324	0.149
	Cholesterol–TC	0.271	**0.553**	**0.874**	0.146	0.187	**0.430**	−0.204	0.174	−0.021	−0.129	0.194	0.106	0.198	0.176	0.117
	Triglycerides–TG	–	0.320	0.018	0.022	−0.016	0.072	−0.104	0.099	0.001	−0.045	**0.427**	0.167	0.035	0.315	−0.061
	HDL		–	0.094	**0.451**	−0.031	0.102	**−0.524**	0.149	−0.008	−0.215	0.118	0.113	0.298	**0.468**	0.189
	LDL			–	−0.068	0.245	**0.454**	0.044	0.116	0.020	−0.036	0.108	0.044	0.070	−0.078	0.050
	Urea-N–BUN				–	0.096	0.025	**−0.580**	**0.417**	−0.097	−0.330	−0.255	−0.305	0.243	**0.480**	−0.121
	Total protein–TP					–	**0.831**	0.323	−0.317	0.225	0.215	0.018	0.228	−0.157	**−0.625**	−0.233
	Albumin–ALB						–	0.311	−0.283	0.114	0.221	0.210	0.265	0.084	**−0.441**	−0.229
F1 pigs 160 kg	Glucose–GLU	−0.057	0.140	−0.330	−0.065	−0.198	**−0.597**	0.242	0.033	0.236	0.182	−0.193	0.104	−0.019	−0.242	−0.223
	Cholesterol–TC	0.237	**0.814**	**0.913**	−0.065	−0.204	0.185	−0.171	0.185	**−0.507**	0.062	0.128	−0.261	0.363	0.307	0.022
	HDL		–	**0.528**	−0.000	−0.001	0.000	0.068	0.088	−0.354	0.025	0.127	−0.119	0.280	0.026	−0.010
	LDL			–	−0.087	−0.265	0.251	−0.243	0.173	**−0.482**	0.048	0.070	−0.337	0.323	**0.383**	0.053
	Urea-N–BUN				–	**0.388**	0.171	−0.017	−0.052	0.170	−0.104	**0.444**	0.117	0.141	0.341	−0.249
	Albumin–ALB						–	−0.183	−0.027	**−0.440**	−0.187	−0.131	−0.079	0.284	0.301	−0.076
Genetic group	**Performance x carcass [2]**	**TG**	**HDL**	**LDL**	**BUN**	**TP**	**ALB**	**CI**	**DFI**	**ABF**	**MBL**	**DB12**	**DB6**	**ALT**	**LIV**	**HEA**
AL pigs	Feed conversion index–CI							–	**−0.498**	**0.327**	**0.630**	0.298	**0.487**	**−0.415**	**−0.697**	−0.227
	Daily feed intake–DFI								–	**−0.318**	**−0.357**	**−0.241**	**−0.393**	−0.018	**0.568**	0.062
	Abdominal fat depots–ABF [3]									–	**0.453**	0.168	**0.384**	**−0.248**	0.111	**−0.327**
	Marbling–MBL [3]										–	**0.394**	**0.737**	**−0.567**	−0.181	**−0.460**
	Dorsal backfat 12 cm –DB12 [3]											–	**0.414**	0.092	0.035	−0.114
	Dorsal backfat, 6 cm–DB6 [3]												–	**−0.671**	0.029	**−0.342**
	Area L. *thoracis*–ALT [3]													–	0.028	**0.327**
F1 pigs	Feed conversion index–CI							–	−0.074	0.229	0.172	−0.106	**0.440**	−0.031	**−0.513**	0.008
	Daily feed intake–DFI								–	−0.160	0.095	0.136	**−0.353**	0.194	**0.519**	0.008
	Abdominal fat depots–ABF [3]									–	**0.398**	0.058	**0.573**	**−0.612**	**−0.249**	−0.114
	Marbling–MBL [3]										–	**0.397**	**0.581**	**0.400**	−0.189	**−0.252**
	Dorsal backfat 6 cm–DB6 [3]												–	**−0.524**	**−0.519**	**−0.267**
	Area L. *thoracis*–ALT [3]													–	**0.371**	−0.037

GLU = Glucose, TC = Total cholesterol, TG = Triglycerides, BUN = Urea-N, TP = Total protein,.
HDL = HDL-cholesterol, LDL = LDL cholesterol, ALB = Albumin.
[1] Correlations with estimate >|0.36| are significant ($P < 0.05$).
[2] Correlations with estimate >|0.20| are significant ($P < 0.05$).
[3] Carcass data obtained at slaughter.

Table 3

- Correlations among biochemical parameters (obtained at 90 kg), feed conversion index, daily feed intake / week, fat depots (abdominal, and dorsal backfat), marbling, area L. *thoracis*, weight of internal organs (liver and heart), Warner-Bratzler Shear Force (WBSF2 and WBSF9 at 2 and 9 d *post mortem*, respectively), color coordinates (L2, A2, B2 and L9, A9, B9 to L* a* b* at 2 d and 9 d *post mortem*), proximate composition (moisture-MOI, ashes-ASH, crude protein-PB, ethereal extract-IMF) and pH (at 2 d *post mortem*) of the pigs intensively finished between 90 and 160 kg of live weight.

Genetic group	Biochemical parameters[1]	WBSF2	WBSF9	L2	A2	B2	L9	A9	B9	MOI	ASH	PB	IMF	pH
AL - 90 kg	Cholesterol–TC	−0.06	0.199	**−0.406**	0.042	−0.166	−0.043	−0.119	−0.181	0.033	**0.362**	0.097	−0.006	0.277
	LDL	−0.054	0.309	**−0.363**	−0.072	−0.145	0.046	−0.184	−0.104	0.106	**0.378**	0.103	−0.025	0.158
	Urea-N–BUN	−0.051	0.118	**−0.409**	0.017	−0.235	−0.258	−0.155	−0.294	−0.085	0.243	0.075	−0.044	0.312
	Total protein–TP	−0.324	−0.086	**−0.451**	0.231	−0.297	0.151	0.218	−0.112	0.153	**0.474**	0.084	−0.094	**0.372**
	Albumin–ALB	0.086	−0.147	**−0.421**	0.100	−0.228	**−0.426**	0.191	−0.263	0.255	0.062	0.223	−0.294	**0.401**
F1 - 90 kg	Glucose–GLU	−0.157	0.262	−0.147	−0.333	−0.116	−0.277	−0.117	−0.205	0.165	−0.317	**0.438**	−0.246	**0.526**
	Albumin–ALB	0.256	0.254	−0.020	−0.226	−0.030	0.145	−0.000	0.071	−0.199	**−0.385**	0.308	−0.030	0.135
AL - 120 kg	Triglycerides–TG	−0.213	−0.146	−0.330	0.165	0.030	−0.327	0.073	**−0.365**	−0.085	0.290	−0.136	−0.088	**0.444**
	Total protein–TP	−0.130	−0.028	**−0.368**	0.237	−0.138	−0.273	0.195	−0.295	0.032	0.338	0.083	−0.209	0.318
	Albumin–ALB	−0.044	−0.011	−0.262	0.330	−0.033	**−0.391**	0.257	−0.285	−0.201	0.039	−0.044	−0.068	0.274
F1 - 120 kg	Triglycerides–TG	−0.270	0.161	−0.029	−0.199	−0.030	−0.005	0.038	0.049	0.259	0.230	0.240	−0.207	**0.396**
AL - 160 kg	HDL	0.042	0.310	0.379	−0.023	**0.467**	**0.455**	−0.176	0.167	−0.107	−0.127	−0.157	0.241	0.173
	Urea-N–BUN	0.078	**0.542**	0.210	0.058	**0.444**	**0.436**	0.009	0.332	0.193	−0.002	0.058	0.017	−0.293
	Albumin–ALB	−0.037	**−0.426**	0.160	0.343	−0.103	−0.150	0.085	0.056	−0.325	0.025	−0.043	**0.465**	0.054
F1 - 160	HDL	−0.315	−0.288	−0.242	−0.283	−0.232	−0.134	**−0.461**	−0.332	−0.172	0.125	0.119	0.108	0.109
	Urea-N–BUN	**0.443**	0.088	0.041	0.080	0.049	0.025	−0.131	0.020	0.011	0.107	0.078	−0.083	0.190
	Total protein–TP	0.093	0.088	0.121	0.320	**0.408**	0.255	0.215	0.373	−0.243	−0.059	−0.140	0.309	−0.038
Genetic group	**Performance x carcass** [2]	**WBSF2**	**WBSF9**	**L2**	**A2**	**B2**	**L9**	**A9**	**B9**	**MOI**	**ASH**	**PB**	**IMF**	**pH**
AL pigs	Feed conversion index	−0.076	**−0.497**	**−0.324**	**0.419**	**−0.468**	**−0.274**	**0.291**	−0.082	−0.039	**0.439**	**0.291**	0.016	0.209
	Daily feed intake	0.148	**0.540**	−0.207	**−0.374**	0.131	0.066	**−0.322**	0.015	**0.388**	**−0.288**	**0.249**	**−0.323**	**−0.215**
	Abdominal fat depots [3]	**0.343**	0.151	−0.060	0.038	−0.085	−0.012	−0.035	−0.103	**0.182**	**0.218**	**0.350**	**−0.297**	−0.080
	Marbling [3]	**−0.262**	−0.160	**−0.446**	0.015	**−0.399**	**−0.273**	0.031	**−0.251**	−0.105	0.176	−0.036	0.055	**0.215**
	Dorsal backfat 12 cm [3]	−0.053	−0.157	−0.134	0.090	−0.068	−0.205	−0.000	−0.178	**−0.398**	−0.014	**−0.271**	**0.292**	0.156
	Dorsal backfat, 6 cm [3]	0.045	0.179	−0.040	**−0.228**	−0.101	0.085	**−0.275**	−0.172	**−0.189**	0.023	−0.118	0.163	**−0.188**
	Area L. *thoracis* [3]	−0.117	**−0.247**	0.157	**0.360**	**0.266**	−0.098	**0.336**	0.144	−0.083	−0.037	0.080	0.052	0.181
	Liver [3]	0.111	**0.509**	−0.015	**−0.235**	**−0.224**	0.172	**−0.196**	−0.001	0.068	−0.143	−0.094	−0.103	−0.177
	Heart [3]	−0.004	0.032	**0.224**	−0.085	0.183	**0.224**	0.118	**0.273**	0.026	−0.037	−0.034	−0.073	−0.109
F1 pigs	Feed conversion index	**−0.244**	0.078	−0.134	0.084	0.090	0.025	**0.265**	**0.242**	0.135	−0.016	0.048	−0.092	0.018
	Daily feed intake	−0.184	**−0.539**	0.056	−0.087	−0.120	0.122	**−0.343**	−0.090	−0.154	**0.323**	−0.104	0.171	**−0.435**
	Abdominal fat depots [3]	**0.260**	**0.352**	**−0.420**	**−0.420**	**−0.429**	**−0.346**	**−0.212**	**−0.291**	**−0.231**	−0.070	0.073	**0.272**	−0.023
	Marbling [3]	0.110	0.164	**−0.517**	**−0.225**	**−0.371**	**−0.280**	−0.178	**−0.294**	**−0.352**	**−0.208**	−0.115	**0.383**	−0.104
	Dorsal backfat 6 cm [3]	**0.364**	**0.401**	**−0.381**	**−0.473**	**−0.340**	**−0.265**	**−0.287**	**−0.216**	**−0.335**	0.012	−0.057	**0.433**	0.023
	Area L. *thoracis* [3]	**−0.366**	**−0.283**	**0.491**	**0.473**	**0.498**	**0.411**	**0.301**	**0.452**	**0.187**	**0.228**	0.014	**−0.328**	0.060
	Liver [3]	**−0.237**	**−0.430**	**0.349**	**0.446**	**0.382**	**0.344**	**0.281**	**0.352**	−0.042	**0.201**	0.113	−0.119	−0.125
	Heart [3]	**−0.373**	**−0.343**	0.127	**0.321**	**0.257**	−0.012	**0.317**	0.143	0.090	0.009	**−0.259**	0.065	0.086

[1] Correlations with estimate >|0.36| are significant ($P < 0.05$).
[2] Correlations with estimate >|0.20| are significant ($P < 0.05$).
[3] Carcass data obtained at slaughter.

(0.87 and 0.91, for AL and F1) and ALB (0.43, for AL). For F1 pigs, high TC levels were related to low ABF deposition (−0.51). At 90 kg of live weight, AL pigs showed positive correlations (P <0.05) between HDL and BUN (0.42), TP (0.45), ALB (0.41), ABF (0. 48), MRB (0.59), DB12 (0.38) and DB6 (0.46); on the other hand, they showed negative correlations between HDL and LDL (−0.47) and HDL and DIF (−0.42). However, in F1 pigs, high levels of HDL were related to high LDL (0.36 and 0.52, in 90 and 160 kg animals). High levels of HDL were associated with low ABF (−0.43) and LIV (−0.42). At 90 kg, both AL and F1 pigs showed a significant and positive correlation (P <0.05) between LDL and TP (0.45 and 0.49, respectively). In AL pigs, high LDL values were also associated with high levels of BUN (0.67, at 90 kg) and ALB (0.45, at 160 kg). In F1 pigs, high LDL were negatively related to ABF (−0.40 and −0.48, at 120 and 160 kg), but positively related to ALT (0.38, at 90 kg) and LIV (0.38, at 160 kg). These coefficients describe the close relationship between total cholesterol, LDL and HDL, since LDL and HDL lipoproteins are associated with cholesterol mobilization in the bloodstream. At 90 kg, for F1 pigs, average HDL values increased when average LDL values rose. In these animals, higher cholesterol values have been associated with lower average values of fat deposition. In AL pigs, on the other hand, higher average LDL values were associated to lower HDL levels and fat deposition, correspondingly, resulting in higher cholesterol deposition. High levels of HDL were correlated with high fat deposition in all deposits analyzed, with r between 0.39 and 0.60 (AL at 90 kg). However, for AL pigs at 120 kg, high amounts of fat deposits were correlated with high serum TG (with r between 0.42 to 0.51), as shown below.

This data suggests that fat deposition on the carcass at an initial finishing stage is associated, in AL pigs, with high cholesterol deposition; however, at 120 kg, fat deposition is related to triglyceride deposition. These indexes show an important association between lipoproteins and fat tissue development.

3.3.4. Total protein (TP), albumin (ALB), and urea (BUN)

Concerning the correlation between TP and ALB, AL pigs showed a similar pattern throughout the experiment, with indexes of 0.51, 0.73 and 0.83, for 90, 120 and 160 kg, respectively. These two metabolites (TP and ALB) were positively correlated with BUN, with indexes of 0.46, 0.43 and 0.39 for AL (90 and 120 kg) and F1 pigs (160 kg). In AL pigs, high BUN was shown to be associated with LDL (0.67 at 90 kg) and ALB (0.45 at 160 kg). In F1 pigs, high levels of LDL were negatively related to the abdominal fat deposits (−0.40 and −0.48, at 120 and 160 kg, respectively), and positively to the ALT (0.39, at 90 kg) and LIV (0.38, at 160 kg). These results indicate that BUN (product of protein excretion involved in lipid transport) was highly correlated with LDL lipoprotein. That is, in the early fattening phase, lipid metabolism involves cholesterol deposition. In F1 pigs, high levels of LDL are negatively associated with fat deposits and positively associated with areas of the *Longissimus thoracis*.

3.3.5. Biochemical parameters and animal performance

Feed conversion index (CI) and daily feed intake measures were correlated with TP. In AL pigs at 90 and 120 kg, the correlation coefficient between TP and CI was 0.40 and 0.42, respectively. In F1 pigs at 90 and 120 kg, correlations between TP and daily feed intake were −0.43 and −0.41, respectively. On the other hand, AL pigs at 160 kg showed negative correlations between BUN and CI (−0.58) and between HDL and CI (−0.52). Data suggests that higher amounts of serum TP are associated with higher CI indexes (lower production efficiency), at 90, 120 and 160 kg. This association of results is confirmed in AL pigs at 160 kg, by the correlations between BUN and CI and between HDL and CI. In AL and F1 pigs, high daily feed intake determined high LIV (with r values of 0.57 and 0.52, respectively). On the other hand, animals with high IC presented lower LIV (with r values of −0.70 and −0.51, respectively). This demonstrates that animals with higher feed consumption (daily feed intake/week) presented higher LIV. However, animals with higher CI (lower feed efficiency) exhibited lower liver weight.

3.3.6. Biochemical parameters and carcass traits

For carcass traits, associated with lipid and protein mobilization, high correlations were found between TP and ABF (r = 0.62), TP and MBL (0.57), TP and DB6 (0.45), and between ALB and ABF (0.49). Similarly, animals with high CI values showed high fat deposition (ABF, MBL and DB6, with r = 0.33, 0.63 and 0.49, respectively). In contrast, high daily feed intake rates coincided with lower fat deposits (ABF, MBL, DB12 and DB6, with r values of −0.32, −0.36, −0.24 and −0.39, respectively). Data shows that in AL pigs at 120 kg, high TP rates provided higher fat deposits. And animals with higher fat deposits showed a higher CI ratio. Additionally, the genetic groups showed opposite behavior patterns between ALB and abdominal fat, resulting in a positive association (0.49) in AL pigs at 120 kg and a negative association (−0.44) in F1 pigs at 160 kg. This opposite behavior was also observed between ALB and LIV, with a positive association for F1 (0.36) and a negative association for AL (−0.44). AL pigs with low HEA values had high TG (−0.40), ABF (−0.33), MBL (−0.46) and DB6 (−0.34). This showed that AL pigs with lower heart weight were associated with high rates of TG and fat deposits (abdominal, marbling and backfat).

3.3.7. Biochemical parameters and meat traits

Regarding meat color in AL pigs at 90 kg, high values of L2 were associated with low TC (−0.41), LDL (−0.36), BUN (−0.41), TP (−0.45) and ALB (−0.42). A similar pattern was observed in AL pigs at 120 kg, with high values of L2 negatively related to BUN (−0.30) and TP (−0.37). In AL pigs at 160 kg, HDL was positively correlated with L9 (0.45) and B2 (0.47), and negatively correlated with A9 (−0.46). Luminosity (L* coordinate) was negatively correlated with TC, LDL, BUN, TP and ALB, at 2 days *post mortem*. However, at 9 d, this negative relationship was only maintained with ALB. Therefore, in AL animals, high serum lipid levels coincided with lower luminosity values on the *Longissimus thoracis* surface, when compared to F1 animals.

In 160 kg AL pigs, at 9 d *post mortem*, WBSF was negatively associated with ALB and CI (−0.43 and −0.50, respectively), but positively with BUN and daily feed intake (0.54 and 0.54, respectively). However, in F1 pigs, WBSF was negatively associated with daily feed intake (−0.54). At both 2 and 9 d *post mortem*, WBSF was negatively related to ALT, LIV and HEA (with an r value between −0.23 and −0.43). These results show that AL pigs at 160 kg with high ALB levels, greater CI, greater LIV weight and greater HEA weight, present lower WBSF (greater tenderness). On the other hand, in AL pigs, animals with higher daily feed intake and larger areas of the *Longissimus thoracis* were associated with higher WBSF (or lower tenderness).

4. Discussion

Differences in fat deposition, *Longissimus thoracis* area and weight of commercial cuts, between AL pigs and F1, that were used for this research work, can be found in (Bressan et al., 2016) and (Almeida et al., 2019). Extensive reviews on lipid metabolism and fat deposition in autochthonous or commercial pigs can be found in (Chapman, 1980; Hausman et al., 2018) and (Poklukar et al., 2020).

In monogastric animals, dietary carbohydrates are converted into glucose (the main source of energy for both oxidation and fat storage). When in a state of positive energy balance, surplus energy is used for synthesizing fat. In our work, glucose rates varied from 4.403 to 5.681 mmol/L. They are within the bounds of reference values for finishing pigs (4.0 to 8.0 mmol/L), according to (Friendship et al., 1984). Compared to the F1, AL pigs had high glucose levels (14% higher), which can be attributed to higher feed intake. The positive relationship between glucose and daily feed intake/week (r = 0.417) was observed in pigs at 120 kg. Regarding the effect of body weight, glucose levels initially showed a reduction of 9.0%, followed by an increase of 22.6%,

when live weights increased from 90 to 120 kg, and from 120 to 160 kg, respectively. The reduction in serum glucose coincided with an increase in triglyceride contents (about 42%). A similar behavior for glucose and triglyceride levels was described by Nakajima et al. (2019) in high backfat pigs (Meishan) and low backfat pigs (Landrace). This glucose reduction may be due to control systems (mediated by insulin), which determine a higher uptake of glucose by adipose and muscle tissue cells.

Quantitatively, the most important plasma lipids are triglycerides. In the present work, TG levels varied from 0.41 to 1.24 mmol/L. Lower values (0.35–0.63 mmol/L) were reported in commercial pigs and AL pigs with live weight between 40 and 110 kg (Kozera et al., 2016; Rauw et al., 2007) . As expected, this parameter was higher in AL pigs, when compared to F1 animals (about 2.55 and 1.15 times, at 120 and 160 kg, respectively). In pigs, minor differences for serum triglyceride between Meishan (high backfat) and Landrace (low backfat) animals, in the order of 23%, were reported by Nakajima et al. (2019). High TG in AL pigs at 120 and 160 kg can be justified by the genetic background associated with the diet. Diets rich in lipids or fast-absorbing carbohydrates can stimulate high levels of triglycerides and cholesterol in the bloodstream. In our study, AL pigs had a total feed intake of 81,102.47 kg/animal and a daily feed intake/week of 4.777 kg, when compared to F1 pigs (408, 501.133 kg and 3.342 kg, respectively). Additionally, when analyzing the daily feed intake/week averages, F1 pigs showed similar averages over the 15 weeks. In AL pigs, however, intake increased gradually during the first 5 weeks, stabilized between the 6th and 10th weeks and decreased slightly towards the 15th week. Comparing triglyceride data with weekly averages of daily consumption, we observed that periods of higher food intake were coincident with higher levels of serum triglyceride. The relationship between high food consumption and high triglyceride rates, in pigs, was described by Rauw et al. (2007).

Total serum proteins (50–65% albumin) support fundamental functions in the body, such as: maintaining osmotic pressure, supplying amino acids for protein synthesis, transporting free fatty acids, cholesterol, bile pigments, hormones and minerals (Piotrowska et al., 2011; Spector, 1975). For total protein and albumin, reference values described in adult pigs (males) are between 5.200 and 8.300 g/dL and 1.900 to 4.200 g/dL, respectively (Friendship et al., 1984). In our research work, mean values found for total protein and albumin ranged from 7.169 to 7.540 g/dL and 3.610 to 3.950 g/dL. When compared to F1 pigs, the AL showed higher values of total protein and albumin, by about 5% and 2.6%, respectively, which can be justified by the high amounts of triglycerides and cholesterol in the blood and the need to transport these lipids (Spector, 1975).

Concerning the effect of body weight, total protein levels in pigs at 160 kg were, as expected, higher (about 3.3%) than for animals at both 90 and 120 kg. In general, biochemical concentrations of total protein and albumin increase with physiological phases or are age-dependent (Piotrowska et al., 2011). In agreement with this, in F1 pigs, serum albumin concentration increased progressively by 4.60% and 4.00% in the 90–120 kg and 120–160 kg ranges, respectively. However, in AL pigs, albumin levels were similar from 90 to 160 kg. Variations in total protein and albumin serum levels generally result from changes in plasma lipid concentrations due to genetic or dietary factors (Cox and García--Palmieri, 1990). However, in AL pigs, albumin levels were similar, although triglycerides increased twofold and total cholesterol increased by 8.6% when body weight increased from 90 to 120 kg. High correlations between albumin and triglycerides, in AL and F1 pigs, were expected, because total proteins are usually involved in triglyceride mobilization.

In the present research work, blood urea ranged from 21.49 to 34.31 mg/dL, and these results are within the 10 to 45 mg/dL reference range (Fernández-Fígares et al., 2018). Urea levels are produced proportionally to dietary protein levels and are considered an indicator of protein metabolism. The urea values found in F1 pigs were as expected. Pigs with high potential for muscle deposition show low plasma urea rates (Coma et al., 1995), due to the efficient use of protein Nitrogen. In literature, serum urea levels are referred to as possible markers of efficient lean tissue growth (Madeira et al., 2016). Supporting this behavior - in AL pigs - high amounts of urea found at 90 and 120 kg were positively related to high rates of lipid mobilization, serum proteins (total protein, albumin, HDL-cholesterol and LDL-cholesterol) and lipids (triglycerides and total cholesterol).

In mammals, cholesterol is essential for membrane biogenesis and steroid hormone biosynthesis. In excess, however, cholesterol can form atheromas and compromise vascular integrity (Röhrl and Stangl, 2018). Thus, the body requires a precise balance between cholesterol synthesis, absorption and excretion. In the bloodstream, cholesterol is transported by lipoproteins, the most important of which are LDL-cholesterol and HDL-cholesterol (Hegele, 2009). In our research work, the close relationships between total cholesterol and LDL and between total cholesterol and HDL are highlighted by the high correlation indexes (between 0.94 and 0.55). In general, endogenous or dietary cholesterol is transported to the regions of use (cells) by LDL-cholesterol, and from the cells to the liver (which removes excess) by HDL-cholesterol, followed by the resulting excretion via bile acids. Changes in cholesterol concentrations are attributed to genetic or dietary factors, as well as metabolic disorders (Nakajima et al., 2019; Pond et al., 1993).

Reference values for cholesterol levels in fattening pigs are between 3.88 and 5.15 mmol/L (Friendship et al., 1984). In our research work, averages ranged from 2.136 to 2.706 mmol/L (or 85.598 mg/dL to 104.640 mg/dL). Closer values, between 2.13 and 2.33 mmol/L, were reported in AL pigs at 28 kg (Fernández-Fígares et al., 2018); values between 81 and 136 mg/dL were obtained in selected pigs, for low or high cholesterol values (Pond et al., 1993). Our data shows that AL pigs presented higher average cholesterol values than F1 pigs (by about 20%). When comparing body weight, animals at 120 kg showed higher cholesterol levels than animals at 90 and 160 kg (higher by about 9% and 5%, respectively). At these body weights, the pigs also had elevated triglyceride levels. In non-obese pigs, the effect of genetic predisposition on cholesterol levels was described by Pond et al. (1993) in a crossbreed population, which presented differences of 15, 27, 40 and 68% between the high and low cholesterol groups, for the first, second, third and fourth generation, respectively. On the other hand (Nakajima et al., 2019) stated that obese pigs (Meishan) showed high values of blood lipids (triglycerides and non-esterified fatty acids), when compared to non-obese pigs (Landrace). The association between high cholesterol and high serum triglyceride concentrations is attributed to the interaction between lipoprotein transcription factors (Bordoni et al., 2021), triggered by dietary changes such as a greater food intake (Rauw et al., 2007) or related to genetic factors (Nakajima et al., 2019). This association between high cholesterol and triglyceride levels seems to be present in AL pigs (120 kg), with a positive correlation of 0.52.

In pigs, HDL-cholesterol levels must represent at least 40% of total cholesterol; values below this percentage are considered undesirable (Winnicka, 2011). However (Kozera et al., 2016) described ratios between 47 and 55% in pigs with live weight between 30 and 110 kg. In the present research work, ratios of HDL to total cholesterol for AL pigs and F1 were 32.6 and 33.8%, respectively, while averages for LDL and HDL varied from 1.275 to 1.619 mmol/L, and from 0.723 to 0.882 mmol/L, respectively. These authors described variations of 1.01 to 1.25 mmol/L and 0.77 to 1.01 mmol/L, for HDL and LDL, respectively, in 1 to 5-months-old pigs from commercial breeds. AL pigs showed higher LDL and HDL than F1 pigs (differences of about 21 and 18%, respectively). These differences between genetic groups for LDL and HDL follow the pattern observed between AL and F1 in terms of total cholesterol (about 20% higher for AL).

Regarding the behavior of lipoproteins LDL and HDL, high cholesterol levels were associated with high LDL and HDL levels in F1 animals (90 kg), which is an expected result. However, in AL pigs (90 kg), high cholesterol values were associated with high LDL levels and low serum HDL levels. Furthermore, in these AL animals, HDL rates were associated with high fat deposition. In general, known functions of HDL-cholesterol

include: reverse cholesterol transport, that removes cholesterol from peripheral tissues to the liver and steroidogenic organs; global cholesterol homeostasis; and promotion of cholesterol efflux from macrophages. However HDL is highly heterogeneous and has many functions, which are not fully understood, unlike LDL-cholesterol (Movva and Rader, 2008; Darabi et al., 2021).

Concerning animal performance, AL pigs showed a correlation between the high feed conversion index (low efficiency) and high fat deposition amounts, when compared to F1 pigs. This was expected, as described by Martins et al. (2019). In these AL pigs (with high feed conversion indexes), high total protein and HDL-cholesterol levels were found at 90 and 120 kg of live weight.

Regarding carcass traits, high glucose serum concentrations were associated with high areas of the *Longissimus thoracis*, low backfat thickness and low marbling index. In the chemical composition of meat, high glucose was related to higher amounts of meat crude protein. This relationship was expected, according to (Choe and Kim, 2014). In our study, triglycerides and carcass traits were correlated differently for each genetic group. In AL pigs at 120 kg, higher triglycerides were related to higher body fat depositions (abdominal fat depots, marbling and backfat). In contrast to the F1 at 90 kg, high triglycerides were related to high areas of the *Longissimus thoracis*. These different relationships between glucose and tissue types can be attributed to genetic differences (Poklukar et al., 2020) In this context (Berg et al., 2003) reported that the Iberian and Landrace breeds differ strongly in growth and carcass traits at 110 kg body weight.

Cholesterol and fat deposits were diversely correlated in the two genetic groups. In AL pigs, high total cholesterol coincided with high abdominal fat deposition. However, in F1 pigs, higher TC coincided with lower abdominal fat deposition (−0.50, −0.55 and −0.50 at 90, 120 and 160 kg, respectively). Relationships between cholesterol levels and tissue deposition vary between pigs with genetic predisposition to obesity and lean pigs (Poklukar et al., 2020). In these animals, during the tissue growth phase, muscle cells and adipocytes interact and influence adipogenesis (such as adipocyte differentiation and proliferation; connective tissue structure incorporating adipocytes) and may be involved in differences in fat content between individuals (Hocquette et al., 2010).

The relationship between serum lipids and fat deposits appeared to differ among fattening phases in AL group pigs. In these animals, high fat deposition was positively correlated with high serum HDL-cholesterol at 90 kg; at 120 kg, it was positively correlated with high triglyceride levels. In our data, the relationship between fat deposition and serum HDL-cholesterol is unexpected. Fat deposition in general is associated with triglyceride deposition or with LDL-cholesterol, as reported by Rauw et al. (2007) in Duroc barrows. On the other hand, considering that the chemical composition of HDL (pigs), by average weight%, features proteins (33.4–54.8%), phospholipids (22.4–38.3%), cholesteryl esters (14.1–27.4%), free cholesterol (2.2–3.9%) and triglycerides (2.0–3.8%) (Chapman, 1980), it is possible that in our research work, differences between animals at 90 and 120 kg may be related to the different stages of adipose tissue development: pre-adipocyte formation, consisting of linear filaments or membranes (rich in cholesterol and phospholipids), followed by triglyceride deposition on the adipocyte (Uezumi et al., 2010).

Meat with high pH value was found in pigs with high serum glucose (F1 pigs at 90 kg) or triglycerides (F1 and AL pigs at 120 kg). In the AL, high total serum protein and albumin were also positively correlated with pH. (Choe and Kim, 2014) stated that pigs with high glucose levels produced meat with higher pH values (darker color), attributed to low glycogen values in muscle tissue. Meat pH is an important determinant of meat quality, as it influences color, water-holding capacity and tenderness. Under normal circumstances, muscle pH declines gradually until the onset of rigor mortis, during *postmortem*. The amount of glycogen stored in the muscle at the time of slaughter is, therefore, decisive for the final pH value of meat. The pH value decreases, in the hours following slaughter, from values close to 7.0, to 6.3 and to 6.1, after 45 min, and then increases from 5.4 to 5.6, after 24 h; (Bertol et al., 2015; Mansutti et al., 2005; Tejeda et al., 2020). On the other hand, a final pH value lower or equal to 5.8 is also important for maintaining the quality traits of meat during shelf life (Holmer et al., 2009).

Regarding meat traits and color, the luminosity (L* value) found on the sample surface was negatively correlated with: cholesterol, LDL-cholesterol, urea-N, total protein and albumin at 2 d *post mortem*. However, at 9 d *post mortem* this negative relationship remained only with albumin. This indicates that AL pigs with high serum lipid levels and high lipid mobilization rates produced darker meat, when compared to F1. Correlations between triglycerides and color coordinates (L*, a*, and b*) showed that high triglyceride rates were correlated with low b* (yellow content), in AL pigs (120 kg). Yellow content is usually associated with carotenoid pigments in the feed (Mancini and Hunt, 2005). High serum triglyceride was associated with low yellowing values, which suggests that high serum TG determines low pigment deposition, possibly due to increased endogenous fat synthesis. Meat with lower tenderness (high WBSF) was found in animals with high rates of BUN, ABF, daily feed intake. However, animals with high ALB showed low mean WBSF (AL-9 d and F1–2 d), and samples of pork with high levels of serum albumin coincided with tenderer meats.

5. Conclusion

The genetic predisposition for fat deposition or muscle deposition defined levels of feed intake as observed when comparing Alentejo (obese) and F1 Landrace x Large white (lean) pigs. In obese pigs, high levels of feed intake determine high plasma triglycerides levels. Compared to F1 pigs, the fat deposition in Alentejan pigs occurs in two distinct phases, the first phase (90 kg) is associated with high levels of HDL-cholesterol, which surprisingly suggests a high deposition of phospholipids, and the second phase (120 kg) is associated, as expected, with high levels of triglycerides. Furthermore, elevated serum albumin levels at an early finishing phase (90 kg) are indicative of animals with the potential to develop obesity. Additionally, pigs with high triglyceride levels showed meat with low luminosity, yellowness and Warner Braztler Shear Force (high tenderness).

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Author contributions

Author contributions: conceptualization, M.C.B. and L.T.G.; data collection and curation, M.C.B., J.M.A., A.T.B., C.B., O.M, A.A., and S.H.; statistical analysis, D.C. and L.T.G.; research, M.C.B., and L.T.G.; writing-original draft preparation, D.C., C.B., and M.C.B.; writing-reviewing and editing, A.T.B., S.H., A.A., and LTG; funding acquisition, M.C.B., and L.T.G. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

None.

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