Blood parameters in fattening pigs from two genetic types fed diet with three different protein concentrations[1]

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ABSTRACT: The study aimed to evaluate possible differences between two genetic groups (**GG**) of pigs, fed diets varying in dietary CP level, in hematological and biochemical plasma profiles. The study was carried out in an experimental farm and involved 36 barrows (average BW 129 ± 11 kg) from two GG: group A (18 Italian Duroc boars × Italian Large White sows) and group D (18 DanBred Duroc), fed three experimental diets: a conventional diet and two low-protein diets (**LP1** and **LP2**). A digestibility/balances trial was carried out on 12 pigs A and 12 pigs D that were housed individually in metabolic cages during four digestibility/balances periods. The experimental design was a factorial design, with 3 diets × 2 GG × 4 periods. The experiment lasted 56 d. Blood was sampled from jugular vein in the morning before feed distribution from all barrows in pens at the start and the end of the experimental period; a supplementary blood sample was collected from the 24 pigs at the end of the four digestibility periods (six pigs per period). Blood was analyzed for hematological and biochemical parameters and serum protein profile using automated analyzers. The GG D showed lower white blood cells (**WBC**), lymphocyte, and monocyte counts than A group. The GG affected several plasma metabolite concentrations: triglycerides, creatinine, Cl, Fe, alkaline phosphatase, and tartrate-resistant acid phosphatase activities were higher in D groups, while urea, albumin, Ca, Na, total bilirubin, and albumin as percentage of total protein were lower than A group. On the contrary, the dietary protein level neither affects WBC nor their populations; only a trend was reported for erythrocytes (red blood cell) and platelets. The diet affected only plasma urea and total bilirubin concentrations.

Keywords: dietary protein, genetic type, hematology, metabolic profile, pig

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INTRODUCTION

The feeding strategy for heavy pig destined for the production of typical Italian hams requires a restricted energy regime (Mordenti et al., 2003) followed by a CP restriction for pigs of high genetic potential for fast lean growth rate, as that available from recent genetic progress (Bosi and Russo, 2004); indeed, their carcass and hams could be evaluated as too lean to be labeled as Protected Designation of Origin (**PDO**) ham (Lo Fiego et al., 2005).

Galassi et al. (2015) evaluated the effect of reducing CP supply on N and energy partitioning

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(including N excretion) during the fattening phase in two different genetic groups (**GG**): a traditional GG obtained from Italian selection (pigs' national breeders association, ANAS, defined as A) by breeding Italian Duroc boars with Italian Large White sows and DanBred Duroc GG (D).

Selection of genetic lines for high daily lean growth seems to influence some hematological traits, as reported by Clapperton et al. (2006). Fisher et al. (2013) reported greater adiposity and higher plasma glucose, LDL-cholesterol and total bilirubin concentrations, alkaline phosphatase (**ALP**) and γ-glutamyltransferase (**GGT**) activities, in young genetically lean female pigs fed from 5 to 21 wk of age a high energy, CP restricted diet compared with ones fed a control diet.

The role of the diet in protein metabolism was well described by Deng et al. (2007) and Zhou et al. (2015) who evidenced how a reduction of dietary CP decreased plasma urea concentration in growing and finishing pigs.

The present research aimed to study the effects of feeding two different GG of pigs with diets varying in dietary CP level on hematological and biochemical plasma profiles to evaluate possible differences between the two GG of barrows in response to a lower dietary CP within a typical fattening dietary regimen for typical Italian's ham production.

MATERIALS AND METHODS

Animals and Diets

All animals were cared for in accordance to the guidelines on animal welfare in animal research of the Italian Legislative Decree no. 116/1992 (Italian Ministry of Health, 1992). The study was carried out at the "Cascina Baciocca" experimental farm of "Università degli Studi di Milano," located in Cornaredo (Italy). As previously described (Galassi et al., 2015), three experimental diets (Table 1) were fed to pigs: a control conventional diet (**CONV**), containing cereal meals (corn, barley and wheat, 69.1%), soybean meal (9.2%), wheat bran (8.0%), wheat middling (6.0%), minerals and supplements, and two low-protein diets (**LP1** and **LP2**) without soybean meal and supplemented with different amounts of crystalline amino acids. The CONV diet was a commercial feed with ingredients and nutritional characteristics comparable with those commonly used in the PDO dry-cured ham production circuit (Mordenti et al., 2012; Gallo et al., 2014).

Table 1. Composition and analysis of the experimental diets (g/kg as fed)

Diet	CONV	LP1	LP2
Corn meal	384.3	382.4	541.3
Barley meal	200.0	200.0	340.0
Wheat meal	106.7	200.9	0.0
Soya bean meal	91.7	0.0	0.0
Wheat bran	80.0	80.0	40.0
Wheat middlings	60.0	60.0	0.0
Cane molasses	40.0	40.0	40.0
Beef tallow	14.0	11.0	8.0
Calcium carbonate	13.5	13.7	11.5
Dicalcium phosphate	2.0	2.2	6.0
Sodium bicarbonate	2.5	2.5	2.5
Sodium chloride	3.0	3.0	3.0
Vitamin and mineral premix*	2.0	2.0	2.0
Choline HCl	0.4	0.4	0.4
L-Lysine HCl	0.0	1.4	2.8
L-Threonine	0.0	0.5	1.5
L-Tryptophan	0.0	0.1	0.5
DL-Methionine	0.0	0.0	0.5
Analyzed nutrient composition:			
DM	882	883	885
CP ($N \times 6.25$)	132	104	97
Starch	443	486	519
NDF	121	129	120
ADF	38	39	36
EE	40	38	35
Ash	44	40	40
Calculated SID amino acid content†			
Lysine	4.4	3.5	4.4
Methionine	1.9	1.6	1.9
Threonine	3.6	3.0	3.8
Tryptophan	1.1	0.8	1.1

*Providing per kilogram of diet: vitamin A 7,200 UI, vitamin D_3 1,600 UI; vitamin E 32 mg; vitamin K_3 1.68 mg; vitamin B_1 1.2 mg; vitamin B_2 3.2 mg; vitamin B_6 2.4 mg; vitamin B_{12} 0.016 mg; d-panthotenic acid 16.2 mg; zinc 105 mg; copper 16 mg; iodine 1.5 mg; iron 182 mg; manganese 75 mg; selenium 0.36 mg.

†According to NRC (2012), SID: SID AA.

The CONV diet was formulated according to the National Research Council (2012) although a moderate lysine deficiency can be evidenced (Table 1). The CONV and LP1 diets had a comparable essential AA content per unit of CP and were formulated to contain per kg of feed, 132 and 104 g of CP, and 4.4 and 3.5 g of standardized ileal digestible (**SID**) lysine, respectively. The LP2 diet was formulated to contain 97 g CP/kg, and this diet had the same content of essential SID AA, compared with the CONV diet, per unit of feed. The pigs had free access to water, and feed was restricted to allow a daily DMI of 6.8% of $BW^{0.75}$, as traditionally done with heavy pigs destined for the production of PDO dry-cured ham.

Two crossbred types were used: a traditional GG (A), obtained from Italian selection (National Pig Breeders Association: ANAS) by breeding Italian Duroc boars with Italian Large White sows, and Danbred Duroc GG (D). The first genetic line was chosen because it received a genetic pressure based on carcass and ham quality traits (Cecchinato et al., 2008; Fontanesi et al., 2012); the second line was chosen because of the high selective pressure to increase daily gain, lean meat percentage (**LMP**), and feed efficiency. The animals of the two GG were acquired from two commercial herds taking care that all the piglets were born within the same week.

Forty barrows were involved in the trial to assess blood differences between the two GG: 20 A pigs and 20 D pigs.

The digestibility/balances trial was carried out on 24 from the 40 barrows (101 ± 8.4 kg BW). The 12 pigs A and 12 pigs D were fed the three experimental diets (four pigs A and four pigs D per each of the three diets), for four consecutive periods. After 3 wk, six pigs (one pig A and one pig D per each of the three diets) were housed individually in metabolic cages for the first of four digestibility/balances periods that lasted 14 days each. The experimental design was a factorial design, with three diets × two GG × four periods.

A blood sample was drawn from the 36 barrows in pens at the start and the end of the experimental period (after 56 d), whereas sampling sessions were carried at the end of each of the four digestibility periods for the six pig at the exit of the cage.

Clinical, Hematological, and Biochemical Parameters

Blood was sampled from jugular vein in the morning before feed distribution. Blood samples for hematological profile were drawn in K_3EDTA treated tube (5 mL, Venoject, Terumo, Leuven, Belgium), and analyzed within 2 h after collection using a Cell-Dyn 3700 hematology analyzer (Abbott Diagnostici, Roma, Italy). The results from the instrument were monitored with whole blood reference control (Cell-Dyn Control). The measurements were total red blood cells number (**RBC**; M/μL), hemoglobin (**Hgb**; g/dL), hematocrit (**HCT**; %), mean corpuscular volume (**MCV**; fL), mean corpuscular hemoglobin (**MCH**; pg), mean corpuscular hemoglobin concentration (**MCHC**; g/dL), width of RBC volume distribution (**RDW**; %), total white blood cells number (**WBC**; K/μL), neutrophils (**NEU**; K/μL and % of WBC), lymphocytes (**LYM**; K/μL and % of WBC), monocytes (**MON**; K/μL and % of WBC), eosinophils (**EOS**; K/μL and % of WBC), basophils (**BAS**; K/μL and % of WBC), total platelet number (**PLT**; K/μL), mean platelet volume (**MPV**; fL), and plateletcrit (**PCT**, %).

Blood samples for serum protein profile were drawn in tubes without anticoagulant and allowed to separate serum, successively frozen at −20 °C until analysis. The serum protein profile was assessed by agarose gel electrophoresis using a standard kit for blood serum proteins (Hydragel 30, Sebia Italia, Firenze, Italy), on an automated multiparametric agarose gel electrophoresis system (Hydrasys, Sebia Italia). The gels were analyzed by a densitometer and dedicated software (Phoresis, Sebia Italia). The obtained fractions were as follows: albumin; alpha (**α**), beta (**β**), and gamma (**γ**) globulins.

Blood samples for biochemical metabolic profile were drawn in Li-heparin-treated tubes (10 mL, Venoject, Terumo, Leuven), cooled immediately, and centrifuged for plasma separation within 2 h after collection; plasma was frozen at −20 °C until analyzed. Plasma glucose, total cholesterol, triglycerides (**TG**), total bilirubin, urea, creatinine, albumin, total protein, Ca, inorganic P, Mg, Na, K, Cl, and Fe contents were determined using an automated analyzer for biochemical chemistry (ILAB Aries, Instrumentation Laboratory, Lexington, MA) working at 37 °C, by colorimetric and enzymatic methods using commercial kits (Instrumentation Laboratory). The concentration of NEFA was measured using commercial kits (NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany; Richmond, VA), adapting them to the analyzer conditions. The selection of markers to assess hepatic condition (in terms of possible injuries) was conducted following the suggestions of Boone et al. (2005). Alanine aminotransferase (**ALT**, EC 2.6.1.2) and aspartate aminotransferase (**AST**, EC 2.6.1.1) were used to assess hepatocellular injury. ALP (EC 3.1.3.1) was considered both for its possible involvement in description of hepatobiliary injury and as marker of osteoblast activity. Total bilirubin and GGT (EC 2.3.2.2) were used as indices of hepatobiliary injury; L-lactate dehydrogenase (**LDH**, EC 1.1.1.27) were considered as markers of muscular tissue damage, and tartrate-resistant acid phosphatase (**TRAP**) was an indirect marker of bone mobilization determined using commercial kits (Instrumentation Laboratory). The enzymatic activities were assessed using commercial kits supplied by the same producer (Instrumentation Laboratory), and enzymatic activities (amylase) were determined using

an automated analyzer for biochemical chemistry (ILAB ARIES, Instrumentation Laboratory) and its dedicated kits. Plasma cation–anion balance (**CAB**) was calculated for each blood sample according to the formula: CAB (meq/L) = Na (meq/L) + K (meq/L) − Cl (meq/L).

Statistical Analysis

The results were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) according to Littell et al. (1998). Data were tested for normal distribution using the Shapiro–Wilk test. Variables not normally distributed were subjected to $\log_{10}$ transformation to obtain a normal distribution of values prior to statistical analysis (Farver, 1997).

Two barrows within each GG were not considered in the statistical analysis because of two for leg-related problems, one for heart-attach, and one to maintain a balance in the experimental design management. All these problems were evidenced and managed before the start of digestibility trial, just few days after the arrival of the pigs at the experimental facility.

A first analysis was performed on data from 36 barrows to test the effect of GG (A, D), time of sampling (**TOS**) in the pen-trial (start, end), and their interaction included in the statistical model, with the pig as random variable. Only simple (SIMPLE) structure of covariance was tested. A second analysis was performed on data from 24 barrows involved in the digestibility trials, to test the effect of diet (CONV, LP1, and LP2), GG (A, D), and their interaction included in the statistical model, with the pig as random variable.

Effects were considered significant when $P < 0.05$; a trend was considered when $P < 0.10$. Simple Pearson's correlations were calculated among variables for the entire period using CORR procedure of SAS and reported when $P < 0.01$.

RESULTS

Hematological Profile

Mean values of hematological parameters in the 36 barrows of groups A and D, the effect of GG, TOS (start vs. end of trial), and their interaction are shown in Table 2.

The GG D showed lower WBCs than A group, essentially due to lower circulating blood LYM and MON counts. The only difference between GG in blood erythrocyte parameters was recorded for the MCHC that was higher in D than A, whereas no difference was evidenced for PLT variables.

Time of sampling affected EOS, LDIF, MDIF, EDIF, RBC, MCV, MCH, and RDW; only a trend was recorded for MON, HGB, and HCT; on the contrary, platelet parameters were not affected by TOS.

Table 3 shows the mean values of hematological parameters in the 24 barrows fed the three different diets (four A and four D pigs per group CONV, LP1, and LP2) and the effect of diet, GG, and their interaction during the digestibility trial.

Diet affected only RDW, with LP1 and LP2 at the two extremes (the lower and the higher, respectively). Dietary protein level or AA supply neither affects WBCs nor their populations; only a trend was reported for RBC and PLT.

Metabolic Profile

Table 4 shows the mean values of plasma biochemical parameters and serum protein fractions in the 36 barrows of groups A and D, the effect of GG, TOS, and their interaction.

The GG affected several plasma metabolite concentrations (TG, urea, creatinine, albumin, Ca, Na, Cl, Fe, total bilirubin) and enzyme activities (ALP, TRAP). Plasma TG concentration was higher in D GG, which also showed a trend for higher plasma glucose concentration. Plasma cholesterol was affected by TOS and by interaction between GG and TOS, with higher plasma cholesterol in D GG at the end of the trial compared with the beginning and also to the value of cholesterol of A GG at the end of the trial ($P < 0.05$). Plasma NEFA decreased in D GG from the first to the second sampling time, whereas the opposite was recorded for A GG (data not shown).

Among the plasma metabolites related to protein and N metabolism, the D GG was characterized by lower urea and albumin concentrations but showed higher creatinine content than A group; a significant GG × TOS interaction was observed for creatinine, where D showed increased values with time and A the opposite (data not shown). Plasma Ca and Na concentrations were lower in D than A, whereas the opposite was reported for Cl and Fe.

The GG did not affect any enzyme activity related to tissues damages (ALT and GGT); however, biochemical plasma markers of bone mineral turnover (ALP and TRAP) were significantly higher in D than A GG. Serum albumin as a percentage of total serum protein was lower in D than A group; a TOS effect was observed for γ-globulins.

Table 2. Effects of different GG (A vs. D) and TOS (start vs. end of trial) on hematological profile in barrows

	GG						
	A (n = 18)		D (n = 18)		P value		
Item	LSMEANS*	SE (CI)*	LSMEANS	SE (CI)	GG	TOS	GG × TOS
WBC, k/µL	18.42	(16.46–20.62)	14.37	(12.81–16.13)	**0.004**	0.992	0.204
NEU, k/µL	4.27	(3.59–5.07)	3.66	(3.07–4.36)	0.209	0.388	0.430
LYM, k/µL	11.09	(9.57–12.86)	8.31	(7.15–9.66)	**0.009**	0.218	**0.055**
NEU:LYM, ratio	0.45	0.07	0.54	0.07	0.350	0.321	0.188
MON, k/µL	1.35	(1.11–1.63)	0.93	(0.76–1.12)	**0.008**	**0.057**	**0.076**
EOS, k/µL	0.72	(0.58–0.89)	0.63	(0.51–0.79)	0.418	**0.006**	0.458
BAS, k/µL	0.20	(0.17–0.25)	0.18	(0.15–0.22)	0.495	0.718	0.279
NDIF, % WBC	24.84	1.85	27.29	1.89	0.360	0.268	0.611
LDIF, % WBC	61.28	2.24	59.41	2.28	0.562	**0.022**	0.125
MDIF, % WBC	7.99	0.56	7.05	0.57	0.246	**0.016**	**0.0003**
EDIF, % WBC	4.66	0.52	4.81	0.53	0.847	**0.020**	0.942
BDIF, % WBC	1.22	0.13	1.45	0.13	0.234	0.605	0.948
RBC, M/µL	6.34	0.12	6.31	0.13	0.900	**0.005**	0.241
HGB, g/dL	12.60	0.20	12.70	0.21	0.733	**0.080**	0.401
HCT, %	35.71	0.56	35.54	0.57	0.831	**0.065**	0.559
MCV, fL	56.58	0.60	56.34	0.62	0.783	**0.0006**	**0.050**
MCH, pg	19.98	0.23	20.14	0.24	0.627	**<0.0001**	0.109
MCHC, g/dL	35.32	0.14	35.75	0.14	**0.036**	0.419	0.798
RDW, %	21.74	0.38	21.72	0.39	0.964	**<0.0001**	0.765
PLT, k/µL	276.1	19.3	256.5	19.6	0.481	0.569	**0.008**
MPV, fL	12.69	1.92	16.68	1.95	0.154	0.236	0.553
PCT, %	0.29	0.03	0.31	0.04	0.748	0.256	**0.032**

Values are reported as LSMEANS and SE or 95% CI. P-values below 0.10 were reported in bold type.

BAS = basophils; BDIF = basophils as percentage of total WBC; EDIF = eosinophils as percentage of total WBC; LDIF = lymphocytes as percentage of total WBC; LSMEANS = least squares means; MDIF = monocytes as percentage of total WBC; MPV = mean platelet volume; NDIF = neutrophils as percentage of total WBC; NEU = neutrophils; PCT = plateletcrit; SE or CI = standard error of the mean (pooled) or confidence interval for the general mean (for variables not normally distributed).

In Table 5, shown is the mean plasma biochemical parameters and serum protein fractions in the 24 barrows fed the three diets during the digestibility trial, the effect of diet, GG, and their interaction. Diet affected only plasma urea and total bilirubin concentrations; urea concentration decreased from CONV to LP1 to LP2 (with LP2 significantly lower than the others), total bilirubin was higher in LP2 compared with LP1. Diet did not affect serum protein profile; a GG effect was observed only for albumin and an interaction Diet × GG for β-globulin percentage.

Pearson's Correlations Among variables

During digestibility trial, plasma urea concentration resulted correlated ($R > 0.56$; $P < 0.05$) with the urinary protein and nitrogen excretion and also with the N excreted as g d^{-1} $BW^{-0.75}$ (R = 0.468, P = 0.021). The blood LYM count in digestibility trial resulted correlated (R = 0.543; P = 0.006) with the N retained as g d^{-1}, across GG and diets.

DISCUSSION

Data on the hematological profile of commercial line of pigs are scarce, despite the increasing interest of this approach in the research on the relationships between genetics, physiology, and productive performance (Clapperton et al. 2005, 2006). Where not specifically discussed according to the experimental thesis, data from this study can be considered within the normal range for hematological features as reported by Klem et al. (2010).

Reiner et al. (2007, 2008) firstly reported the presence of QTL for RBCand WBC in pigs. Clapperton et al. (2006) demonstrated how GG selected for high-lean tissue BW daily gain were associated to a higher level of circulating WBC and LYM. This was not our case, considering how DanBred genetics is generally associated to high level of daily lean tissue deposition but here, reporting a lower WBC, LYM, and MON than ANAS GG.

The lack of dietary effects on blood leukocytes and their populations agrees with a lack of evidence

Table 3. Effects of different diet (CONV, LP1, LP2) and GG (ANAS vs. DanBred) during the digestibility trial on hematological profile in barrows

	Diet						*P* value		
	CONV (*n* = 8)		LP1 (*n* = 8)		LP2 (*n* = 8)				
Item	LSMEANS	SE (CI)	LSMEANS	SE (CI)	LSMEANS	SE (CI)	Diet	GG	Diet × GG
WBC, k/µL	17.64	(14.91–20.87)	16.96	(14.22–20.24)	14.47	(12.31–17.02)	0.202	**0.060**	0.550
NEU, k/µL	4.19	(3.02–5.82)	4.99	(3.53–7.03)	3.34	(2.44–4.59)	0.229	0.617	0.527
LYM, k/µL	10.57	(8.72–12.80)	8.49	(6.94–10.38)	8.60	(7.15–10.35)	0.202	0.191	0.526
NEU:LYM, ratio	0.48	0.10	0.70	0.10	0.43	0.10	0.152	0.875	0.866
MON, k/µL	1.24	(0.94–1.64)	1.44	(1.07–1.92)	1.05	(0.80–1.37)	0.278	**0.001**	0.895
EOS, k/µL	0.77	(0.58–1.04)	0.94	(0.69–1.27)	0.69	(0.52–0.91)	0.330	0.693	0.904
BAS, k/µL	0.18	(0.14–0.23)	0.21	(0.16–0.28)	0.20	(0.15–0.26)	0.662	0.953	**0.004**
NDIF, % WBC	25.59	3.12	31.75	3.27	24.52	3.00	0.240	0.735	0.660
LDIF, % WBC	61.16	3.46	51.50	3.63	60.16	3.33	0.125	0.654	0.772
MDIF, % WBC	7.53	0.99	8.94	1.03	8.29	0.95	0.618	**0.008**	0.503
EDIF, % WBC	4.63	0.94	6.42	0.98	5.44	0.90	0.430	0.986	0.602
BDIF, % WBC	1.09	0.18	1.38	0.19	1.60	0.18	0.162	0.239	0.061
RBC, M/µL	6.14	0.19	5.82	0.20	6.46	0.18	**0.080**	0.500	0.828
HGB, g/dL	12.54	0.34	11.98	0.35	12.82	0.32	0.220	0.435	0.919
HCT, %	35.21	0.95	33.69	0.99	35.99	0.91	0.243	0.781	0.954
MCV, fL	57.51	0.84	57.95	0.88	55.95	0.81	0.221	0.283	0.306
MCH, pg	20.47	0.35	20.62	0.36	19.97	0.33	0.387	0.823	0.630
MCHC, g/dL	35.59	0.23	35.58	0.25	35.65	0.23	0.970	**0.086**	0.379
RDW, %	21.00	0.50	19.63	0.53	21.62	0.48	**0.029**	0.795	0.559
PLT, k/µL	267.0	26.7	320.3	28.0	234.9	25.8	**0.096**	0.259	0.510
MPV, fL	12.38	1.53	12.73	1.61	14.43	1.48	0.592	0.190	0.381
PCT, %	0.29	0.05	0.31	0.06	0.24	0.05	0.674	0.121	0.730

Values are reported as LSMEANS and SE or 95% CI.

BAS = basophils; BDIF = basophils as percentage of total WBC; EDIF = eosinophils as percentage of total WBC; LDIF = lymphocytes as percentage of total WBC; LSMEANS = least squares means; MDIF = monocytes as percentage of total WBC; MPV = mean platelet volume; NDIF = neutrophils as percentage of total WBC; NEU = neutrophils; PCT = plateletcrit; SE or CI = standard error of the mean (pooled) or confidence interval for the general mean (for variables not normally distributed).

in our references about this topic. This confirms the lack of differences in inflammatory problems recorded in the differently fed pigs in the trial.

Differences in erythrocyte features among GG were identified to be associated to a selection for a different residual feed intake (**RFI**), as reported by Jégou et al. (2016), with higher RBC, HGB, and HCT in line with lower RFI. These items were not affected in our trial, where the only difference in MCHC between the two GG was substantially low and of small biological relevance. The lack of differences in the other erythrocyte and thrombocytary features evidences how the genetic selection did not affect the QTL for these traits, as reported by Reiner et al. (2007), in the two considered GG. Their mean values were just below the range reported by Kleim et al. (2010) for RBC, while those for MCHC were higher than those reported by the same Author.

The differences in RDW due to dietary treatment did not lead to values outside the range reported as referenced by Kleim et al. (2010). The biological value of this difference, however, is limited; in fact, it seems unreasonable to suppose the way by which a restricted amount of protein, but well integrated by AA, might have determined, in the short time, an increased wideness of erythrocyte size in comparison with the other two experimental groups.

Differences in the energy and lipid metabolism between GG can be suggested by the performance reported by Schiavon et al. (2015). In their paper, they compared four commercial crossbreeds evidencing how ANAS and DanBred resulted the two extreme types for the shift from fat to lean cut yield but with very similar performance in ADG, feed intake, and gain:feed (Schiavon et al., 2015).

The trend for a higher plasma glucose concentration in D GG than in A agrees with the previous comparison between a GG with high-lean deposition (Landrace) and an obese GG (Iberian) reported by Fernández-Fígares et al. (2007). In fact, D is well known for its potential to depot high

Table 4. Effects of different GG (A vs. D) and TOS (start vs. end of trial) on biochemical metabolic profile in barrows

	GG						
	A (n = 18)		D (n = 18)		P value		
Item	LSMEANS	SE (CI)	LSMEANS	SE (CI)	GG	TOS	GG × TOS
Glucose, mmoL/L	4.19	0.07	4.37	0.07	**0.070**	**<0.0001**	**0.077**
Total cholesterol, mmoL/L	2.47	0.08	2.51	0.08	0.726	**0.0001**	**<0.0001**
TG, mmoL/L	0.319	0.019	0.419	0.019	**0.0007**	**0.004**	0.897
NEFA, meq/L	0.045	0.004	0.036	0.004	0.139	0.566	**0.019**
Urea, mmoL/L	3.89	0.16	3.36	0.16	**0.028**	**0.013**	0.457
Creatinine, µmoL/L	163.9	16.5	242.9	16.8	**0.002**	0.307	**0.001**
Total protein, g/L	67.92	0.84	65.58	0.86	**0.060**	**<0.0001**	0.941
Albumin, g/L	33.53	0.44	32.13	0.44	**0.031**	**0.004**	0.364
Globulins, g/L	34.38	0.67	33.45	0.68	0.338	**<0.0001**	0.298
Ca, mmoL/L	2.51	0.02	2.36	0.02	**<0.0001**	**<0.0001**	0.368
P, mmoL/L	2.56	0.04	2.66	0.04	**0.085**	**<0.0001**	**0.034**
Mg, mmoL/L	0.78	0.01	0.80	0.02	0.259	**0.004**	0.449
Na, mmoL/L	142.4	0.4	140.5	0.4	**0.003**	**0.013**	0.836
K, mmoL/L	5.04	0.15	5.23	0.15	0.382	0.806	0.972
Cl, mmoL/L	102.4	0.3	103.8	0.3	**0.005**	**0.042**	0.413
Fe, µmoL/L	16.28	0.95	19.07	0.97	**0.049**	**0.002**	0.195
Amylase, U/L	2,212	144	1,895	148	0.134	0.508	0.247
ALT, U/L	47.22	1.97	48.97	2.02	0.539	**0.013**	**0.0004**
GGT, U/L	26.45	2.53	30.04	2.60	0.329	0.571	0.888
Total bilirubin, µmoL/L	0.96	0.14	0.53	0.14	**0.040**	**0.066**	0.855
ALP, U/L	112.65	12.68	159.33	12.98	**0.015**	**<0.0001**	0.621
TRAP, U/L	10.87	2.25	19.19	2.32	**0.015**	0.800	0.535
LDH, U/L	891	75	1,058	76	0.127	0.731	0.767
Serum protein fractions, % of total proteins							
Albumin	45.05	1.11	41.39	1.12	**0.027**	0.807	0.532
α-globulins	21.13	0.90	22.05	0.91	0.502	0.545	0.915
β-globulins	14.09	0.82	15.57	0.83	0.209	**0.092**	0.918
γ-globulins	19.76	0.46	20.39	0.47	0.340	**0.002**	0.287

Values are reported as LSMEANS.

LSMEANS = least squares means; SE or CI = SE of the mean (pooled) or CI for the general mean (for variables not normally distributed).

ADG of lean tissue, and A GG is characterized by a more balanced attitude to depot lean and fat tissue, namely in the fattening phase.

Pond et al. (1997) reported how the selection of divergent lines for plasma total cholesterol affected BW gain and some carcass traits, jointly with the level of TG and ALP activity. They interpreted the increased plasma activity of this enzyme as a reflection of a greater rate of bone growth of pigs selected for high cholesterol. Among the other enzyme activities, ALT and AST were also modified (reduced) in those line (Pond et al., 1997). In the present trial, however, ALT and GGT were unaffected by GG, suggesting the lack of significant differences in liver susceptibility in pigs with different genetics. Compared with Fisher et al. (2013), our trial did not evidence significant interactions between reduced dietary CP supply and genetics on total cholesterol, ALP, GGT, and total bilirubin; however, LP2 pigs had higher total bilirubin than LP1 pigs. Plasma LDH activity was not responsive to dietary protein supply, differently from the results by Xie et al. (2015).

The different pattern in plasma total cholesterol for the two GG is not comparable with other reported studies. The mentioned paper by Fernández-Fígares et al. (2007) with two divergently selected breeds did not report differences in plasma levels of lipid and energy metabolism–related metabolites.

The genetic basis for a different feed efficiency was mirrored in plasma metabolite pattern around meal in pigs differently selected for RFI (Montagne et al., 2014), where pigs with lower RFI tended to have greater plasma NEFA. In our trial (not reported in the Tables), the pattern of plasma NEFA changed across GG in time, with a decrease in time for D and an increase in time for A; therefore, it is

Table 5. Effects of different diet (CONV, LP1, LP2) and GG (ANAS vs. DanBred) during the digestibility trial on biochemical metabolic profile in barrows

	Diet								
	CONV (n = 8)		LP1 (n = 8)		LP2 (n = 8)		P value		
Item	LSMEANS	SE (CI)	LSMEANS	SE (CI)	LSMEANS	SE (CI)	Diet	GG	Diet × GG
Glucose, mmoL/L	3.98	0.14	4.00	0.15	4.16	0.14	0.619	**0.046**	0.853
Total cholesterol, mmoL/L	2.62	0.12	2.58	0.13	2.64	0.12	0.933	**0.021**	0.237
TG, mmoL/L	0.334	0.027	0.342	0.029	0.345	0.026	0.962	**0.006**	0.863
NEFA*, mmoL/L	0.031	0.006	0.043	0.006	0.044	0.006	0.285	**0.016**	0.826
Urea, mmoL/L	3.93[b]	0.22	3.64[b]	0.24	2.87[a]	0.22	**0.006**	**0.016**	0.876
Creatinine, µmoL/L	188.4	31.0	208.4	32.5	237.5	29.9	0.525	**0.0008**	0.698
Total protein, g/L	69.25	1.26	68.92	1.32	68.39	1.21	0.885	0.114	0.318
Albumin, g/L	32.49	0.67	31.39	0.70	32.23	0.64	0.499	**0.026**	0.825
Globulins, g/L	36.76	0.92	37.53	0.96	36.17	0.88	0.586	0.603	0.265
Ca, mmoL/L	2.40	0.03	2.34	0.03	2.36	0.03	0.423	**0.002**	0.570
P, mmoL/L	2.50	0.06	2.54	0.06	2.48	0.06	0.743	**0.009**	0.299
Mg, mmoL/L	0.81	0.02	0.81	0.02	0.85	0.02	0.320	0.757	0.567
Na, mmoL/L	140.6	0.7	139.5	0.7	141.7	0.7	0.114	**0.054**	0.760
K, mmoL/L	4.93	0.27	5.00	0.29	5.39	0.26	0.433	0.519	0.531
Cl, mmoL/L	103.3	0.7	103.0	0.7	104.2	0.7	0.467	0.146	0.154
Fe, µmoL/L	14.86	1.78	15.38	1.86	17.50	1.71	0.533	0.429	0.999
Amylase, U/L	1,880	197	2,177	215	2,105	189	0.561	0.245	0.524
ALT, U/L	43.42	2.46	47.82	2.58	47.69	2.37	0.370	**0.026**	**0.096**
GGT, U/L	23.50	3.47	29.63	3.63	33.54	3.51	0.140	0.410	0.408
Total bilirubin, µmoL/L	0.90[ab]	0.20	0.52[a]	0.21	1.29[b]	0.19	**0.040**	**0.076**	0.959
ALP, U/L	108.67	15.11	122.62	15.84	131.93	14.56	0.545	**0.011**	0.321
TRAP, U/L	12.63	3.90	19.43	4.09	13.60	3.95	0.444	**0.045**	0.586
LDH, U/L	857	117	1,001	122	1,097	113	0.345	0.274	0.703
Serum protein fractions, % of total proteins									
Albumin	43.59	0.92	42.53	0.96	43.77	0.88	0.603	**0.019**	0.207
α-globulins	21.80	0.82	21.86	0.86	21.41	0.79	0.238	0.375	0.729
β-globulins	14.77	0.94	13.67	0.98	13.16	0.90	0.464	0.248	**0.008**
γ-globulins	19.84	0.86	21.95	0.90	21.66	0.82	0.190	0.212	0.403

Values are reported as LSMEANS. [a, b]Values without the same designator differed for P <0.05.

LSMEANS = least squares means; SE or CI = SE of the mean (pooled) or CI for the general mean. *NEFA = non esterifed fatty acids

not plausible, basing on these data, to attribute a relationship between NEFA and feed efficiency in our conditions.

Plasma glucose, total cholesterol, TG, and NEFA were not affected by dietary treatment, and this is in accordance with the results reported by Deng et al. (2007). In their trial, they compared plasma energy metabolites in barrows with dietary protein content decreasing from 18.2% to 13.6 % CP on dietary DM (Deng et al., 2007).

The GG affected plasma urea. The DanBred genetics is associated to a high-lean growth potential. Genetic lines with high-lean growth potential were found to be characterized by a lower plasma urea level than lines with more fat in their ADG. Among the first papers reporting this observation, Coma et al. (1995b) suggested how the concentration of these metabolites in plasma was directly related to its rate of synthesis and, therefore, inversely related to the efficiency of N deposition into lean growth. More recently, Madeira et al. (2016) compared two GG (one for high-lean tissue deposition and one with more fat deposition); they confirmed the lower plasma urea in the first GG as a marker of efficient lean tissue growth. The Pearson's correlations here evidenced between plasma urea and N excretion during the balance trial period confirmed how a low plasma urea concentration characterizes a higher efficiency in N utilization for lean growth.

The role of genetics to determine plasma urea level was assessed by the USDA group in Nebraska (Klindt et al., 2006). The same group (Lents et al., 2013) further evidenced the association between low-fat growth lines and low plasma urea. In the

present trial, plasma urea did agree with those results, evidencing specifically for the first time; to our knowledge, this pattern in the fattening barrow within the dietary system for typical Italian ham production.

The GG affected plasma creatinine, with D GG reporting significantly higher concentration than A GG. By metabolomics approach, Rohart et al. (2012) reported how plasma creatinine can be considered a reliable marker of LMP between different GG. In their first model, the LMP measured at slaughter was positively linked to circulating creatinine measured between 60 and 70 kg BW. A similar result was obtained in the comparison between Iberian (as obese) and Landrace (as lean) genetics conducted by Fernández-Fígares et al. (2007). The same creatinine is directly linked to the muscular mass and as such is correlated to the total AA catabolism in muscle (Fernández-Fígares et al., 2007).

The higher plasma Fe concentration in D pigs had no counterpart in the hematological profile, considering the similarity of erythrocyte features between the two GG, with the only exception of MCHC. Plasma Fe concentration is essentially considered a marker of inflammatory processes that lower its values (Elbers et al., 1992). In our trial, there were no differences in acute inflammatory processes recorded; from the hematological profile, there was a higher WBC in A pigs but essentially due to a higher LYM count. Considering also the scarcity of reference for these variables in fattening barrows, no conclusive sentence can be formulated to define an effect from GG per se.

The level of dietary protein and AA supply did affect plasma urea concentration, where LP2 reported the lower values of this metabolite compared with LP1 (intermediate) and CONV.

Coma et al. (1995a) reported how plasma urea level was a good marker of the lysine adequacy in the diet, either for barrows and gilts; they also evidenced how quick was the response of this plasma metabolite to the dietary level of lysine in time. The lower plasma urea level in LP2 may be related to an improved Lys availability due to the supplementation of its crystalline form.

This result confirms how plasma urea in pig represents a measure of the efficiency of dietary nitrogen supply. In fact, as reported in our previous paper (Galassi et al. 2010, 2015), the LP2 group reported also the lower level of N excretion as consequence of reduced dietary N but also of an adequate AA (lysine included) supply. Our results agree with those reported in other studies (Zervas and Zijlstra, 2002; Deng et al., 2007; Zhou et al., 2015), confirming how plasma urea is a good marker of protein quality and AA supply also in fattening barrows. A specific trial with reduced dietary protein in D barrows, even not with a direct comparison with another GG, was performed by Gómez et al. (2002), who confirmed the significant decrease within GG with diet well balanced in AA supply.

Plasma ALP and TRAP activities were higher in the GG with greater lean growth potential, the D type. If ALP interpretation in growing pig was already interpreted as a marker of dietary P availability, with high value associated to an inadequate bone mineralization (Boyd et al., 1983), the only available data on TRAP in growing swine are those from our previous paper (Abeni et al., 2015), where the effect of a reduced movement due to metabolic cage led to lower plasma activities of either ALP and TRAP. In the present paper, the simultaneous and coincident change in these two enzyme activities suggests a probable different degree of bone mineralization activities in D compared with A GG.

Madeira et al. (2016) also reported higher plasma ALP levels in leaner pigs compared with a fat line, but they did not attribute a specific meaning to this difference between GG.

DanBred pigs had also lower plasma Ca than ANAS, and this result is consistent with the difference in plasma albumin.

Results from serum protein profile agree with those obtained by Elbers et al. (1992). The lower serum albumin in D then A was not previously reported. To our knowledge, a comparison with other papers reporting different GG or breeds is not possible.

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