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# Effects of additional organic micro-minerals and methionine on carcass composition, gait score, bone characteristics, and osteochondrosis in replacement gilts of different growth rate

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ABSTRACT

Osteochondrosis (OC) is a multifactorial defective endochondral ossification that causes lameness and early culling in gilts and sows. Previous research suggested that nutrition and growth rate could influence OC development and progression. As part of a broader study [n = 360 gilts; 28.8 ± 8.8 kg body weight (BW)] designed to evaluate the effect of 4 dietary treatments: 1) basal diet (CON); 2) CON plus organic micro-minerals (MIN, copper, manganese and zinc at 10, 20 and 50 mg/kg, respectively); 3) additional methionine (MET, at 102% methionine:lysine); and, 4) the organic micro-minerals plus the additional methionine (MM), on lameness and performance, a sub-sample of 40 heavy replacement gilts (10 gilts/treatment, 171.5 ± 8.1 kg of BW) was used. Within treatment, gilts were classified for final average daily gain (ADG) as low (LG, 838 ± 36.3 g/day; n = 20) or high (HG, 922 ± 31.1 g/day; n = 20). Dietary treatment and growth classification were the fixed effects to evaluate gait, OC, tibia bending measures, metacarpal mineralization; and using computerized tomography, the carcass composition, bone size, and whole bone density (WBD). The WBD was expressed as volume of Hounsfield values (HU), where higher values indicate increased density. A porcine reproductive and respiratory syndrome virus outbreak occurred during this trial. It differentially affected MM gilt performance and consequently may have influenced the results for this treatment. Gilts fed MIN diet had 0.75 cm larger tibia than CON ($P < 0.05$), and 10% increase of WBD > 140 HU compared to CON and MET ($P < 0.05$). The volume of high dense bones (> 1000 HU) was also increased in MIN and MET compared with CON ($P < 0.05$). Tibia bending moment and breakage strength were greater ($P < 0.05$) in MIN than in CON, with MET and MM intermediate. Metacarpal ash, Ca, and P content, but not proportions, were higher in gilts fed MIN than CON ($P < 0.05$). Total score of OC lesions was lower in MM gilts compared to CON ($P < 0.05$). The OC total score increased with ADG from 35.8 to 109.8 kg BW ($R^2 = 0.10$; $P < 0.10$). However, between 109.8 and 171.5 kg BW OC score increased with decreased ADG ($R^2 = 0.14$; $P < 0.05$). In conclusion,

*Abbreviations:* ADG, average daily gain; BF, backfat; BW, body weight; Ca, calcium; CI, confidence interval; CON, control basal-diet; Cu, copper; HG, high-growth rate group; HU, Hounsfield Units; LG, low-growth rate group; Lys, lysine; Met, methionine; MET, additional Met dietary treatment; MIN, additional trace minerals dietary treatment; MM, additional Met and trace minerals dietary treatment; Mn, manganese; OC, osteochondrosis; P, phosphorus; PRRS, porcine reproductive and respiratory syndrome; RMSE, root-mean-square error; SE, standard error; SID, standarized ileal digestible; Thr, threonine; TM, trace minerals; WBD, whole bone density; Zn, zinc

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supplementing growing gilts with MIN enhanced bone strength and bone density, MET increased the proportion of highly dense bone ( > 1000 HU), and MM dietary treatment reduced OC lesion score compared with CON.

## 1. Introduction

Osteochondrosis (OC) is focal disturbance in endochondral ossification seen in growing animals (Olstad et al., 2015). The cartilage superficial OC lesions fracture is suggested to be a major cause of lameness in gilts (de Koning et al., 2015), and lameness is a primary reason for gilt failure (Engblom et al., 2008). The OC prevalence is variable and can be high (41–100%) although by 6 months of age healing is described as being above 50% (Busch and Wachmann, 2011; Olstad et al., 2014; Van Grevenhof et al., 2012). Osteochondrosis is the result of a blood supply failure to the epiphyseal growth cartilage and ischemic chondronecrosis. The originating cause is controversial and may be influenced by genetics, growth rate, nutrition, conformation, and mechanical stress (Ytrehus et al., 2007; de Koning et al., 2014; Olstad et al., 2015; Quinn et al., 2015; Le et al., 2016). For instance, Busch and

**Table 1**
General composition of experimental diets (phases I, II and III) offered to growing gilts (as-fed basis).

| | Phase I (1-14 day) | | | | Phase II (15-91 day) | | | | Phase III (92 -134 day) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment[1] | | | | | | | | | | | |
| Ingredient, g/kg | CON | MIN | MET | MM | CON | MIN | MET | MM | CON | MIN | MET | MM |
| Corn | 250 | 250 | 250 | 250 | 250 | 250 | 253 | 253 | 250 | 250 | 250 | 250 |
| Wheat | 100 | 100 | 100 | 100 | 112.5 | 112.5 | 97.5 | 97.5 | 300 | 300 | 298 | 298 |
| Barley | 224 | 224 | 283 | 283 | 250 | 250 | 250 | 250 | 240 | 240 | 233 | 233 |
| Soybean meal | 236 | 236 | 238 | 238 | 193 | 193 | 195 | 195 | 103 | 103 | 105 | 105 |
| Sunflower seed meal | – | – | – | – | – | – | – | – | 65 | 65 | 65 | 65 |
| Bakery byproduct | 100 | 100 | 27 | 27 | 100 | 100 | 100 | 100 | – | – | – | – |
| Wheat middling | – | – | – | – | 26 | 26 | 28 | 28 | – | – | – | – |
| Fat | 40 | 40 | 43 | 43 | 21 | 21 | 21 | 21 | 3.0 | 3.0 | 3.0 | 3.0 |
| Calcium Carbonate | 7.7 | 7.7 | 7.4 | 7.4 | 10.3 | 10.3 | 9.6 | 9.6 | 13.5 | 13.5 | 14.2 | 14.2 |
| Di-calcium phosphate | 13.5 | 13.5 | 13.2 | 13.2 | 14.1 | 14.1 | 14.5 | 14.5 | 12.3 | 12.3 | 12.3 | 12.3 |
| Salt | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Hydroxy-analogue Met | 1.8 | 1.8 | 11.6 | 11.6 | 0.3 | 0.3 | 8.6 | 8.6 | – | – | 6.3 | 6.3 |
| L-Lys HCl | 7.7 | 7.7 | 7.5 | 7.5 | 4.1 | 4.1 | 4.1 | 4.1 | 3.9 | 3.9 | 3.9 | 3.9 |
| L-Thr | 2.1 | 2.1 | 2.1 | 2.1 | 0.8 | 0.8 | 0.8 | 0.8 | 0.4 | 0.4 | 0.4 | 0.4 |
| L-Trp | 0.3 | 0.3 | 0.3 | 0.3 | – | – | – | – | – | – | – | – |
| Aplomotec Plus[1] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Premix[2] | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Analyzed composition | | | | | | | | | | | | |
| Moisture, g/kg | 113 | 111 | 116 | 117 | 116 | 114 | 120 | 118 | 127 | 126 | 124 | 129 |
| Net energy[3], MJ/kg | 10.7 | 10.7 | 10.7 | 10.7 | 10.2 | 10.2 | 10.2 | 10.2 | 9.67 | 9.67 | 9.67 | 9.67 |
| Crude protein, g/kg | 181 | 180 | 178 | 177 | 165 | 166 | 162 | 163 | 139 | 141 | 137 | 140 |
| Crude fat, g/kg | 65.8 | 64.2 | 60.7 | 60.3 | 41.5 | 12.5 | 38.8 | 40 | 19.9 | 20.3 | 19.6 | 20 |
| Crude fiber, g/kg | 33.9 | 33.4 | 32.3 | 33.0 | 35.9 | 34.0 | 35.0 | 35.8 | 45.3 | 44.9 | 45.2 | 45.5 |
| aNDFom, g/kg | 150 | 150 | 157 | 157 | 140 | 140 | 139 | 139 | 150 | 150 | 150 | 150 |
| ADFom, g/kg | 79.8 | 79.7 | 83.9 | 83.9 | 72.6 | 72.6 | 72.3 | 72.3 | 70.1 | 70.0 | 69.9 | 69.9 |
| Lysine, g/kg | 12.5 | 12.6 | 12.4 | 12.5 | 10 | 9.8 | 9.7 | 10.1 | 7.7 | 7.5 | 7.8 | 7.7 |
| Methionine (Met), g/kg | 4.3 | 4.1 | 13 | 12.8 | 2.7 | 2.5 | 9.8 | 10.2 | 2.6 | 2.4 | 5.1 | 5.4 |
| Hydroxy-analogue Met, g/kg | 1.6 | 1.4 | 10.3 | 10.2 | 1.9 | 2.3 | 7.3 | 7.6 | – | – | 10.2 | 9.9 |
| Total calcium[3], g/kg | 7.5 | 7.5 | 7.5 | 7.5 | 8.9 | 8.9 | 8.9 | 8.9 | 9.6 | 9.6 | 9.6 | 9.6 |
| Total phosphorus[3], g/kg | 5.6 | 5.6 | 5.6 | 5.6 | 5.9 | 5.9 | 5.9 | 5.9 | 5.7 | 5.7 | 5.7 | 5.7 |
| Copper, mg/kg | 15.6 | 26.3 | 15.2 | 25.1 | 15.5 | 25.4 | 15.9 | 25.8 | 16.4 | 26.1 | 16 | 26.5 |
| Manganese, mg/kg | 66.4 | 85.2 | 62.1 | 82.7 | 66.1 | 86 | 68.5 | 88.2 | 65.3 | 84.4 | 67.1 | 84.7 |
| Zinc, mg/kg | 122.5 | 173.4 | 121.1 | 176.4 | 120.4 | 172.8 | 125.1 | 175.5 | 123.4 | 174.1 | 124.1 | 173.9 |

[1] Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control; MIN, mineral treatment which provided the diet with additional 10, 20 and 50 mg/kg of chelated copper, manganese, and zinc, respectively (1 g/kg; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, including additional methionine (Met) at 1.02 Met:Lys; or MM, including mineral and methionine treatments combination.

[2] Vitamin-minerals premix provided per kg of feed: vitamin $B_2$, 3.5 mg; vitamin $B_{12}$, 0.035 mg; nicotinamide, 20 mg; folic acid, 1.25 mg; vitamin $D_3$, 2000 UI; vitamin A, 10, 000 IU; vitamin E, 30 mg; vitamin $K_3$, 1 mg; vitamin $B_1$, 1 mg; vitamin $B_6$, 2.4 mg; D-calcium pantothenate, 14 mg; biotin, 0.125 mg; choline chloride, 400 mg; iron (from $FeSO_4.H_2O$), 120 mg; iodine (from $Ca(IO_3)_2$), 0.5 mg; copper (from $CuSO_4{\cdot}5H_2O$), 10 mg; manganese (from $MnO_2$), 40 mg; zinc (from $ZnO_2$), 110 mg; selenium (from $Na_2SeO_3$), 0.4 mg; phytase EC 3.1.3.26, 1500 FTU; and butylhydroxytoluene, 25 mg.

[3] Calculated values.

Wachmann (2011) described that every 100 g increase in wean-to-finish average daily gain (ADG) increased risk of OC by 20%.

Some nutrients are essential for bone and cartilage development and if supplemented may reduce OC and enhance healing (Van Riet et al., 2013). Zinc (Zn) impacts bone mass and is important for matrix quality (i.e. key in insulin-like growth factor-1, osteoblast, parathyroid hormone activations; Matsui and Yamaguchi, 1995; Veum et al., 2009)Matsui and Yamaguchi, 1995; Veum et al., 2009). Manganese (Mn) and copper (Cu) also participate in bone and cartilage matrix formation by being involved with proteoglycans and lysyl oxidase, respectively (Van Riet et al., 2013).

The availability of inorganic trace minerals (TM) is often variable or unknown (Reese and Hill, 2010), which may lead to deficiency. A common practice to prevent this is to increase dietary levels above recommendations, which results in extra cost and environmental impact (Creech et al., 2004; Suttle, 2010). Organically bound minerals, may have higher retention compared with inorganic minerals, could minimize those problems and reduce content in the manure (Liu et al., 2016). Additionally, the European Union recently limited the inclusion of Zn to 150 mg/kg (European Commission, 2016), and Cu to 25 g/kg in pigs above 12 weeks of age (European Commission, 2003) in complete feeds.

Other nutrients, such as methionine (Met) and threonine (Thr), have shown potential to reduce OC severity in growing pigs (Frantz et al., 2008). Such effects were attributed to Met, which is a source of sulfur known to enhance osteoblast differentiation and increase osteocalcin (Bottiglieri, 2002; Ouattara et al., 2016), and therefore, would benefit collagen and bone formation.

We hypothesized that supplementing Cu, Mn, and Zn with additional Met would enhance bone and cartilage development. In addition, it was hypothesized that among heavy gilts, lower growth rate may include less risk of joint lesions and interact with dietary treatment. Therefore, the objective of this study was to assess the effect of supplementing the diet with organic TM (Cu, Mn, and Zn), Met or the combination on carcass composition, gait score, bone density, and OC in replacement gilts of different growth rate.

## 2. Materials and methods

The animals used were produced and housed in commercial swine facilities. The Ethical Committee on Animal Experimentation at the Universitat Autònoma de Barcelona reviewed and approved the procedures and protocols for the experiment according to the guidelines of the European Union (European Commission, 2010).

### 2.1. Animals, experimental design, housing, and dietary treatments

This study was part of a broader experiment designed to evaluate the effect of 4 dietary treatments on lameness and performance (Fabà et al., 2018). A total of 360 young gilts [28.8 ± 8.8 kg body weight (BW)]; DanAvl Dania Hybrid line, Landrace × Yorkshire, DanBred Internacional, Sant Cugat del Vallés, Spain) were blocked and randomly assigned to 1 of 4 treatments: 1) control (CON, basal diet); 2) CON plus organic minerals (MIN, Cu, Mn, and Zn at 10, 20 and 50 mg/kg respectively; Aplomotec Plus, Tecnología & Vitaminas, S.L, Alforja, Spain); 3) additional Met [MET, at 102% Met:lysine (Lys)]; and 4) MET plus MIN (MM). The experimental diets were formulated for 3 different phases of 14, 75, and 45 day to include 10.7, 10.2, and 9.67 MJ net energy/kg and 11.5, 9.0, and 7.0 g/kg standarized ileal digestible (SID) Lys, respectively. For each experimental period, feeds were formulated to meet or exceed nutrient requirements (FEDNA, 2013) for proper gilt growth (see Table 1). Feed was pelleted and provided *ad libitum* using dry feeders with 1 space for 134 days. Gilts had free access to fresh water and enrichment items (biting iron chains and solid plastic balls).

Forty of the 360 gilts were used in the study described herein. Initially, gilts were distributed into 36 pens and 3 blocks of BW (as 10 animals/pen and 0.90 $m^2$/gilt with 60% slatted and 40% solid floor in each pen). At the end of rearing, gilts were selected (171.5 ± 8.12 kg of BW and 221 ± 7.68 day of age) as 10 gilts per treatment among the 120 heaviest gilts in the study. For each treatment, gilts were originally provided from 4 pens with 4 gilts from 1 pen and the other 6 gilts from 3 different pens. Within each dietary treatment, the 10 gilts were proportionally divided according to growth rate as low growth (LG, 838 ± 36.3 g/day of ADG) or high growth (HG, 922 ± 31.1 g/day of ADG). The criteria to choose heavy gilts and ADG classification was that heavy animals with HG gilts were a population with higher risk of OC (Busch and Wachmann, 2011). The 40 gilts were slaughtered in a commercial slaughterhouse and their left half carcass studied on a similar BW to gilts at first service.

### 2.2. Measurements and sampling

#### 2.2.1. Chemical analysis in feeds

Chemical composition of feeds for moisture determination (method 925.09), crude protein (method 968.06), crude fat (method 920.39), crude fiber (method 962.09), and acid detergent fiber (ADFom) expressed exclusive of residual ash (method 973.18) were determined according to AOAC (2000). Neutral detergent fiber (aNDFom) was assayed using sodium sulfite with a heat stable amylase and expressed exclusive of residual ash (Mertens, 2002). Furthermore, Lys and Met were determined [method 982.30 E(a); AOAC International, 2006]. The hydroxy-analogue Met was analyzed using the HPLC method described by Wauters et al. (1990), and minerals Cu, Mn, and Zn using ICP-OES spectrometry (Perkin-Elmer, model Optima 4300DV; MA, USA).

#### 2.2.2. Growth, carcass traits and whole bone density by computerized tomography

Measures of BW collected were on day 1, 41, 67, and 134 of the experimental period. The backfat (BF) and loin muscle depth were determined at 6 cm from the midline and at the level of the last rib using an ultrasound scanner (AV-3000 V Digital Handheld Electronic B Ultrasound Scanner, AMBISEA Technology Corp., Ltd; Hong Kong, China). The 40 gilts were fasted for 12 h and transported to a local slaughterhouse where exsanguination occurred post $CO_2$ stunning. Within 15 min after slaughter, hot carcass

weight was recorded to calculate dressing percentage ( = 100 × hot carcass weight / live weight), and then placed in a chilling room (4 °C).

Left half carcasses were transported to IRTA-Monells research institute (Institut de Recerca i Tecnologia Agroalimentaria, Monells, Spain). Carcasses were prepared following the European Reference Method (Walstra and Merkus, 1996) and were scanned with the General Electric HiSpeed Zx / I computed tomography device (GE Healthcare, Madrid, Spain). Acquisition parameters were 140 kV, 145 mA, 10 mm-thick, helical, 512 × 512 matrix, displayed field of view 500 mm and reconstruction algorithm STD+ (Font-i-Furnols et al., 2009). From the images of the whole half carcasses, it was obtained the distribution of volume by Hounsfield (HU) values with Matlab [Version 7.5.0.342 (R2007b) The MathWorks, Inc., MA, USA]. Using the distribution of HU values, it was calculated the lean tissue percentage of the carcass using prediction equations previously developed by Font-i-Furnols et al. (2009).

The HU distribution was also used to determine the whole bone density (WBD), which was calculated in 2 manners: 1) estimated by the formula developed by Picouet et al. (2010), and 2) considering bone density at HU > 140 as previously described (Font-i-Furnols et al., 2015). For 2) method, whole half carcass HU values higher than 140, 500, 1000, and 1500 were obtained, thus marrow is excluded. The use of these cut-off ranges were defined according to Gaudré et al. (2014), and therefore, used to compare proportions of different density threshold. The higher the HU value, the higher the density of the bone (Batawil and Sabiq, 2016). Hence, volume and (or) proportion of bone from different ranges of HU values is associated with higher or lower bone density. The density for specific bones was not measured and WBD refers to entire half left carcass.

From the collected images and using Horos (2016), femur and tibia length were measured. Furthermore, a transversal image from the middle of the femur and tibia bones provided the cortical bone area (total bone area minus marrow area).

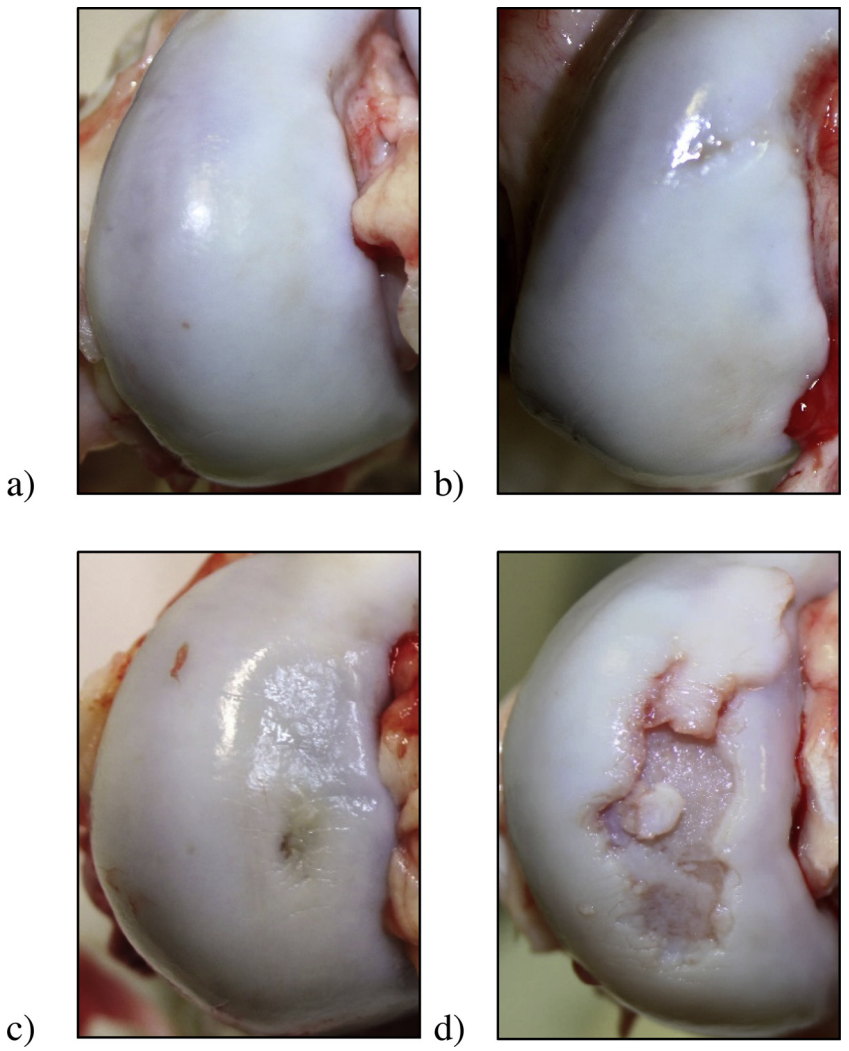


**Fig. 1.** Illustration of femur lateral condyle to macroscopically scoring the severity of osteochondrosis lesions: a) none (0); b) small (1), when the lesion involved less than 5% of the epiphysis cartilage surface; c) moderate (2), when the lesion was 5–10%, and d) severe (3) fragmented cartilage and lesion exceeded 10% of the cartilage surface.

*2.2.3. Tibia bending test*

Bending test of fresh tibia was determined using an MTS Material Testing Apparatus (Model 810, MTS Systems Corporation, Minneapolis, USA) in Escola Tècnica Superior d'Enginyeria Industrial de Barcelona (Departament de Resistència de Materials i Estructures; Universitat Politècnica de Catalunya, Spain) according to methodology described by Veum and Ellersieck (2008). Tibia was held on 2 supports spaced 120 mm apart and force was applied to the midpoint with crosshead speed constant at 6 mm/min. The bending maximum force as breakage strength (kN), bending depth (mm), total energy (area under the curve, kN), and bending moment (kN – mm) were reported. Bending moment, defined as applied force adjusted for the distance over which it is applied (Crenshaw et al., 1981), was calculated using the formula: bending moment = (F × L)/4, where F is a measure of the maximum load (kN) and L is the distance between the bottom 2 fulcra (mm).

*2.2.4. Analysis of minerals in third metacarpal and serum*

The 3rd metacarpal of the left half carcass was collected, systematically boiled and cleaned of adherent tissue, diagonally cut in 2 pieces, weighed, dried (109 °C 12 h), chemically cleaned (in acetone for 24 h), dried (109 °C 12 h), weighed again, and burned in a muffle-oven overnight (550 °C). The ash percentage was calculated and the metacarpal content of calcium (Ca), phosphorus (P), Zn, Mn, and Cu were analyzed using atomic absorption spectrophotometry at proper wavelength ICP-MS (Perkin-Elmer, model Optima 4300DV; MA, USA). Blood samples (10 ml) were collected at exsanguination post stunning (using siliconized blood-collecting tubes) and levels of Zn and Cu were analyzed in serum using ICP-OES spectrometry (Perkin-Elmer, model Optima 4300DV; MA, USA).

*2.2.5. Macroscopically assessment of joint lesions*

The elbow, carpal, femoral-iliac, knee, and tarsal joints of the left half carcass were opened and examined. All articular faces were gross evaluated for external abnormalities (defects in cartilage surface) as OC lesions on the articular cartilage. The joints were macroscopically evaluated by a board-certified veterinary pathologist, based on the methodology described by Kirk et al. (2008), and without knowledge of the dietary treatment assignment on the individual samples as blind assessment. Different locations were examined, and the presence of erosions, ulcerations, repair reactions, marginal osteophytes, and infolding of the joint were accounted as OC lesions (see the aforementioned study for the description and figures of lesion characteristics). Joint faces were scored for presence of lesions and their severity. The proportion of gilts with moderate or severe lesions was calculated; furthermore, the number of faces with lesions and total score of OC were collected for each gilt. The presence of lesions was scored as presence (1) or absence (0) of lesion on each articular face. The severity on each face was as scored as: none (0), small (1) when the lesion involved less than 5% of the cartilage surface, moderate (2) when the lesion was 5–10%, and severe (3) when fragmented cartilage was visible and lesion exceeded 10% of the cartilage surface (Fig. 1). Proportion of moderate or severe lesions was the number of gilts with at least 1 moderate or severe lesion, respectively. The number of faces with lesion was the sum of faces with lesions for each gilt. Finally, total OC score was calculated as the sum of severities accumulated throughout all evaluated joint faces (0–9). Because none of the left carcasses evaluated showed more than 3 articular faces with lesions, the classification was defined as: articular face of tibia, femur, and “other joints” (gilts with elbow, carpal, or tarsal OC lesions).

*2.2.6. Locomotor capacity*

Even though gilts were selected to be non-lame (no limp), gait was evaluated and scored using an adapted methodology from Mustonen et al. (2011) as: 0, no-difficulty; 1, slight-difficulty and slower exercise; 2, moderate-difficulty with shortened stride and some problems to exercise; and 3, severe-difficulty with evident limp lameness and exercising problems (none was selected at this level).

*2.3. Statistical analysis*

The procedures were performed using software SAS Institute Inc. (2011). Gilt was the experimental unit. The normality and homoscedasticity of variables was evaluated using the Shapiro-Wilk test and examining the normal plot. Data with a normal distribution (BW, BF, loin depth, bone characteristics and WBD assessed with computerized tomography, ash and minerals in third metacarpal, and serum minerals) were analyzed using the general linear model (PROC GLM). The OC lesions scorings were evaluated using Fisher's Exact test for presence or absence of lesions (PROC FREQ). The gait score, OC severity, number of lesions, and total lesion score were tested using non-parametric Binomial or Poisson models (PROC GENMOD). Also, the *t*-test was used to compare severity between knee joint faces (femur vs. tibia) and between femur or tibia face and other joint grouped severity (PROC TTEST). The main fixed effects of the model were dietary treatment and growth rate groups. In the general model, there was no evidence of dietary treatment × growth rate group interactions and was removed from the model. In all parametric analysis, Tukey–Kramer adjustments were used to determine significant ($P < 0.05$) differences. Linear regression was used to study the relationship between ADG for different phases (day 1 to 41, day 1 to 67, day 41 to 67, day 41 to 134, day 67 to 134 and day 1 to 134) and total OC lesion score using PROC REG.

## 3. Results

The levels of Cu, Mn, and Zn were formulated to 10, 40 and 110 mg/kg, respectively, in the basal diet with an additional 10, 20, and 50 mg/kg added, respectively, to dietary treatments MIN and MM. The analyzed levels, which included the feed ingredients contribution, resulted between 10% and 50% higher levels than the formulated values (Table 1).

An outbreak of porcine reproductive and respiratory syndrome (PRRS) virus occurred during this trial. Once it was detected, control measures such as vaccination and prolong the rearing phase until complete 90 days post-vaccination were applied (Fabà et al., 2018). However, for 3 weeks during the outbreak, MM gilts reduced feed intake by 24% compared with other dietary treatments, and consumed 5% less feed overall than CON. Hence, the results for this treatment need to be interpreted with caution.

### 3.1. Growth, carcass traits and whole bone density by computerized tomography

Performance, carcass characteristics, bone measures, and WBD results are presented in Table 2. There was no evidence of ADG differences across dietary treatments. Because ADG was the variable used for growth classification, HG had higher slaughter BW and carcass weight than LG ($P = 0.002$). Carcass weight (132.7 ± 6.10 kg) and dressing percentage (77.4 ± 3.29 kg/100 kg) were not different among dietary treatments. Nevertheless, gilts from CON group had 3.4 mm greater BF depth compared to MIN, while MET and MM did not differ and had intermediate values [standard error (SE) = 0.85; $P = 0.041$]. Similarly, through computerized tomography, it was observed that MIN and MET gilts had 4.3 kg/100 kg leaner carcass percentage than CON; and MM was intermediate and not different (SE = 1.07; $P = 0.013$). The BF was 2.10 to 2.99 mm lower at day 67 for MIN, MET and MM compared to CON (SE = 0.82; $P = 0.003$). For BW and loin measures collected at day 1, 41, and 67 there were no differences across dietary treatments.

**Table 2**
Effect of dietary treatment provided to rearing gilts and growth rate group on growth performance, carcass and bone characteristics.

| | Treatment[1] | | | | Growth[2] | | | *P*-value | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | MIN | MET | MM | LG | HG | RMSE[3] | Diet[1] | Growth[2] |
| n | 10 | 10 | 10 | 10 | 20 | 20 | | | |
| Final performance | | | | | | | | | |
| Age, day | 219 | 222 | 222 | 222 | 222 | 221 | 7.41 | 0.782 | 0.607 |
| Body weight, kg | 171 | 174 | 170 | 172 | 167 | 177 | 5.3 | 0.175 | < 0.001 |
| Average daily gain, g/day | 874 | 899 | 877 | 873 | 838 | 922 | 27.9 | 0.189 | < 0.001 |
| Carcass | | | | | | | | | |
| Hot weight, kg | 134 | 132 | 131 | 134 | 131 | 136 | 5.18 | 0.840 | 0.002 |
| Dressing, kg/100 kg | 78.5 | 76.0 | 77.1 | 77.8 | 78.4 | 76.4 | 3.01 | 0.360 | 0.066 |
| Backfat depth, mm | 19.1$^{a}$ | 15.7$^{b}$ | 17.2$^{ab}$ | 16.5$^{ab}$ | 17.2 | 17.1 | 2.47 | 0.041 | 0.861 |
| Loin depth, mm | 70.1 | 69.7 | 70.0 | 70.2 | 68.1 | 71.9 | 6.09 | 0.998 | 0.439 |
| Lean tissue, kg/100 kg | 47.3$^{b}$ | 51.6$^{a}$ | 51.6$^{a}$ | 51.3$^{ab}$ | 49.9 | 51.0 | 3.18 | 0.013 | 0.307 |
| Bone measures[4] | | | | | | | | | |
| Femur length, cm | 20.3 | 20.9 | 20.6 | 20.6 | 20.4 | 20.7 | 0.51 | 0.124 | 0.096 |
| Femur area, cm$^2$ | 9.46 | 9.25 | 9.09 | 9.31 | 9.01 | 9.50 | 0.88 | 0.979 | 0.119 |
| Tibia length, cm | 17.8$^{b}$ | 18.6$^{a}$ | 18.1$^{ab}$ | 17.9$^{b}$ | 17.8 | 18.4 | 0.64 | 0.007 | 0.003 |
| Tibia area, cm$^2$ | 5.91 | 6.01 | 5.88 | 5.90 | 5.83 | 6.02 | 0.47 | 0.874 | 0.243 |
| Tibia bending test[5] | | | | | | | | | |
| Maximum force, kN | 5.59$^{b}$ | 6.38$^{a}$ | 5.95$^{ab}$ | 5.83$^{ab}$ | 5.87 | 6.01 | 0.51 | 0.022 | 0.404 |
| Bending moment, kN/mm | 168$^{b}$ | 191$^{a}$ | 179$^{ab}$ | 175$^{ab}$ | 176 | 180 | 15.4 | 0.022 | 0.409 |
| Bending depth, mm | 2.77 | 2.75 | 2.75 | 2.52 | 2.52 | 2.87 | 0.53 | 0.726 | 0.058 |
| Total energy, kN | 17.9$^{b}$ | 22.6$^{a}$ | 17.2$^{b}$ | 19.4$^{ab}$ | 19.4 | 19.1 | 3.80 | 0.026 | 0.848 |
| Bone density[6] | | | | | | | | | |
| Density, g/dm$^3$ | 1.53 | 1.55 | 1.55 | 1.53 | 1.55 | 1.54 | 0.029 | 0.141 | 0.144 |
| Volume HU > 140, dm$^3$ | 3.52$^{b}$ | 3.98$^{a}$ | 3.62$^{b}$ | 3.69$^{ab}$ | 3.56 | 3.85 | 0.27 | 0.008 | 0.004 |
| Volume HU ≥ 500, dm$^3$ | 0.73$^{b}$ | 0.89$^{a}$ | 0.84$^{ab}$ | 0.75$^{ab}$ | 0.81 | 0.80 | 0.12 | 0.029 | 0.940 |
| Volume HU ≥ 1000, dm$^3$ | 0.059$^{b}$ | 0.071$^{a}$ | 0.071$^{a}$ | 0.060$^{ab}$ | 0.066 | 0.064 | 0.011 | 0.048 | 0.698 |
| Bone HU ≥ 500, % | 20.8 | 22.4 | 23.3 | 20.4 | 22.6 | 20.9 | 2.83 | 0.142 | 0.086 |
| Bone HU ≥ 1000, % | 1.66 | 1.80 | 1.97 | 1.63 | 1.85 | 1.68 | 0.29 | 0.092 | 0.140 |
| Bone HU ≥ 1500, % | 0.014 | 0.014 | 0.028 | 0.006 | 0.021 | 0.009 | 0.016 | 0.051 | 0.046 |

$^{a-b}$Values within a row with different superscripts differ significantly at $P < 0.05$.

[1] Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control formulated to include 10, 40 and 110 mg/kg of copper (Cu), manganese (Mn), and zinc (Zn), respectively, and 0.32 methionine (Met): lysine (Lys); MIN, mineral treatment which provided additional 10, 20 and 50 mg/kg of chelated Cu, Mn, and Zn, respectively (1 g/kg; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, including additional 1.02 Met:Lys; or MM, including mineral and methionine treatments combination.

[2] Growth = classification of the 40 gilts into 2 groups within the 4 dietary treatments according to average daily gain (ADG): low growth (LG, 838 ± 36.3 g/day ADG) or high growth (HG, 922 ± 31.1 g/day ADG).

[3] RMSE = root-mean-square error.

[4] Measurements performed using computerized tomography.

[5] Maximum force (kN) was the breakage strength and the highest load of force; Bending moment (kN – mm) was calculated using Crenshaw et al. (1981) formula as: bending moment = (F × L)/4, where F is the maximum load (kN) and L is the distance between 2 supports (mm). Total energy was calculated as area under the curve: force (kN) x distance (mm).

[6] Bone density was calculated as the distribution of volume according to their Hounsfield (HU) values. Density value was also estimated applying the formula developed by Picouet et al. (2010) considering HU > 140 (Font-i-Furnols et al., 2015). Bone density was also expressed as the proportion of HU > 500, HU > 1000, and HU > 1500 with respect to the volume with bone density HU > 140.

Estimated WBD using Picouet et al. (2010) equation showed no evidence of differences across dietary treatments, but the volume (proportion) of bone above different levels of WBD (with HU values) presented some patterns. Computed tomography HU values increasing indicate higher density. The carcass WBD or proportion of bone as ≥140 HU, was higher for MIN than for CON and MET ($P < 0.010$) dietary treatments; with MM being not different and intermediate. In addition, gilts fed MIN diet had increased volume of WBD above 500 HU than CON ($P = 0.029$); whilst MET and MM did not differ. Similarly, MIN and MET had 0.012 $dm^3$ (17%) denser bone (≥1000 HU) than CON (SE = 0.004; $P < 0.050$), and MM was intermediate and not different. Comparing growth groups, the WBD volume ≥ 140 HU was higher for HG than LG ($P = 0.004$). The LG had higher percentage of bone ≥ 1500 HU than HG ($P = 0.046$). Bone measurements indicated that length and area of the femur were not different across dietary treatments; however, MIN gilts had 0.7 cm larger tibia than CON and MM (SE = 0.20, $P = 0.007$); whilst Met was intermediate.

### 3.2. Tibia bending test

Results from tibia bone bending test indicated that breakage strength and bending moment were higher ($P < 0.050$) in tibia bones from MIN dietary treatment than CON; while MET and MM were not different. Total energy was greater ($P = 0.026$) in MIN than CON and MET dietary treatments, with MM intermediate. Contrarily, bending depth was similar across treatments. Between growth groups there were no significant differences, although HG gilts tended to have greater bending depth than LG ($P = 0.058$). Growth classification did not result in other bone bending differences.

### 3.3. Analysis of minerals in third metacarpal and serum

Metacarpal length was not different amongst dietary treatments nor growth group (Table 3). Conversely, the de-fatted dry content of MIN was greater ($P = 0.020$) than that of CON, with MET and MM being intermediate and not different. Additionally, ash, Ca, and P contents in the metacarpal were greater in MIN than in CON ($P = 0.006$, $P = 0.007$, and $P = 0.010$; respectively); being not different than MET and MM. Nevertheless, the ash proportion in dry matter basis and the Ca and P proportion in ash basis were not different amongst dietary treatments. Comparing the growth groups, it was observed that HG had increased metacarpal de-fatted dry weight ($P = 0.029$), ash content ($P = 0.040$), and not statistically different for content of Ca ($P = 0.099$) and P ($P = 0.065$) than LG gilts. There was no evidence of differences for Zn, Cu, and Mn content and proportion in metacarpal bone amongst dietary treatments or growth groups. Similarly, no differences were observed for serum levels of Cu and Zn among dietary treatments or growth groups.

**Table 3**
Effect of dietary treatment provided to rearing gilts and growth rate group on ash percentage and mineral content in the third metacarpal and serum.

| | Treatment[1] | | | | Growth[2] | | | *P*-value | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | MIN | MET | MM | LG | HG | RMSE[3] | Diet[1] | Growth[2] |
| n | 10 | 10 | 10 | 10 | 20 | 20 | | | |
| Metacarpal | | | | | | | | | |
| Length, mm | 7.90 | 8.07 | 7.95 | 7.97 | 7.95 | 7.99 | 0.180 | 0.243 | 0.442 |
| De-fatted dry content, g | 18.8$^{b}$ | 20.7$^{a}$ | 19.3$^{ab}$ | 19.3$^{ab}$ | 19.1 | 20.0 | 1.11 | 0.020 | 0.029 |
| Ash content, g | 11.8$^{b}$ | 13.1$^{a}$ | 12.3$^{ab}$ | 12.4$^{ab}$ | 12.2 | 12.7 | 0.698 | 0.006 | 0.040 |
| Ash[4], g/kg | 639 | 637 | 638 | 645 | 642 | 637 | 15.2 | 0.752 | 0.367 |
| Calcium content, g | 4.32$^{b}$ | 4.84$^{a}$ | 4.50$^{ab}$ | 4.58$^{ab}$ | 4.48 | 4.64 | 0.232 | 0.007 | 0.099 |
| Calcium[5], g/kg | 366 | 368 | 366 | 368 | 369 | 366 | 5.12 | 0.685 | 0.124 |
| Phosphorous content, g | 2.09$^{b}$ | 2.29$^{a}$ | 2.14$^{ab}$ | 2.16$^{ab}$ | 2.13 | 2.21 | 0.113 | 0.010 | 0.065 |
| Phosphorous[5], g/kg | 174 | 174 | 174 | 174 | 174 | 174 | 1.60 | 0.907 | 0.322 |
| Zinc content, mg | 2.59 | 2.82 | 2.62 | 2.81 | 41.3 | 43.6 | 0.262 | 0.245 | 0.222 |
| Zinc[5], mg/kg | 215 | 214 | 214 | 226 | 218 | 218 | 17.8 | 0.502 | 0.810 |
| Copper content, µg | 6.00 | 7.26 | 6.15 | 8.30 | 6.23 | 7.23 | 2.710 | 0.292 | 0.133 |
| Copper[5], mg/kg | 5.23 | 5.63 | 5.15 | 6.61 | 5.24 | 6.06 | 2.172 | 0.459 | 0.239 |
| Manganese content, µg | 9.83 | 10.2 | 8.12 | 10.3 | 10.1 | 9.14 | 2.287 | 0.231 | 0.265 |
| Manganese[5], mg/kg | 8.08 | 7.92 | 6.71 | 8.25 | 8.14 | 7.34 | 1.732 | 0.174 | 0.101 |
| Serum | | | | | | | | | |
| Copper, mg/L | 2.77 | 2.77 | 2.75 | 2.58 | 2.69 | 2.75 | 0.276 | 0.436 | 0.517 |
| Zinc, mg/L | 1.56 | 1.44 | 1.83 | 1.52 | 1.58 | 1.60 | 0.422 | 0.287 | 0.907 |

$^{a-b}$Values within a row with different superscripts differ significantly at $P < 0.05$.

[1] Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control formulated to include 10, 40 and 110 mg/kg of copper (Cu), manganese (Mn), and zinc (Zn), respectively, and 0.32 methionine (Met): lysine (Lys); MIN, mineral treatment which provided additional 10, 20 and 50 mg/kg of chelated Cu, Mn, and Zn, respectively (1 g/kg; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, including additional 1.02 Met:Lys; or MM, including mineral and methionine treatments combination.

[2] Growth = classification of the 40 gilts into 2 groups among the 4 dietary treatments according to average daily gain (ADG): low growth (LG, 838 ± 36.3 g/day ADG) or high growth (HG, 922 ± 31.1 g/day ADG).

[3] RMSE = root-mean-square error.

[4] Bone dry matter basis.

[5] Bone ash basis.

### 3.4. Macroscopically assessment of joint lesions

Results from the joint evaluation and OC lesions detected in the joints are presented in Table 4. Articular lesions of OC were detected in 80% of gilts. Twenty percent of gilts had only small lesions, while 45% of gilts had moderate lesions, and 15% had severe lesions. Lesions were highly detected in the knee joint (68.8%) compared to elbow (21.8%), tarsal (6.3%), carpal (3.1%), and femoral-iliac joints (0%). In the knee joint, 21.8% of lesions were on the femur face and 87.5% on the tibia face. On the other hand, the severity was higher ($P < 0.001$) in the femur face [1.05, confidence interval (CI) = 0.798, 1.302] than on the tibia face (0.18, CI = 0.007, 0.427). Similarly, severity was higher ($P = 0.037$) in "other joints" (0.55; for elbow, carpal, and tarsal joints together CI = 0.232, 0.726) than on tibia (0.18, CI = 0.007, 0.427).

Occurrence of OC lesions and their severity were not statistically different across dietary treatments for the faces of tibia and femur. However, the OC severity for the variable "other joints" grouped was highest ($P = 0.020$) for gilts fed CON diet; and not different amongst MIN, MET, and MM. The MM dietary treatment did not have any OC lesions in the tibia. At the animal level, the CON group had a higher total OC lesion score than MM ($P = 0.030$); whilst MIN and MET were intermediate and not different. Comparing growth rate groups (LG and HG), no evidence of differences was detected in OC lesions.

Linear regressions between total score of OC and ADG within experimental periods (day 1 to 67, 67 to 134, and 1 to 134) were established. The regression results indicated that ADG from day 1 to 67 (35.8 ± 5.92 to 109.8 ± 6.12 kg of BW) may have a positive relationship with lesions. As growth rate increased, the total OC score increased (total OC score = 0.006 × ADG, g/day – 4.661; $R^2 = 0.10$; $P = 0.059$). Thereafter, ADG from day 67 to 134 (up to 171.5 ± 8.05 kg of BW) showed the inverse relationship and ADG decreased with increasing total OC score (total OC score = -0.0047 × ADG, g/day + 5.943; $R^2 = 0.14$; $P = 0.018$). When assessing the whole growing period (day 1 to 134), no relationship between total OC score and growth rate was observed (total OC score = -0.0044 × ADG, g/day + 5.660; $R^2 = 0.02$; $P = 0.301$).

### 3.5. Locomotor ability

The gait did not differ across dietary treatments or growth rate groups. Without signs of lameness, this score was maximum at 2 for a 3-point scale (10%) and was positively related to the total OC score (OC total score = 1.2095 × gait score + 1.2598; $R^2 = 0.27$; $P = 0.001$).

**Table 4**
Effect of dietary treatment provided to rearing gilts and growth rate group on gait score, osteochondrosis (OC), prevalence and severity scores on the cartilage faces of the knee (tibia and femur), elbow, carpal and femoro-iliac joints from the left half carcass.

| | Treatment[1] | | | | Growth[2] | | | *P*-value | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | MIN | MET | MM | LG | HG | RMSE[3] | Diet[1] | Growth[2] |
| n | 10 | 10 | 10 | 10 | 20 | 20 | | | |
| Gait Score | 0.8 | 0.7 | 0.3 | 0.4 | 0.45 | 0.65 | 0.63 | 0.550 | 0.504 |
| OC prevalence[4], 0-1 | | | | | | | | | |
| Tibia | 0.1 | 0.2 | 0.4 | 0.0 | 0.2 | 0.15 | 0.70 | 0.162 | 0.407 |
| Femur | 0.6 | 0.9 | 0.8 | 0.5 | 0.65 | 0.75 | 0.74 | 0.165 | 0.462 |
| Other joints[5] | 0.4 | 0.2 | 0.2 | 0.2 | 0.25 | 0.25 | 0.74 | 0.682 | 0.998 |
| OC severity[6], 0-3 | | | | | | | | | |
| Tibia | 0.1 | 0.2 | 0.4 | 0.0 | 0.2 | 0.15 | 0.70 | 0.110 | 0.705 |
| Femur | 1.3 | 1.3 | 1.1 | 0.5 | 0.9 | 1.2 | 0.70 | 0.194 | 0.354 |
| Other joints | 1.0[a] | 0.4[b] | 0.4[b] | 0.4[b] | 0.6 | 0.6 | 0.60 | 0.020 | 0.672 |
| Articular faces with lesion, n | 1.4 | 1.5 | 1.5 | 0.7 | 1.3 | 1.3 | 0.69 | 0.103 | 0.606 |
| Gilts with moderate lesion, 0-1 | 0.7 | 0.5 | 0.6 | 0.1 | 0.9 | 1.0 | 0.70 | 0.082 | 0.999 |
| Gilts with severe lesion, 0-1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 1.06 | 0.998 | 0.999 |
| Total Score[7], 0-9 | 2.4[a] | 1.9[ab] | 1.9[ab] | 0.9[b] | 1.7 | 1.85 | 0.50 | 0.030 | 0.596 |

[a−b]Values within a row with different superscripts differ significantly at $P < 0.05$.

[1] Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control formulated to include 10, 40 and 110 mg/kg of copper (Cu), manganese (Mn), and zinc (Zn), respectively, and 0.32 methionine (Met): lysine (Lys); MIN, mineral treatment which provided additional 10, 20 and 50 mg/kg of chelated Cu, Mn, and Zn, respectively (1 g/kg; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, including additional 1.02 Met:Lys; or MM, including mineral and methionine treatments combination.

[2] Growth = classification of the 40 gilts into 2 groups within the 4 dietary treatments according to average daily gain (ADG): low growth (LG, 838 ± 36.3 g/day ADG) or high growth (HG, 922 ± 31.1 g/day ADG).

[3] RMSE = root-mean-square error.

[4] Prevalence at each articular face as absence (0) or presence of lesion (1).

[5] Other joints = gilts with elbow, or carpal, or tarsal OC lesions were put together due to lower incidence.

[6] Severity on each articular face macroscopically evaluated: none (0), small (1) when the lesion involved less than 5% of the cartilage surface, moderate (2) when the lesion was 5–10%, and severe (3) when the lesion exceeded 10%.

[7] Total Score as sum of severities accumulated throughout all evaluated joint faces (Fig. 1).

## 4. Discussion

The hypothesized interaction between dietary treatment and growth rate was not observed, hence removed from the models and final data was analyzed as main factors as described in the statistical analysis methods.

### 4.1. Trace minerals

Dietary TM can influence bone and joint quality, but these effects have been mainly reported when TM were fed below requirements (Owen et al., 1973; Ott and Asquith, 1989; Veum et al., 2009; Muszyński et al., 2018). Above requirements, performance maintains, excretion increases, and effects on bone development are null or controversial (Orth, 1999; Creech et al., 2004; Gowanlock et al., 2013; Olstad et al., 2015; Liu et al., 2016). Compared with CON, the dietary treatment MIN increased tibia bending moment and force, resulted in heavier metacarpal, and higher WBD. These effects could be attributed to dietary Zn, which positively correlates with bone mass (Ovesen et al., 2009). High Zn is related with extracellular matrix quality and bone development by mediating with several of its components (i.e. osteoblast) or as cofactor for metalloproteinases and growth factors (Ovesen et al., 2009). This may have enhanced bone growth and trabecular thickness. Muszyński et al. (2018) did improve bone geometry, yield, and ultimate strengths, by supplementing Cu or added phytase under dietary deficiency in broilers. Conversely, Cu deficiency is not contemplated herein. Evidence of bone changes from Mn supplementation are not available and likely Zn is the TM influencing bone characteristics herein. Increasing dietary Zn to 100% of NRC (1998) and Cu to 160% of NRC (1998) linearly improved bone ash and bone strength in a 28-d study (Veum et al., 2009). Yet, the base-line of TM used herein (10, 40 and 110 mg/kg of Cu, Mn, and Zn) were remarkably higher than NRC (2012) recommendations of 3.0 to 5.0 mg/kg for Cu, 2.0 to 3.0 mg/kg for Mn, and 50 to 80 mg/kg for Zn (as BW ranges in this study). Additionally, MIN and MM levels were considerably higher and provided for a longer time (134-d trial) than previous mentioned research, which make direct comparisons difficult.

Other authors reported effects on bone metabolism with increasing TM availability or supplementation. Revy et al. (2004) observed that supplements of Zn or phytase increased *alkaline phosphatase,* and added phytase, increased Zn in metacarpal and serum, bone strength, and ash content. They reported that Zn from organic source and without phytase increased P percentage in metacarpal. This supports the positive effect on bone development when Zn is increased. Liu et al. (2016) described that TM from organic sources increased the activity of *Cu, Zn-superoxide dismutase, alkaline phosphatase*, and *glutathione peroxidase* enzymes in finishing pigs. This would enhance bone development and reduce bone loss from reactive oxygen species (Altindag et al., 2008; Clarke, 2008; Smietana et al., 2011). However, the present study did not compare TM sources and whether any benefit comes from the type of source is unknown. Inconsistency across studies suggests that present results may have occurred purely by chance, or otherwise, potential of supplementing TM is limited and interact with other factors (i.e. actual risk of lameness).

Metacarpal Cu and Mn were not affected by treatment, likely because bone is not the primary reservoir pool (Ma et al., 2018), but Zn was expected to increase in MIN and MM. Already high dietary base-line levels may have plateaued Zn bone content. Comparisons are difficult because TM bone content is not so often measured, and previous studies in growing pigs compare high vs. low or deletion. All present levels were high.

Similar to metacarpal weight and ash content, gilts fed the MIN diet had 0.75 cm larger tibia than CON, with MET and MM having intermediate values. Obviously, initial length of the tibia was unknown but all groups were homogeneous in BW and age. Weremko et al. (2013) reported that there was a positive relation between bone size and mineralization after feed, protein, or minerals restriction. However, the present difference in tibia length is small and restriction was not applied. The higher WBD for MIN may not be strictly related to bone length but to bone growth and quality. In fact, HG had 0.60 cm larger tibia than LG, but without WBD differences; while the MIN dietary treatment maintained higher WBD than CON above 500 and 1000 HU. Recently, Lagos et al. (2018) reported that Ca and P requirements are higher for mineralization than for growth and a similar conclusion was suggested earlier for TM requirements in turkey (Ferket et al., 2009). Nonetheless, this should be further investigated with a dose response approach.

According to Orth et al. (1999) and Ytrehus et al. (2007), long bone growth is influenced by a number of growth factors, dietary factors, and other cellular signals involved in chondrocyte differentiation. Under such level of interactions, minimum intervention is attributed to diet supplements (Olstad et al., 2015; Tóth et al., 2016). Although limited, the present results suggest improvements and contrast with abovementioned research. It could be that supplementing TM only present some benefits under challenging prevalence of lameness and severity, as CON gilts exhibited 14.8% lameness and lameness reduced final BW by 7.06 kg and ADG by 80 g (Fabà et al., 2018). However, little is reported regarding lameness during rearing of gilts and its consequences; Kilbride et al. (2009) reported 11.8% prevalence.

### 4.2. Methionine

Bone properties from gilts fed MET diet were not different from MIN, and both increased dense bone (WBD > 1000 HU) compared with CON. However, MET had lower total bending energy than MIN. Slight increase of highly dense bone (> 1000 HU; 1.76% total proportion of bone) seems not to directly translate to bone strength, and greater proportion of dense bone (> 500 HU; 21.7% total proportion of bone) appears more important. The role of Met in bone development seems limited and was attributed to its sulfur donator capacity (Frantz et al., 2008; Huang et al., 2014), which may be more linked to cartilage development through proteoglycan formation. Deficiency of Met delays bone differentiation and results in smaller and thinner bones (Huang et al., 2014; Ouattara et al., 2016). Whether a pathological process in the bone such as OC induce Met deficiency is unknown. However,

supplementing Met induces bone tissue improvements during osteoporosis (Vijayan et al., 2014). These authors reported that Met supplementation down-regulates TLR4/MyD88/NF-κB signaling in osteoclast precursors and reduces bone loss during osteoporosis. Other sulfur sources (i.e. coenzyme A, S-adenosyl methionine, glutathione, sulfate etc.) may also supply dietary sulfur with a similar objective. However, sources may have different availability and Met was selected because of previous evidence. Comparing levels above sufficiency (0.29 to 0.32 Met:Lys) with supplementing 1.10 Met:Lys, Frantz et al. (2008) also observed improvements on OC scores with Met above requirements. Therefore, greater effects than the present study may be expected under Met deficiency as current CON levels were above requirements (0.29 to 0.34 Met:Lys ratio).

### 4.3. Trace minerals and methionine

The dietary treatments MIN and MET improved WBD ≥ 500 compared to CON, however, the combination MM was intermediate, which also had intermediate values for bone strength and suggest some inconsistency. The reason is unknown but could be related with the PRRS outbreak. During the outbreak, MM gilts from Fabà et al. (2018) reduced feed intake compared with other treatments, and overall ADFI than CON, which would have lowered total mineral intake. Effects of PRRS include lethargy, anorexia, and immune system activation with increasing energy and amino acids demands (Badaoui et al., 2013). Such effects, if greater in MM gilts, could also be associated with the lower feed intake and BF in MM than CON. The reason behind such reduction on feed intake in MM gilts was unknown, but can reduce mineralization (Van Riet et al., 2013; Weremko et al., 2013) and minimize fat deposition (Whittemore, 1986), and because of this, MM gilts would show intermediate WBD, bone strength, and lower BF.

### 4.4. Osteochondrosis

The prevalence of OC (80%) is in agreement with previous reports (Busch and Wachmann, 2011; Van Grevenhof et al., 2012; de Koning et al., 2014). Progression of OC undertakes 3 stages: OC latens, manifesta, and dissecans; and only the last includes clinical signs (Ytrehus et al., 2007). Absence of lameness was criteria for gilt selection, therefore, 100% of OC was subclinical, still, gait scoring indicated that mild difficulties in gait moderately correlated with increased severity of OC. Furthermore, 2 gilts had macroscopically severe lesions of OC dissecans without evident lameness (i.e. Fig. 1d). Other authors also observed low correlation between OC and clinical lameness (Crenshaw et al., 2013; Etterlin et al., 2015). In this trial, most of the lesions were classified as moderate or small (81.3%). Likewise, Ytrehus et al. (2004) observed 10 times more prevalence of OC manifesta than dissecans in heavy pigs.

Total OC score reduced when combining the organic TM and Met (MM); whilst MIN and MET remained intermediate. In contrast, Frantz et al. (2008) observed a reduction of total OC score when supplementing Met, a combination of Cu and Mn, or all combined. In humans, providing dietary S-Adenosyl Met metabolite to osteoarthritis patients improved joint health and functionality (Najm et al., 2004). The use of heavy, grown gilts (221 ± 7.68 day of age) may have reduced the odds to find severe OC because as gilts become older, the possibility of OC healing increases (Aasmundstad et al., 2013; Olstad et al., 2014). Contrarily, limited meaning may be attributed to data from gilts fed MM diet due to greater PRRSv interaction with this treatment as above and previously discussed (Fabà et al., 2018). Variability was high and more sample size is required to validate these findings.

Initially, ADG was positively associated with total OC score (from 35.8–109.8 kg BW), and subsequently, negatively (from 109.8–171.5 kg BW). This relationship was weak, although it explains why our growth classification (overall ADG) missed any relationship of ADG to OC. Some studies reported that higher ADG increases risk of OC (Busch and Wachmann, 2011). Other authors could not find this relationship or observed interactions with the diet (Ytrehus et al., 2004, 2007; Quinn et al., 2015; Tóth et al., 2016). Present association is weak but in agreement with Van Grevenhof et al. (2012), as ADG positively influenced OC up to a BW thershold (90–109 kg). In the broader experiment by Fabà et al. (2018), lameness (7.7%) was detected between 106.8 to 129.7 kg of BW, increased with BW, and was associated with lower growth after gilts became clinically lame. Pain and disconfort are known to weaken feeding behavior and reduce ADG (Weary et al., 2009), and even before lameness, OC may lower ADFI by 25% (Munsterhjelm et al., 2015).

The vascular failure origin of OC is thought to be related with problems when incorporating blood vessels into the advancing ossification, therefore, little support is currently given to other previously suspected factors such as trauma and nutrition (Olstad et al., 2015). Yet intervening causes on OC progression, healing, or heritable factors are still controversial. Present results, together with the previous discussed suggest a role of TM and Met on OC occurrence in grown gilts.

Faba et al. (2018) reported that mildly lame gilts, which entered the sow herd, weaned 1.2 fewer piglets at first parity compared to none-lame gilts. Culling due to lameness (10 of 81; 12.3%), was 9 times higher in gilts than first parity sows. This, being high for CON (7 of 24) and MM (3 of 15), while null for MIN (0 of 22) and MET (0 of 20). Insufficient incidence and sample size prevent firm conclusions but our data suggest that dietary treatment during rearing likely reduces lameness and enhances both performance and longevity.

## 5. Conclusions

The dietary treatment did not interact with growth rate groups, but supplementing alone organic trace mineral copper, manganese, and zinc for 134 days increased bone growth, bone density and tibia strength of developing gilts compared with the control diet. Supplementing high methionine alone also increased bone density and had intermediate values of bone bending and breakage strength. The combination trace minerals and additional methionine, presented intermediate bone mineralization and density

compared with additional trace minerals, but may reduce osteochondrosis total score compared to control. Finally, the present study supports that growth rate can influence osteochondrosis total score but explains only 10% of its variance and does not negate that other factors may be more important.

## Declaration of Competing Interest

E. Vilarrasa is employed by the feed supplier for the experiment but played no role in analysis of data. All authors declare no potential conflicts of interest.

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