

# Effects of a high carbohydrate diet and arginine supplementation during the rearing period of gilts on osteochondrosis prevalence at slaughter

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## ABSTRACT

Osteochondrosis (OC) is a consequence of necrotic growth cartilage formation early in life and suggested to be associated with lameness and premature culling of sows. Higher insulin, glucose, and insulin-like growth factor-1 (IGF-1) are associated with increased OC in horses and are affected by carbohydrates. If dietary composition can affect OC through metabolic parameters in sows, it could be a tool in practice to reduce OC prevalence. This study examined if OC prevalence in rearing gilts can be influenced by dietary carbohydrates and/or arginine by affecting IGF-1, insulin, glucose, and nitric oxide (NO) levels. Gilts ( $n=212$ ; Dutch Large White  $\times$  Dutch Landrace) were acquired after weaning (4 weeks of age). At 6 weeks of age, gilts were subjected to a  $2 \times 2$  factorial treatment design of dietary carbohydrate and arginine level scale fed at pen level. Carbohydrate level consisted of 12.5% cornstarch and 12.5% dextrose added to a basal diet (C+) versus an isocaloric diet in which cornstarch and dextrose were replaced with 8.9% soya bean oil (C−). Arginine supplementation consisted of 0.8% arginine supplemented to a basal diet (A+) versus 1.64% alanine as the isonitrogenous control (A−). At 24 weeks of age, blood samples of in total 34 gilts around feeding were taken and assessed for insulin, glucose, IGF-1, and NO levels. After slaughter at 25 weeks of age, OC was scored on the elbow, knee, and hock joints. Gilts in the C− treatment had higher glucose and insulin levels 90 min after feeding onwards and higher IGF-1 levels than gilts in the C+ treatment ( $P < 0.05$ ). Arginine supplementation did not significantly affect metabolic parameters. Arginine supplementation tended to decrease OC prevalence ( $P=0.07$ ) at the animal level (all joints combined) and in the knee joint. Carbohydrate treatment affected prevalence of OC only in the knee joint in which gilts in the C− treatment had a higher odds ratio (OR) to have OC (OR=2.05, CI: 1.18–3.58) than gilts in the C+ treatment. Additionally, body weight at slaughter was significant when added to the statistical model ( $P < 0.01$ ) in the knee joint and the animal level (per 10 kg increase OR=1.33, CI=1.11–1.6 and OR=1.17, CI=1.05–1.31, respectively). This study found effects of carbohydrates on OC prevalence in gilts at slaughter. The dietary treatment effects found in the current study likely have been mediated through effects on body weight.

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## 1. Introduction

Formation of necrotic cartilage due to vascular disruption at young age in the epiphyseal growth cartilage is the first step in

osteochondrosis (OC) development and suggested as a cause of lameness and premature culling in sows (Yazdi et al., 2000; de Koning et al., 2015). Reparative attempts by chondrocytes and vasculature have been suggested to occur (Ytrehus et al., 2004a; Olstad et al., 2007). Feeding practices may affect OC prevalence in sows by affecting chondrocyte functioning or growth.

Diets differing in carbohydrate content could influence OC through metabolic parameters such as glucose, insulin, and insulin-like growth factor-1 (IGF-1) as shown in horses (Ralston, 1996; Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Pagan, 2001). Insulin and

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IGF-1 affect survival and proliferation of chondrocytes (Hunziker et al., 1994; Henson et al., 1997) and are involved in growth (Balage et al., 2001; Laron, 2001). Arginine through nitric oxide synthase is a nitric oxide precursor, which has been indicated as an angiogenesis signal in hypoxic tissues through its required interaction with vascular endothelial growth factor (Murohara et al., 1998; Duan et al., 2000; Dulak et al., 2000; Milkiewicz et al., 2005; Hazeleger et al., 2007; Liu et al., 2012; Wu et al., 2012). Considering that OC involves local hypoxia and that reparative responses to OC involve proliferation of chondrocytes and vasculature towards the necrotic cartilage, hypothetically, insulin, IGF-1, and arginine may aid in reparative responses of chondrocytes and vasculature to OC. However, insulin and IGF-1 can increase growth and body weight (BW) gain that in turn can increase OC prevalence (de Koning et al., 2013). Carbohydrate rich diets affect metabolic parameters such as glucose and insulin and is correlated with IGF-1 in pigs (Wientjes et al., 2012, 2013). It may be possible that both a carbohydrate rich diet and supplementation with arginine affect the prevalence of OC, where arginine supplementation could aid reparative responses and reduce OC prevalence, and carbohydrates may aid reparative responses reducing OC prevalence or increase growth and thereby increasing OC prevalence.

The aim of the study was to assess if increased dietary carbohydrate and arginine levels in the rearing period of breeding gilts decrease OC prevalence at 6 months of age. If OC prevalence is affected, this could be used as a dietary strategy to reduce OC prevalence in practice.

## 2. Materials and methods

### 2.1. Ethical note

Osteochondrosis can cause lameness affecting welfare of the gilts. Gilts were daily inspected for impairments of welfare. Severely lame or wounded gilts were taken out of the experiment and euthanized. The experiment and all measurements were approved by the Animal Welfare Committee of Wageningen University and Research center in compliance with Dutch law on animal experimentation.

### 2.2. Animals

The experiment was performed using 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired after weaning at 27 (2.6 SD) days of age and 7.0 (1.5 SD) kg of BW from a commercial breeding company (TOPIGS, Veldhuizen Wehl, Wehl, The Netherlands). Previous research showed an OC prevalence within this line of animals of up to 60% at approximately 6 months of age (de Koning et al., 2013, 2014). Gilts were housed in a 8.37 m<sup>2</sup> pen with a conventional floor consisting of 60% slatted floor (twisted metal bars) and 40% solid floor (epoxy-coated concrete). Enrichment items were provided at all times (such as biting chains, burlap sacks, solid plastic balls, rubber mats) and different items were made available within pens every 2–3 days. Gilts were weighed every 2 weeks after weaning until slaughter at on average 176 (4.4 SD) days of age. Gilts had continuous access to water through a drinking nipple. For approximately the first 2 weeks after weaning, all gilts received a similar commercial weaning diet administered ad libitum to adapt to housing conditions after weaning.

### 2.3. Treatments

Gilts were assigned to 1 of 4 treatment groups and 1 of 32 pens of 6–7 individuals after weaning, based on an equal distribution of BW measured 1 week before the start of the experiment. Littermates were distributed across treatment groups as much as possible to prevent that 1 litter received only 1 treatment. Pens were divided

over 4 departments (8 pens per department) with an equal distribution of treatments within each department. Treatments were administered from approximately 40 (2.6 SD) days of age onwards weighing on average 9.5 (2.0 SD) kg of body weight. Treatments consisted of a 2 × 2 factorial design of dietary carbohydrate and arginine supplementation on a commercial basal diet (diets produced by Research Feed Plant, ForFarmers BV, Heijen, The Netherlands), which remained similar for all animals (see Tables 1 and 2 for the dietary component composition). The carbohydrate level consisted of a diet with 12.5% cornstarch and 12.5% dextrose added to the commercial basal diet (high carbohydrate diet; C+) versus a diet in which the cornstarch and dextrose was replaced with an isocaloric (based on MJ NE/kg) 8.9% soya bean oil (low carbohydrate diet; C−). This contrast of carbohydrate level was determined from previous experiments as the largest possible contrast conceivable in commercial diets in terms of food uptake and digestion (Wientjes et al., 2013). Arginine (L-arginine, Daesang Corp., Seoul, South Korea) supplementation consisted of 0.8% arginine added to the commercial basal diet (A+) versus 1.64% alanine (L-alanine, Omya Hamburg GmbH, Hamburg, Germany) supplemented to the basal diet as the isonitrogenous control (A−) as described by others (He et al., 2011; Shan et al., 2012; Zheng et al., 2013). All dietary components are described as fed. The 2 × 2 factorial treatment design resulted in the following treatment combinations termed treatment groups: C+A+, C+A−, C−A+, C−A−. The dietary treatments fed were isocaloric and were initially administered at approximately 95% of ad libitum energy uptake determined in a previous experiment using the same line of gilts (de Koning et al., 2013). During the current experiment, it was observed that the amount of feed presented to the high carbohydrate treatment group resulted in regular feed residuals (see Fig. 1), which could cause differences in energy uptake with the isocalorically fed low carbohydrate diets. Therefore, the amount of feed administered to all groups was lowered to approximately 85 to 90% of the estimated ad libitum intake. All gilts received feed in 2 portions per day (8:00 h and 16:00 h) in 2 troughs with ample feeding space and with metal bars separating individual feeding places. Feed residuals were collected and weighed after each day in the morning before the first feeding bout.

After weaning, all gilts received first the pelleted weaning diet (10.3 MJ NE/kg, 171 g/kg CP, 12.7 g/kg ileal digestible lysine). Subsequently, gilts changed to 3 successive diets in time each containing 1 of the 4 dietary treatments, adapted to their feed uptake and age (Tables 1 and 2). Diets were switched gradually over a 2 day period in which diets were offered as a 50% mixture of the old and new diets. Gilts were switched at 40 days of age to a pelleted grower diet (based on 1 kg of the C+ diet: 10.1 MJ NE/kg, 12.1 g/kg ileal digestible lysine; 6.3 g/kg calcium; 5.5 g/kg phosphorus); at 77 days of age to a pelleted rearing diet 1 (based on 1 kg of the C+ diet: 9.7 MJ NE/kg, 10.0 g/kg ileal digestible lysine; 8.0 g/kg calcium; 5.5 g/kg phosphorus); at 116 days of age to a pelleted rearing diet 2 (based on 1 kg of the C+ diet: 9.5 MJ NE/kg, 6.5 g/kg ileal digestible lysine; 8.0 g/kg calcium; 5.5 g/kg phosphorus).

### 2.4. Blood sampling

All blood sampling was performed using EDTA as an anticoagulant. To determine if dietary treatments affected glucose and insulin profiles, blood samples were taken at the end of the experiment at 167 (2.5 SD) days of age of 10 randomly selected gilts per treatment group. Blood sampling occurred through cannulation of the ear vein. In 6 gilts cannulation was not successful as the veins were too small (number of gilts sampled: C+A+ n=8; C+A− n=10; C−A+ n=8; C−A− n=8). The insulin and glucose profiles could not be obtained at younger age, as the veins in the ear are still too small for the cannulation procedure. Cannulation of the ear vein was performed as described in Wientjes et al.

**Table 1**  
Dietary composition (%) for the 3 experimental successive diets<sup>a</sup>.

Component	Grower diet	Rearing diet 1	Rearing diet 2
<i>Basal diet</i>			
Barley	25.00	30.00	40.00
Maize	15.18	11.74	14.13
Wheat bran	3.00	–	–
Wheat	–	3.43	–
Rapeseed meal extracted (CP < 380)	–	5.00	7.36
Hamlet Protein	2.00	–	–
Soy bean meal (CF 45–70 g/kg)	18.50	20.00	9.41
Soya oil	0.77	0.37	–
Monocalciumphosphate	1.06	0.92	0.98
Limestone	0.55	0.84	0.83
Salt (NaCl)	0.21	0.39	0.18
Na bicarbonate	0.58	0.14	0.45
PRESAN-FX <sup>b</sup>	0.20	0.15	0.10
Piglet premix <sup>b</sup>	1.00	–	–
Pig premix <sup>b</sup>	–	1.25	1.25
Lactose	4.15	–	–
Coconut oil	0.75	–	–
DL-Methionine 99%	0.36	0.14	0.03
L-Threonine 98%	0.32	0.10	0.02
L-Valine 96.5%	0.28	0.04	–
L-Tryptophan 98%	0.08	0.01	–
L-Lysine 98%	0.73	0.35	0.20
PHYZYME XP 5000 TPT	0.01	0.01	–
Vitamin E 50% adsorb	0.24	0.10	0.06
Choline Chloride 50%	0.03	–	–
Total basal diet <sup>c</sup>	75.00	75.00	75.00
<i>C+ diets addition</i>			
Corn starch	25.00	25.00	25.00
Dextrose	25.00	25.00	25.00
<i>C– diets addition</i>			
Soya bean oil	8.90	8.90	8.90
<i>A+ diets addition</i>			
L-Arginine	0.8	0.8	0.8
<i>A– diets addition</i>			
L-Alanine	1.64	1.64	1.64

<sup>a</sup> Gilts received 3 successive diets based on their age and weight development. Dietary treatments were imposed per diet consisting of a 2 × 2 factorial design of carbohydrates and arginine. C+ = high carbohydrate, low fat diet; C– = low carbohydrate, high fat diet; A+ = diets supplemented with arginine; A– = diets supplemented without arginine but with alanine as the isonitrogenous control. Components are represented as fed.

<sup>b</sup> PRESAN-FX by Selko Feed Additives (Tilburg, The Netherlands) includes: E 200 sorbic acid, E414 Acacia, sodium and calcium salt of butyric acid, pure distilled coconut/palm acid, soy oil, palm fat, E551a Silicic acid, E526 Sepiolite. Piglet Premix product no. 10011475 by Trouw Nutrition Nederland BV. (Putten, The Netherlands) includes (per kg feed): 1.5 g Sepiolite, 8000 IU vitamin A, 2000 IU vitamin D3, 30 IU vitamin E, 1.5 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 12 mg panthothenic acid, 20 mg niacinamide, 1 mg vitamin B6, 0.3 mg folic acid, 30 mcg vitamin B12, 50 mg betaine, 100 mg FeSO<sub>4</sub>, 1.0 mg CaI<sub>2</sub>, 16 mg CuSO<sub>4</sub>, 30 mg MnO, 100 mg ZnSO<sub>4</sub>, 0.3 mg Na<sub>2</sub>O<sub>3</sub>Se. Pig Premix product no. 10011495 by Trouw Nutrition Nederland BV. includes (per kg feed): 1.9 g Sepiolite, 8000 IU vitamin A, 2000 IU vitamin D3, 30 IU vitamin E, 1.5 mg vitamin K3, 1 mg vitamin B1, 4 mg vitamin B2, 12 mg panthothenic acid, 20 mg niacinamide, 1 mg vitamin B6, 0.3 mg folic acid, 20 mcg vitamin B12, 50 mg betaine, 100 mg FeSO<sub>4</sub>, 1.0 mg CaI<sub>2</sub>, 15 mg CuSO<sub>4</sub>, 30 mg MnO, 100 mg ZnO, 0.3 mg Na<sub>2</sub>O<sub>3</sub>Se, 0.15 mg CoCO<sub>3</sub>.

<sup>c</sup> Basal diet composition was fed equally between treatment groups. The contrast in carbohydrate content was fed isocalorically. Amino acid composition as fed of the grower diet (per kg dry matter): 12.4 g lysine, 5.5 g methionine, 2.1 g cysteine, 10.5 g threonine, 2.3 g tryptophan, 5.3 g isoleucine, 9.0 g arginine, 6.5 g phenylalanine, 3.4 g histidine, 10.0 g leucine, 4.5 g tyrosine, 6.0 g valine, 5.7 g alanine, 12.3 g aspartic acid, 25.1 g glutamic acid, 5.2 g glycine, 8.9 g proline, 6.3 g serine, 7.6 g methionine + cysteine. Amino acid composition as fed of the rearing diet 1 (per kg dry matter): 10.6 g lysine, 3.8 g methionine, 2.6 g cysteine, 7.0 g threonine, 1.9 g tryptophan, 6.3 g isoleucine, 10.5 g arginine, 7.6 g phenylalanine, 4.0 g histidine, 11.5 g leucine, 5.3 g tyrosine, 7.1 g valine, 6.5 g alanine, 14.1 g aspartic acid, 30.1 g glutamic acid, 6.2 g glycine, 10.6 g proline, 7.3 g serine, 6.4 g methionine + cysteine. Amino acid composition as fed of the rearing diet 2 (per kg dry matter): 7.0 g lysine, 2.3 g methionine, 2.2 g cysteine, 4.4 g threonine, 1.3 g tryptophan, 4.6 g isoleucine, 7.4 g arginine, 5.7 g phenylalanine, 3.0 g histidine, 9.0 g leucine, 3.9 g tyrosine, 5.5 g valine, 5.1 g alanine, 9.4 g aspartic acid, 24.1 g glutamic acid, 4.8 g glycine, 9.4 g proline, 5.4 g serine, 4.5 g methionine + cysteine. Amino acid composition presented exclude the experimental % supplementation of arginine and alanine and as calculated for standardized ileal digestibility according to Centraal Veeboederbureau (CVB, 2007).

(2012) for adult sows. Gilts were individually housed in a 2.6 m<sup>2</sup> pen 2 days before blood sampling, serving as a habituation period to individual housing. Gilts were cannulated one day before blood sampling and were fixated in a nose sling during the cannulation procedure. One ear was scrubbed with disinfectant and a sterile medical PVC catheter with an inner diameter of 0.8 mm and outer diameter of 1.6 mm (Rubber BV, Hilversum, The Netherlands) was inserted 40 cm into a suitable (broad and straight) ear vein. The catheter at the site of cannulation was taped to the ear of the gilt and the ear was fixated against the head of the gilt. The other end of the catheter was secured with a one-way luer-lock stopcock<sup>®</sup>

(Vygon, Veenendaal, The Netherlands). The catheter was externally placed and secured in a fabric pouch fixated with medical tape on the back of the gilt. After the procedure, the gilts received 0.4 mg kg<sup>−1</sup> Novem<sup>®</sup>20 (containing meloxicam). Blood samples were taken 1 day after cannulation around the morning (08:00 h) feeding time at −24, −12, 0 (feeding time), 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, 372, and 444 min relative to feeding. Gilts were allowed to eat an isocaloric feed amount (11.4 MJ NE) for a maximum of 30 min to ensure profiles of glucose and insulin were measured as close to a short feeding bout as possible. Only 1 gilt from the C+A+ treatment had a substantial amount of feed left

**Table 2**

Contents as fed of the high and low dietary carbohydrate treatments for the 3 successive diets<sup>a</sup>.

Diet	Component <sup>b</sup>				
	GE (MJ)	DM (g)	CP (g)	CF (g)	Carb+Sug (g)
<b>Grower diet</b>					
C+A+	15.98	882.2	169.1	29.4	594.0
C-A+	15.70	752.6	165.3	116.5	343.2
C+A-	15.99	885.7	169.7	28.7	594.0
C-A-	15.63	752.2	166.6	111.8	343.2
<b>Rearing diet 1</b>					
C+A+	15.89	894.1	167.1	19.5	574.0
C-A+	15.46	755.6	167.6	107.1	323.0
C+A-	15.76	891.8	167.0	18.0	574.0
C-A-	15.35	754.3	167.3	105.7	323.0
<b>Rearing diet 2</b>					
C+A+	15.67	889.7	139.8	15.0	617.0
C-A+	15.40	758.5	135.2	109.3	366.6
C+A-	15.64	892.8	139.5	14.9	617.0
C-A-	15.20	754.1	135.0	101.2	366.6

<sup>a</sup> Gilts received 3 successive diets based on their age and weight development. Dietary treatments were imposed per diet consisting of a 2 × 2 factorial design of carbohydrates and arginine. C+ = high carbohydrate, low fat diet; C- = low carbohydrate high fat diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine but with alanine as the isonitrogenous control.

<sup>b</sup> The content of the components are as fed: for the C+ diets per 1 kg of C+ diet, for the C- diets per 839 g of C- diet (the isocaloric amount of feed). Carbohydrate and sugar content according to the Centraal Veevoederbureau (CVB 2007). GE=gross energy (ISO-9831, 1998); DM=dry matter (ISO-6496, 1999); CP=crude protein (ISO-5983-2, 2005); CF=crude fat (ISO-6492, 1999).

over after 30 minutes of approximately 40%. Statistical analyses were performed with and without this gilt and indicated as such in the results section. All sampling time points were used to determine glucose and insulin profiles. Plasma IGF-1 levels of the gilts were determined 228 min after feeding. Plasma nitric oxide (NO) levels were determined in 30 randomly selected gilts per treatment group at approximately 70 (2.5 SD) days of age by puncture of the jugular vein and at the cannulation procedure. Plasma NO was determined as NO is a product of arginine metabolism. Blood samples were collected in EDTA tubes and were centrifuged for 10 min at 3000 × g at 4 °C to obtain plasma and was stored at -20 °C until use.

## 2.5. Plasma analysis

Plasma glucose, insulin, and IGF-1 analyses were performed as described in [Wientjes et al. \(2012\)](#) using commercial kits following manufacturers' instructions. Briefly, plasma glucose levels (in mg·dL<sup>-1</sup>) were assessed in duplicate for all sampling time points during cannulation using a glucose oxidase-peroxidase kit (Roche Diagnostics Nederland BV, Almere, The Netherlands) after protein precipitation of 50 µl plasma using 500 µl 0.3 M trichloroacetic acid. Plasma insulin levels (in µU·mL<sup>-1</sup>) were assessed in duplicate for all sampling time points during cannulation using a RIA kit (PI-12K Porcine Insulin RIA-kit<sup>®</sup>, Millipore, St. Charles, MO, USA). Plasma IGF-1 levels (in ng·mL<sup>-1</sup>) were assessed in duplicate using an IRMA kit (IRMA IGF-1 A15729<sup>®</sup>, Immunotech, Marseille, France) after ethanol/HCL extraction. Plasma total NO levels (in µmol·L<sup>-1</sup>) were determined by indirect assessment of nitrite concentration (where the nitrate present was converted to nitrite) and were assessed in duplicate for the blood samples taken at 10 and 24 weeks of age using a total NO kit (Parameter<sup>™</sup> Total nitric oxide KGE001, R&D Systems Inc., Minneapolis, MN, USA) after ultrafiltration of plasma samples using a 10 K MW filter and centrifugation for 40 minutes at 16,000 × g (Pierce<sup>®</sup> Concentrator PES 88,513, Thermo Scientific, Rockford, IL, USA).

## 2.6. Osteochondrosis assessment

Osteochondrosis assessment was performed after slaughter as described in [de Koning et al. \(2013, 2014\)](#). One half of the gilt population was slaughtered within 2 days at 173 (2.7 SD) days of age, the other half of the gilt population was slaughtered within 2 days at 180 (2.6 SD) days of age with an equal distribution of treatments on each day of slaughter. After slaughter, carcasses were stored for 1 day at 4 °C after which the legs were collected by dissection through the shoulder and hip joints and stored at -20 °C for a maximum of 5 days. After thawing for 2 days, the hock, knee, and elbow joints were dissected in 1 day for each half of the population in random order of gilts. After dissection, the joint surfaces were macroscopically scored for any irregularities indicative of OC on a total of 22 locations (see [de Koning et al. \(2013\)](#)). Scoring of OC was performed using a 5-point grading scale from 0 to 4 with score 0 indicating no OC and score 4 indicating the severest form of OC as described previously ([van Weeren and Barneveld, 1999](#)). Osteochondrosis was scored by one veterinarian specialized in orthopedics, experienced in judging OC, and unaware of the treatments.

## 2.7. Statistical analysis

Statistical analyses were performed for BW progression and growth rate, feed residuals, feed efficiency, blood sampling parameters, and OC scores. Successive measurements were taken for several of these parameters and therefore observations cannot be considered independent. Repeated measurement analyses were performed and suitable variance-covariance structures were evaluated using Akaike's corrected information criterion and mentioned below where repeated measurement analysis was performed. Within each model the carbohydrate treatment (C+, C-), arginine treatment (A+, A-), the time points at which measurements were performed for the repeated measurement models, and their interaction effects were assessed as fixed class effects in the statistical model. Where possible, the departments in which the gilts were housed were added as fixed class effects and a random effect of the experimental unit pen (32 pens) nested within treatments and department was added to assess the treatment effects on pen level. In each of the statistical models, non-significant higher order interaction effects were removed from the model using backward elimination until either the (higher order) interaction effects were significant ( $P < 0.05$ ) or only main effects remained in the model.

### 2.7.1. Body weight and growth rate

The average daily growth rate was calculated for each interval between BW measurements by taking the increase in weight within the interval divided by the number of days of the interval. The analyses of growth rates and BW were performed using PROC MIXED in SAS 9.2 (SAS Inst. Inc., Cary, NC). The repeated measurement analysis was performed applying a heterogeneous first order auto-regressive variance-covariance structure for the BW model and an ante-dependent variance-covariance structure for the growth rate model. The model was not convergent when fixed effects of department were added to the model and assessed in four way interaction effects of fixed class effects. Three way interaction effects of the treatments with measurements and department were assessed, but did not improve the model and were therefore not included in the final model. Addition of dam effect (38 dams) did not result in further improvement of the model. Significant results are displayed as the least squares means and the corresponding SE.



### 2.7.2. Feed residuals

Feed residuals were calculated as the average daily feed residuals per week and per pen and expressed as the proportion of daily feed given during the corresponding week. Feed residuals were analyzed from the start of administering dietary treatments until 88.8 (2.6 SD) days of age. After 88 days of age, the number of pens having feed residuals were too few for statistical analysis (on average 4 pens per week until the end of the experiment) and were restricted mainly to the C+A+ treatment. As feed residuals were calculated as a proportion, the data was analyzed using binomial logistic regression with a logit link using PROC GLIMMIX in SAS 9.2. The repeated measurement analysis was performed applying a heterogeneous first order auto-regressive variance-covariance structure. As with the analysis of BW progression, the same issues and outcomes were encountered for the feed residuals when department was added to the model. Significant results are displayed as the inverted logit least squares means with corresponding CI.

### 2.7.3. Feed efficiency

Total energy consumed (in mega joule per net energy, MJ NE) over the entire experiment was determined by calculating the sum of the average feed eaten per gilt in a pen per day multiplied by the estimated energy within each feed. Total weight gain over the experimental period was determined. The energy required for 1 kg of BW gain was calculated by dividing the total consumed energy by the total weight gain. This indicates whether group-housed fed gilts are equally efficient in growing with the same amount of energy consumed. Feed efficiency was analyzed with PROC MIXED in SAS 9.2. Dams (38 dams) were added as a random effect. Significant results are displayed as the least squares means and the corresponding SE.

### 2.7.4. Glucose, insulin, and insulin-like growth factor-1

Levels of glucose, insulin, and IGF-1 were statistically analyzed using PROC MIXED in SAS 9.2. As the gilts were individually housed for blood sampling, the gilts were the experimental unit and department effects were not assessed. To approximate normality, the natural logarithms of glucose and IGF-1 values were calculated, and the base 10 logarithms of the insulin values were calculated. For glucose and insulin, a repeated measurement analysis was performed with gilts as the subject applying a first order ante-dependent variance-covariance structure. Significant results are displayed as the least squares means and the corresponding SE on their respective logarithm scales.

### 2.7.5. Nitric oxide

Levels of NO at 10 and 24 weeks of age were statistically analyzed using PROC MIXED in SAS 9.2. To approximate normality, the natural logarithms of NO values were calculated. For the statistical model at 10 weeks of age, dam (36 dams) was added as a random effect. Significant results are displayed as the least squares means and the corresponding SE on a natural logarithm scale.

### 2.7.6. Osteochondrosis scores

Osteochondrosis was scored on several locations within a joint on an ordinal scale of 0–4 for each joint separately. However, ordinal logistic regression was deemed not appropriate and could not be performed validly because of a low number of observations of each combination of treatment and OC score (Stokes et al., 2000). As a consequence, OC scores were grouped as a 0 and 1 variable to accommodate binary logistic regression as described previously (de Koning et al., 2013, 2014), where 0 indicates no abnormalities (OC score 0) and 1 indicates an OC score greater than 0. The OC scores were analyzed using PROC GLIMMIX in SAS 9.2 using a binary distribution with a logit link. Statistical analyses were not performed for the elbow joint as the prevalence of OC

was low (<8%). Dams (38 dams) were added as a random effect. The repeated measurement analysis was performed applying a variance component structure. Lastly, in a separate model after the assessment of treatment effects on OC prevalence, the effect of BW of the gilts at slaughter was also assessed to determine whether BW at slaughter had any explanatory effect on OC. Body weight was added as a fixed covariate to the statistical model. Results are displayed as odds ratios (OR) and their CI to indicate effect size.

## 3. Results

A total of 11 gilts were euthanized before the end of the experiment due to health and welfare problems that were deemed unrelated to treatment effects such as diarrhea after weaning, fighting, pneumonia, inflamed wounds on claws, severe tail biting, and an atrial septal defect. The number of gilts euthanized before the end of the experiment were 5 gilts from the C+A+ group (of which 2 were euthanized before dietary treatments were given), 4 gilts from the C–A– group (of which 1 was euthanized before dietary treatments were given), 1 gilt from the C–A+ group, and 1 gilt from the C+A– group. The joints of these animals were assessed and taken into account in analyses where appropriate.

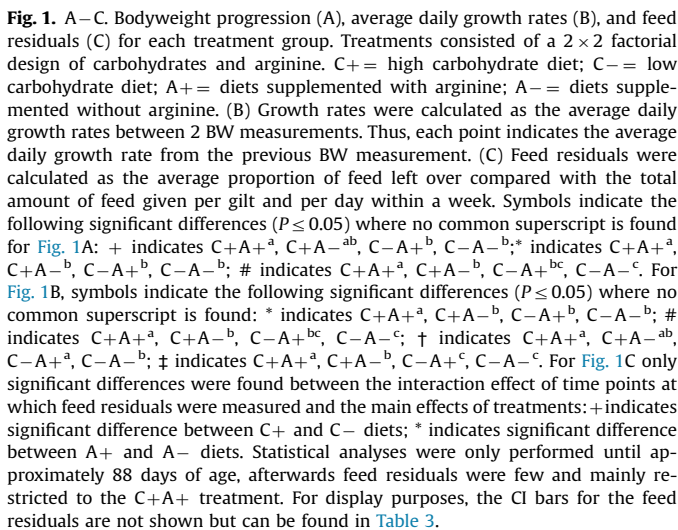
### 3.1. Growth performance

Bodyweight and growth rates were analyzed with a repeated measurement analysis (biweekly measurements) at pen level for fixed main effects of the carbohydrate treatment (C+, C–), arginine treatment (A+, A–), and their interaction effects. Feed residuals were analyzed from the start of administering dietary treatments until 88.8 (2.6 SD) days of age. After 88 days of age, the number of pens having feed residuals were too few for statistical analysis. Feed residuals were analyzed with a repeated measurement analysis (weekly feed residuals) at pen level for fixed main effects of the carbohydrate treatment (C+, C–), arginine treatment (A+, A–), and their interaction effects. Feed efficiency was analyzed for fixed main effects of the carbohydrate treatment (C+, C–), arginine treatment (A+, A–), and their interaction effects.

#### 3.1.1. Bodyweight and growth rate

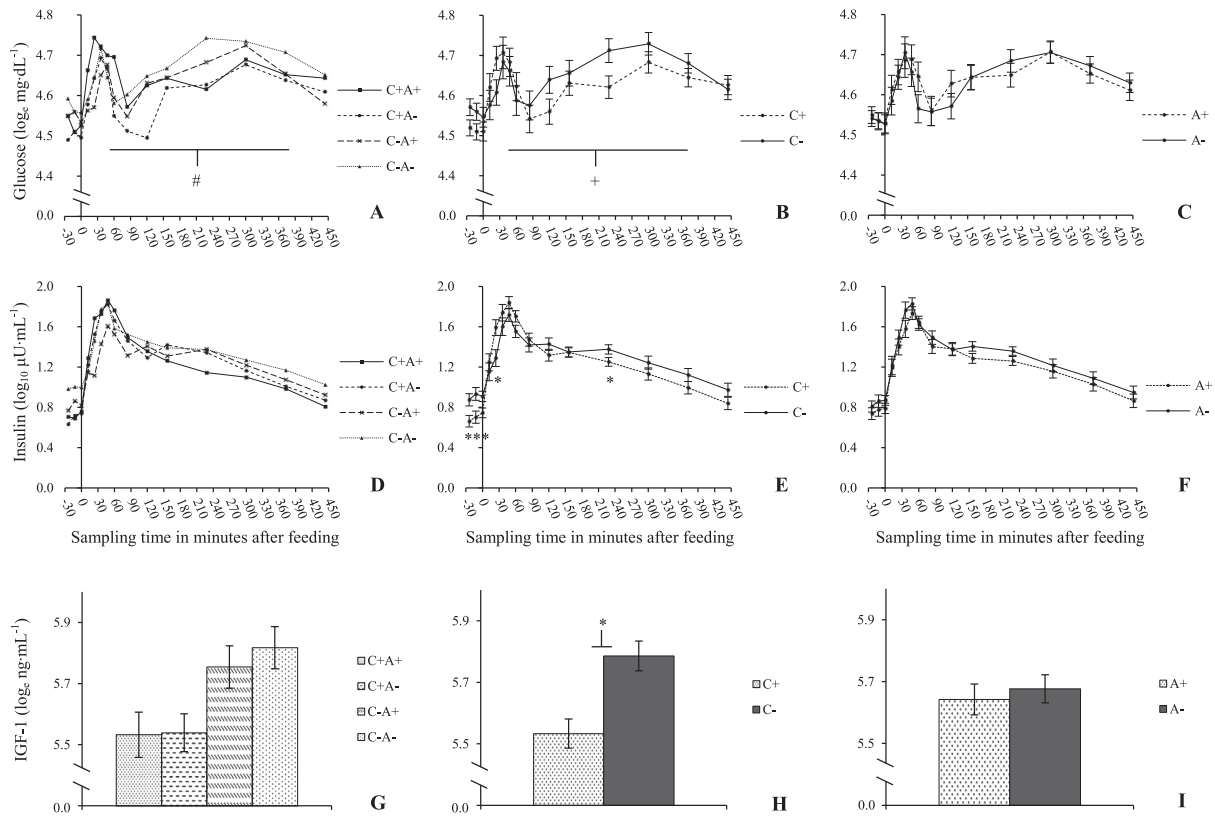
The progression of BW, average daily growth rate, and feed residuals per treatment are shown in Fig. 1A–C. No significant differences between treatments were present in BW until 68 days of age. A significant interaction effect between carbohydrate and arginine treatments was present after approximately 68 days of age until the end of the experiment. After 68 days of age, gilts in the C+A+ treatment had a lower BW ( $P < 0.01$ ) when compared with gilts in the other treatment groups (Fig. 1A), which likely follows from higher feed residuals being present for the C+A+ treatment throughout the experiment (Fig. 1C). Additionally, at approximately 138 days of age until the end of the experiment, the gilts in the C+A– treatment had a lower BW than gilts in the C–A– treatment ( $P < 0.05$ ).

A significant interaction effect between carbohydrate and arginine treatments was present for the average daily growth rates (Fig. 1B). Overall, gilts in the C+A+ treatment had lower growth rates ( $P < 0.05$ ) than gilts in the other treatment groups. These were lower in the 2-week periods from 40 to 82 days of age and from 110 to 169 days of age. Additionally, gilts in the C–A– treatment had the highest growth rates, which were higher ( $P < 0.03$ ) than for gilts in the treatments C+A– at 54–68 days of age, C–A+ at 82–96 days of age, and C+A– from 110 to 151 days of age (Fig. 1B). The BW of the gilts at the last measurement at the end of the experiment was for the C+A+ treatment 85.2 (1.5 SE) kg, for the C+A– treatments 95.7 (1.5 SE) kg, for the C–A+



An increase in feed residuals was present one week after the start of the dietary treatments (at approximately 46 days of age),

Glucose levels peaked at 36 min after feeding, declined thereafter until 84 min after feeding, and then increased again (Fig. 2 A–C). No interaction effects between carbohydrate, arginine, and sampling points were present ( $P > 0.05$ ; Fig. 2A). An overall interaction effect ( $P = 0.03$ ) between carbohydrate and arginine treatments indicated that, overall, gilts in the C–A– treatment had higher glucose levels than gilts in the C+A– treatment ( $P < 0.01$ ), while the other treatment groups did not differ

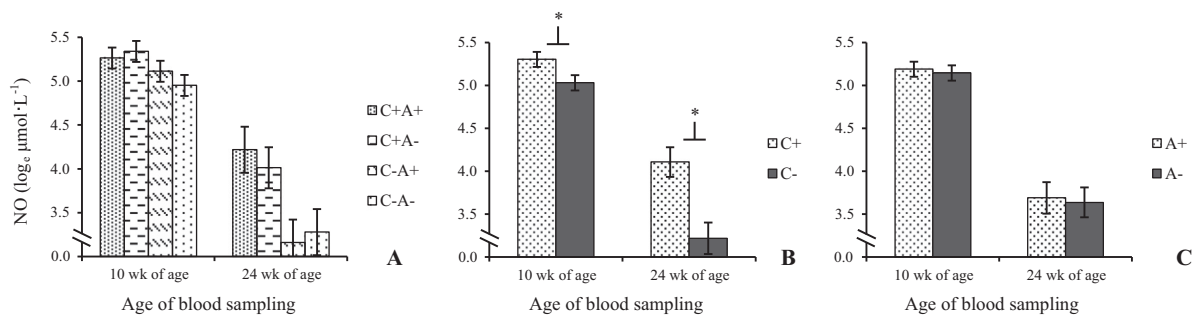


**Fig. 2.** A–I. Profiles of glucose (Fig. A–C), insulin (Fig. D–F), and level of insulin-like growth factor-1 (IGF-1; Fig. G–I) in gilts at 24 weeks of age ( $n=34$ ). Blood sampling was performed at regular intervals before feeding (sampling time  $-24$ ,  $-12$ ,  $0$  min before feeding) and after feeding (sampling time  $12$ ,  $24$ ,  $36$ ,  $48$ ,  $60$ ,  $84$ ,  $120$ ,  $156$ ,  $228$ ,  $300$ ,  $372$ , and  $444$  min after feeding). Treatments consisted of a  $2 \times 2$  factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine. The profiles and levels of glucose, insulin, and IGF-1 are shown for each treatment group (Fig. A, D, and G, respectively), for each main effect of carbohydrate treatment (Fig. B, E, and H, respectively), and for each main effect of arginine treatment (Fig. C, F, and I, respectively). Significant differences are indicated with symbols and specify the following significant differences were no common superscript is found: \* indicates C+A<sup>a</sup>, C-<sup>b</sup>; # indicates overall interaction effect C+A+<sup>ab</sup>, C+A-<sup>a</sup>, C-A+<sup>ab</sup>, C-A-<sup>bc</sup> where C-A- has higher levels overall then C+A-; + indicates overall main effect C+A<sup>a</sup>, C-<sup>b</sup> where C- has higher levels overall then C+. For display purposes, SE bars are not shown for the treatment groups for insulin (figure A) and glucose (Fig. D). Pooled SE of glucose for C+A+ is  $0.04$  SE, C+A- is  $0.04$ , C-A+ is  $0.04$  SE, and C-A- is  $0.04$  SE. Pooled SE of insulin for C+A+ is  $0.09$  SE, for C+A- is  $0.08$ , C-A+ is  $0.09$  SE, and C-A- is  $0.09$  SE.

significantly from each other (Fig. 2A). This interaction effect is reflected in the main effect ( $P=0.03$ ) of carbohydrate treatment, which indicated that gilts in the C- treatment had overall higher levels of glucose than gilts in the C+ treatment and is particularly evident in the period between  $90$  min and  $444$  min after feeding (Fig. 2B). As stated before, one gilt from the C+A+ treatment had a relatively large amount of feed left  $30$  min after feeding. When this gilt was excluded from the statistical model, only the main effect of carbohydrate treatment remained with similar results. Arginine treatment did not have an effect ( $P>0.05$ ) on glucose levels (Fig. 2C).

### 3.2.2. Insulin

Insulin levels peaked at  $48$  min after feeding and steadily declined afterwards (Fig. 2D–F). No interaction effect between carbohydrate and arginine treatments was present ( $P>0.05$ ; Fig. 2D). An effect of carbohydrate treatment ( $P=0.02$ ) was present for insulin levels (Fig. 2E). This effect indicated that before feeding, gilts in the C- treatment showed higher levels of insulin ( $P\leq 0.02$ ) when compared with gilts in the C+ treatment at  $-24$  min,  $-12$  min, and  $0$  min. After feeding, gilts in the C+ treatment showed a higher insulin response than the gilts in the C- treatment and this was significantly different at  $24$  min



**Fig. 3.** A–C. Levels of nitric oxide (NO) in gilts at 10 ( $n=117$ ) and 24 weeks of age ( $n=34$ ). Treatments consisted of a  $2 \times 2$  factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine. The levels of NO are shown for each treatment group (figure A), for each main effect of carbohydrate treatment (Fig. B), and for each main effect of arginine treatment (Fig. C). Significant differences are indicated with symbols and specify the following significant differences were no common superscript is found: \* indicates C+A<sup>a</sup>, C-<sup>b</sup>.

after feeding ( $P=0.01$ ). Insulin steadily declined after the insulin peak. However, gilts in the C– treatment showed higher insulin levels from 120 min onwards after feeding and this was significantly different at 228 min after feeding ( $P=0.05$ ). Removal of the single gilt with large feed residuals 30 min after feeding (see above) did not influence statistical outcome. Arginine treatment did not have an effect ( $P>0.1$ ) on insulin levels (Fig. 2F).

### 3.2.3. Insulin-like growth factor-1

No interaction effect ( $P>0.05$ ) between carbohydrate and arginine treatments was present for IGF-1 levels (Fig. 2G). A main effect of carbohydrate treatment ( $P<0.01$ ) was present for IGF-1 levels (Fig. 2H), which indicated that gilts in the C– treatment had higher levels of IGF-1 than gilts in the C+ treatment (Fig. 2H). Removal of the single gilt with large feed residuals 30 min after feeding (see above) did not influence statistical outcome. No effect ( $P>0.05$ ) of arginine on IGF-1 level was present (Fig. 2I).

### 3.2.4. Nitric Oxide

Levels of plasma NO are shown in Fig. 3A–B. No interaction effect ( $P>0.05$ ) between carbohydrate and arginine treatments were found at either 10 weeks of age or 24 weeks of age (Fig. 3A). A main effect ( $P<0.03$ ) of carbohydrate treatment was present at both ages, which indicated that gilts in the C+ treatment had higher levels of plasma NO than gilts in the C– treatment (Fig. 3B). Arginine treatment did not affect ( $P>0.05$ ) NO levels at either age (Fig. 3C).

## 3.3. Osteochondrosis Prevalence

Osteochondrosis prevalence was analyzed with a repeated measurement analysis (locations assessed for OC) at pen level for fixed main effects of the carbohydrate treatment (C+, C–), arginine treatment (A+, A–), and their interaction effects. Afterwards,

slaughter weight was added as an additional statistical model to determine the effect of weight at slaughter on OC prevalence.

Osteochondrosis was found in the elbow joint on the bilateral homologues of the medial and lateral humeral condyle, and on the anconeal process only on the left elbow joint; in the hock joint on the bilateral homologues of the medial and lateral trochlea of the talus; and in the knee joint on the bilateral homologues of the medial and lateral femoral condyle. The prevalence of OC in the elbow joint was relatively low ( $<8\%$ ; Table 4) and the majority of OC was found on the lateral humeral condyle (77.8% of the total prevalence of OC scores greater than 0). Osteochondrosis in the hock joint was predominantly found on the medial trochlea of the talus (69.8% of the total prevalence of OC scores greater than 0). Osteochondrosis in the knee joint was predominantly found on the medial femoral condyle (97.3% of the total prevalence of OC scores greater than 0). The prevalence of severe OC lesions (scores 3 and 4) was low in all joints assessed (7.2% in the elbow joint; 1.5% in the hock joint; 2.9% in the knee joint; Table 4).

Descriptive statistics at the animal level indicate that gilts in the C+ treatment showed a lower prevalence of OC compared with gilts in the C– treatment (55.2% versus 68.0% respectively) and gilts in the A+ treatment showed a lower prevalence of OC compared with gilts in the A– treatment (59.6% versus 63.5%). The order of the treatments groups from lowest to highest OC prevalence was C+A+ (50.0%), C+A– (60.4%), C–A– (66.7%), and C–A+ (69.2%). Significant effects of treatments were not present ( $P>0.05$ ) for the hock joint and the animal level (the elbow joint was not statistically assessed due to low prevalence of OC). Only a main effect ( $P=0.01$ ) of carbohydrate treatment was found at the knee joint and indicated that gilts in the C– treatment had significantly higher odds (OR = 2.05, CI: 1.18–3.58) to be affected by OC in the knee joint than gilts in the C+ treatment. A tendency was found for arginine treatment and suggested that gilts in the

**Table 4**  
Prevalence of osteochondrosis (OC) scores for the total number of locations assessed ( $n_{loc}$ )<sup>a</sup> and for gilts with the greatest OC score present ( $n_{gi}$  and % total number of gilts)<sup>b</sup> per treatment<sup>c</sup>.

	C+			C–			A+			A–		
	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%
Elbow	516	98	93.3	506	94	91.3	513	98	94.2	509	94	90.4
0												
1	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0
2	0	0	0.0	1	1	1.0	0	0	0.0	1	1	1.0
3	4	3	2.9	3	3	2.9	4	3	2.9	3	3	2.9
4	5	4	3.8	5	5	4.9	3	3	2.9	7	6	5.8
<b>Total<sup>d</sup></b>	<b>9</b>	<b>7</b>	<b>6.7</b>	<b>9</b>	<b>9</b>	<b>8.7</b>	<b>7</b>	<b>6</b>	<b>5.8</b>	<b>11</b>	<b>10</b>	<b>9.6</b>
Hock	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%
0	374	71	67.6	372	72	69.9	375	71	68.3	371	72	69.2
1	27	18	17.1	23	17	16.5	20	15	14.4	30	20	19.2
2	18	15	14.3	15	12	11.7	19	16	15.4	14	11	10.6
3	0	0	0.0	2	2	1.94	1	1	1.0	1	1	1.0
4	1	1	1.0	0	0	0.0	1	1	1.0	0	0	0.0
<b>Total</b>	<b>46</b>	<b>34</b>	<b>32.4</b>	<b>40</b>	<b>31</b>	<b>30.1</b>	<b>40</b>	<b>33</b>	<b>31.7</b>	<b>45</b>	<b>32</b>	<b>30.8</b>
Knee	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%
0	377	76	72.4	340	51	49.5	369	69	66.4	348	58	55.8
1	35	23	21.9	55	37	35.9	36	25	24.0	54	35	33.7
2	4	3	2.9	11	9	8.7	7	6	5.8	8	6	5.8
3	2	2	1.9	3	3	2.9	2	2	1.9	3	3	2.9
4	2	1	1.0	3	3	2.9	2	2	1.9	3	2	1.9
<b>Total</b>	<b>43</b>	<b>29</b>	<b>27.6</b>	<b>72</b>	<b>52</b>	<b>50.5</b>	<b>47</b>	<b>35</b>	<b>33.7</b>	<b>68</b>	<b>46</b>	<b>44.2</b>
Animal <sup>e</sup>	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%	$n_{loc}$	$n_{gi}$	%
0	1267	47	44.8	1218	33	32.0	1257	42	40.4	1228	38	36.5
1	62	31	29.5	78	36	35.0	56	30	28.9	84	37	35.6
2	22	16	15.2	27	20	19.4	26	20	19.2	23	16	15.4
3	6	5	4.8	8	7	6.8	7	6	5.8	7	6	5.8
4	8	6	5.7	8	7	6.8	6	6	5.8	10	7	6.7
<b>Total</b>	<b>98</b>	<b>58</b>	<b>55.2</b>	<b>121</b>	<b>70</b>	<b>68.0</b>	<b>95</b>	<b>62</b>	<b>59.6</b>	<b>124</b>	<b>66</b>	<b>63.5</b>



Elbow	C+A+			C+A–			C–A+			C–A–			Overall		
	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%
0	255	48	92.3	261	50	94.3	258	50	96.2	248	44	86.3	1022	192	92.3
1	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0
2	0	0	0.0	0	0	0.0	0	0	0.0	1	1	2.0	1	1	0.5
3	3	2	3.9	1	1	1.9	1	1	1.9	2	2	3.9	7	6	2.9
4	2	2	3.9	3	2	3.8	1	1	1.9	4	4	7.8	10	9	4.3
<b>Total<sup>d</sup></b>	<b>5</b>	<b>4</b>	<b>7.7</b>	<b>4</b>	<b>3</b>	<b>5.7</b>	<b>2</b>	<b>2</b>	<b>3.8</b>	<b>7</b>	<b>7</b>	<b>13.7</b>	<b>18</b>	<b>16</b>	<b>7.7</b>
Hock	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%
0	189	37	71.2	185	34	64.2	186	34	65.4	186	38	74.5	746	143	68.8
1	8	6	11.5	19	12	22.6	12	9	17.3	11	8	15.7	50	35	16.8
2	10	8	15.4	8	7	13.2	9	8	15.4	6	4	7.8	33	27	13.0
3	0	0	0.0	0	0	0.0	1	1	1.9	1	1	2.0	2	2	1.0
4	1	1	1.9	0	0	0.0	0	0	0.0	0	0	0.0	1	1	0.5
<b>Total</b>	<b>19</b>	<b>15</b>	<b>28.8</b>	<b>27</b>	<b>19</b>	<b>35.8</b>	<b>22</b>	<b>18</b>	<b>34.6</b>	<b>18</b>	<b>13</b>	<b>25.5</b>	<b>86</b>	<b>65</b>	<b>31.3</b>
Knee	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%
0	196	43	82.7	181	33	62.3	173	26	50.0	173	25	49.0	717	127	61.1
1	11	8	15.4	24	15	28.3	25	17	32.7	25	20	39.2	90	60	28.8
2	1	1	1.9	3	2	3.8	6	5	9.6	6	4	7.8	15	12	5.8
3	0	0	0.0	2	2	3.8	2	2	3.9	2	1	2.0	5	5	2.4
4	0	0	0.0	2	1	1.9	2	2	3.9	2	1	2.0	5	4	0.5
<b>Total</b>	<b>12</b>	<b>9</b>	<b>17.3</b>	<b>31</b>	<b>20</b>	<b>37.7</b>	<b>35</b>	<b>26</b>	<b>50.0</b>	<b>35</b>	<b>26</b>	<b>51.0</b>	<b>115</b>	<b>81</b>	<b>38.9</b>
Animal <sup>e</sup>	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%	n <sub>loc</sub>	n <sub>gi</sub>	%
0	640	26	50.0	627	21	39.6	617	16	30.8	601	17	33.3	2485	80	38.5
1	19	13	25.0	43	18	34.0	41	17	32.7	41	19	37.3	140	67	32.2
2	11	8	15.4	11	8	15.1	12	12	23.1	12	8	15.7	49	36	17.3
3	3	2	3.9	3	3	5.7	4	4	7.7	4	3	5.9	14	12	5.8
4	3	3	5.8	5	3	5.7	5	3	5.8	5	4	7.8	16	13	6.3
<b>Total</b>	<b>36</b>	<b>26</b>	<b>50.0</b>	<b>62</b>	<b>32</b>	<b>60.4</b>	<b>62</b>	<b>36</b>	<b>69.2</b>	<b>62</b>	<b>34</b>	<b>66.7</b>	<b>219</b>	<b>128</b>	<b>61.5</b>

<sup>a</sup> Prevalence of OC expressed as the number of OC scores present for the total number of locations assessed for the elbow joint, hock joint, knee joint, and animal level.

<sup>b</sup> Prevalence of OC scores expressed as the number of gilts with the greatest OC score present and as the percentage of the total number of animals per treatment.

<sup>c</sup> Treatments consisted of a 2 × 2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C– = low carbohydrate diet; A+ = diets supplemented with arginine; A– = diets supplemented without arginine.

<sup>d</sup> 'Total' indicates the total number of animals affected with any form of OC.

<sup>e</sup> 'Animal' is the total number of OC lesions for all joints combined.

A+ treatment had a lower prevalence of OC in the knee joint and at the animal level ( $P=0.07$ ). When BW at slaughter was assessed in a separate model as a covariate, treatment effects were no longer present ( $P>0.13$ ). However, BW at slaughter became significant at the knee joint and the animal level ( $P<0.01$ ), which indicated that each 10 kg increase in BW at slaughter resulted in higher odds to be affected with OC at both the knee joint (OR=1.33, CI=1.11–1.6) and the animal level (OR=1.17, CI=1.05–1.31).

#### 4. Discussion

The aim of this study was to investigate whether dietary carbohydrate and arginine levels have an effect on OC prevalence during the rearing period of breeding gilts.

##### 4.1. Bodyweight, feed residuals, and feed efficiency

Feed residuals for all treatments were high the first week after administering the dietary treatments and may represent habituation to the new feed. After the first week, feed residuals steadily declined and approached 0% at approximately 80 days of age, except for gilts in the C+A+ treatment. Body weight differences were present between treatments and were especially evident in the C+A+ treatment. Gilts in the C+A+ treatment showed a higher overall prevalence of feed residuals throughout the experiment, which is reflected by the smaller increase in BW compared with the other treatments. Although no significant interaction effect of carbohydrate and arginine treatments were found for feed residuals,

the high proportion of feed residuals by gilts in the C+A+ treatment may have influenced the significant main effects found for arginine and carbohydrate treatment. The reason for higher feed residuals present in gilts in the C+A+ treatment is unknown. Previous studies using arginine have reported decreased (Southern and Baker, 1982; Anderson et al., 1984), increased (Hernandez et al., 2009; Tan et al., 2009; He et al., 2011), or no significant effects on weight gain (He et al., 2009; Ma et al., 2010). However, the studies that do not find an effect of arginine on BW started treatments at 40+ kg, while the studies that found effects applied arginine supplementation shortly after weaning for (less than) a month. Perhaps effects of arginine on BW are only evident in a short time frame immediately after weaning. Edmonds et al. (1987) indicated that excess arginine supplementation (4%) reduced weight gain due to amino acid imbalance. This is not likely to have occurred in the current study as we supplemented diets with far less arginine (0.8%). The mechanism by which a prolonged period of high carbohydrate and arginine supplementation in the diet results in negative consequences on BW gain are, as of yet, unknown.

After approximately 144 days of age, feed residuals were slightly higher in the C+A– treatment, which may explain the slightly lower BW of this treatment at the end of the experiment compared with the C–A– treatment. Previously, we showed that growing gilts show a quick reaction in BW when feed intake is adjusted (de Koning et al., 2013). Therefore, minor differences in feed intake at the end of the experiment may explain the minor BW differences between the C+A– treatment and the C–A– treatment. However, when looking at the feed efficiency, the gilts in the C+ treatment were the most inefficient group of gilts in terms of gaining BW and were 17–20% less efficient than the gilts in the C– treatment. This

could also explain the differences in BW and might be explained by the differences found in metabolic parameters (see below). The amount of feed provided could not be further decreased, as 3 treatment groups (C+A–, C–A+, and C–A–) had relatively few feed residuals during the major part of the experiment. Further lowering the feed likely would have caused unrest and agitation in the treatment groups that showed a relatively good feed intake, which could result in differences in overall activity of the animals and hence affect OC prevalence on its own, as shown in a previous study (de Koning et al., 2014).

#### 4.2. Treatment effects on glucose, insulin, insulin-like growth factor-1, and nitric oxide

Dietary treatments affected several plasma components measured in the current study. Significant effects were only found for the carbohydrate treatment, whereas arginine treatment did not show any significant effects on any of the plasma components analyzed.

##### 4.2.1. Glucose and insulin

Insulin and glucose profiles showed that although gilts in the C+ treatment presented with (numerically) higher responses shortly after a meal (short term response), gilts in the C– treatment showed a higher level of insulin and glucose before feeding and at longer term (more than 2 h after feeding). These higher insulin and glucose levels in the carbohydrate treatment coincide with higher IGF-1 levels in gilts receiving the C– treatment. Previous experiments from literature with insulin stimulating diets (high carbohydrates) report similar results for glucose and insulin on the short term (van den Brand et al., 1998, 2000; Wientjes et al., 2012, 2013). In comparison to the C+ treatment, the C– treatment had less glucogenic compounds in the feed and the increase in glucose on the longer term for gilts in the C– treatment may reflect glycogenolysis to increase glucose levels for the citric acid cycle (van Knegsel et al., 2005).

##### 4.2.2. Insulin like growth factor

The associations of insulin and IGF-1 responses between treatments are confirmed by associations found between insulin and IGF-1 in other studies (van den Brand et al., 2001; Wientjes et al., 2013; and reviewed by Thissen et al. (2004)). However, the same studies report that dietary treatments did not significantly affect IGF-1 levels. The previously mentioned studies applied dietary treatments during a short time (lactation or weaning to estrous interval) in adult sows, whereas the current study applied dietary treatments for a much longer time in much younger gilts, which might explain these differences. In humans it is reported that (prolonged) dietary fat is associated with higher IGF-1 levels (Kaklamani et al., 1999; Gunnell et al., 2003; Heald et al., 2003), as is the case in the current study. Considering that dietary fat is associated with higher IGF-1 levels, one may wonder whether this effect also explains the differences in growth curves found in the gilts and their feed efficiency. It has been indicated that IGF-1 reduces protein breakdown (Asakawa et al., 1992; Hussain et al., 1993; Cioffi et al., 1994) and increases protein synthesis (Fang et al., 1997; and reviewed by Schiaffino et al., 2013). Possibly, the gilts in the C– treatment, that have higher IGF-1 levels than gilts in the C+ treatment, can more efficiently grow due to the protein synthesis effects of IGF-1. However, this does not fully explain why gilts in the C+A+ treatment showed a much lower BW gain. Studies have shown that arginine decreases protein turnover by decreasing protein synthesis and breakdown (i.e. turnover) during fetal growth in sheep (de Boo et al., 2005), or increases both protein synthesis and breakdown during and after endotoxemia in pigs (Bruins et al., 2002). Perhaps this could mean that the effect of

lower IGF-1 in C+ treatments on protein synthesis is attenuated even more when arginine is presented in the diet, although the catabolic state as seen in endotoxemia is likely not comparable to the situation of growing animals.

##### 4.2.3. Nitric oxide

Although NO is a product of arginine metabolism (Wu and Morris, 1998), this study was unable to find significant differences in plasma NO in the arginine treatment. Others were able to find effects of arginine treatment on NO production, but those were localized to areas of hypoxia at tissue level (Schwarzacher et al., 1997; Duan et al., 2000). Therefore, in the current study, even when NO production would have been affected in osteochondrotic areas, it may have been impossible to detect such an effect at systemic level. Gilts in the C– treatment had significantly lower levels of NO. A high fat diet (C– treatment) has consistently been shown to reduce NO availability by reducing NO synthase activity or increasing reactive oxygen species limiting NO present in the body (Roberts et al., 2000; Bender et al., 2007; Yang et al., 2007; Huang et al., 2011), which seems to have occurred also in the current study. This NO reducing effect of a fatty diet may have reduced the effects of arginine treatment on NO levels. It can be concluded at any rate that the dietary treatments used were effective in eliciting a difference in metabolic responses.

#### 4.3. Osteochondrosis prevalence

Prevalence of gilts affected with OC (OC score > 0) was approximately 61%, which is roughly equal to the prevalence found in our earlier studies using the same line of gilts (de Koning et al., 2013, 2014) and is comparable with other studies (Kadarmideen et al., 2004; Ytrehus et al., 2004b; Jørgensen and Nielsen, 2005; Busch and Wachmann, 2011). However, the prevalence of severe lesions (OC score 3 and 4) was low (12% in the current study) when compared with our previous studies (ranging from 22 to 28%). Especially the hock joint and the knee joint showed a low prevalence of severe lesions. The elbow joint had a low prevalence of any OC score, which is similar to our previous studies. In contrast, the prevalence of minor lesions (OC scores 1 and 2) was higher in the current study (49.5%) compared with our previous studies (30% to 40%). Differences in feed intake between studies may explain some of these results, although animals that are continuously restricted (80% of ad libitum) in feed intake (de Koning et al., 2013) showed a lower overall prevalence of OC when compared with gilts with similar feed intake in the current study in the C+A– treatment (44% versus 60%, respectively) but a higher prevalence of severe lesions of OC (14% versus 11%, respectively). The relatively low prevalence of severe lesions compared with our previous experiments and the high incidence of minor lesions of OC in the current study cannot be readily explained at this moment.

#### 4.4. Treatment effects on osteochondrosis

Osteochondrosis involves the formation of necrotic cartilage due to vascular disruption (local hypoxia) within the growth cartilage at young age in pigs. After the formation of necrotic growth cartilage, reparative attempts by viable chondrocytes and vasculature ensue that may or may not be successful. The level of lesion formation combined with the successfulness of reparative attempts determine final clinical outcome (reviewed by Laverty and Girard (2013); McCoy et al. (2013); Olstad et al. (2015)). This study was only able to find significant effects of carbohydrate treatment on OC prevalence in the knee joint. Gilts in the C+ treatment had lower odds to be affected with OC than gilts in the C– treatment. One may wonder whether the significant carbohydrate effect and

the tendency of the arginine effect is determined by the BW difference, which was a consequence of the C+A+ treatment. A higher BW may increase loading on the joints, which is a suggested risk factor for OC development by increasing risk for vascular disruption or fracturing necrotic growth cartilage (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004a). Indeed, descriptive statistics show that gilts in the C+A+ treatment had the lowest prevalence of OC in the knee joint and at the animal level. However, no significant interaction effect between carbohydrate and arginine treatments could be found. Additionally, the effect of BW at slaughter was significantly associated with OC prevalence, while treatment effects became non-significant. In contrast to studies that could not find associations of BW with OC (Woodard et al., 1987; Jørgensen, 1995; Ytrehus et al., 2004b), the results of BW association with OC in the current study agrees with other studies (Carlson et al., 1988; van Grevenhof et al., 2011; de Koning et al., 2013; Quinn et al., 2015). These studies indicate that animals fed (up to 20%) below ad libitum feed level show a reduction in OC prevalence. In a previous study, we fed gilts ad libitum or 80% of ad libitum feed level and saw a reduced prevalence of OC for gilts fed 80% of ad libitum feed level (de Koning et al., 2013), which corresponds to the current study. Therefore, the reduced prevalence of OC in the C+A+ treatment group likely is a result of a reduction in growth rate over the experimental period. Our initial hypothesis was that a carbohydrate contrast through glucose, insulin, and IGF-1 levels affect OC prevalence as higher levels of these parameters were associated with higher OC levels in horses (Ralston, 1996; Pagan, 2001; Semevolos et al., 2001; Verwilghen et al., 2009) and could affect chondrocytes through proliferation or survival (Hunziker et al., 1994; Henson et al., 1997). Additionally, studies have indicated that arginine affects formation of blood vessels during hypoxic conditions through NO (Murohara et al., 1998; Duan et al., 2000; Hazeleger et al., 2007). Due to the unexpected effects of the diets on body weight, we cannot conclusively state that these metabolic parameters affected OC prevalence by directly affecting chondrocytes.

A complicating factor in this study is the relatively low prevalence of severe OC, probably due to a reduction in growth rates, as mentioned above. The effects of dietary carbohydrates and arginine may only be evident during the development of severe lesions of OC where a more prominent role for reparative responses undertaken by chondrocytes and vasculature would be expected. Although it would be beneficial to undertake a study in a group of animals that develop severe lesions, this is likely impossible as one cannot predict beforehand whether a group of gilts selected at 4 weeks of age will develop a sufficient prevalence of severe OC lesions.

## 5. Conclusion

Dietary carbohydrate levels, but not arginine supplementation, during the rearing period were found to affect metabolic parameters at approximately 24 weeks of age. Low carbohydrate (fatty) diets increased insulin, glucose, and IGF-1 levels compared with carbohydrate rich diets. Carbohydrate rich diets were associated with increased NO levels at 10 and 24 weeks of age. This study could not find significant effects of arginine supplementation during the rearing period of gilts on OC prevalence at approximately 25 weeks of age. Dietary carbohydrate levels were significantly associated with OC in the knee joint. However, dietary treatments affected BW development and results indicate that weight at slaughter supersedes treatment effects on OC, in which heavier gilts at slaughter have a higher odds to be affected with OC. Likely, dietary treatments affected OC prevalence indirectly by affecting BW development.

## Conflict of interest statement

The work presented here contained no conflicts of interests.

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