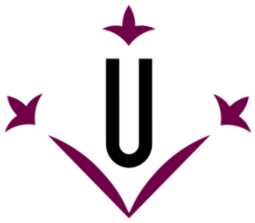

Effects of dietary roughage on organic pig performance, behaviour and antioxidants accretion in perirenal adipose tissue

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ABSTRACT

This study aimed at investigating the effects of oat silage and sex on the growth performance, behaviour and antioxidants accretion in perirenal adipose tissue of organic pigs. In total, ninety-seven crossbred pigs (51.8±7.6 kg) from two sexes (entire males *vs*. females) were assigned to two dietary roughage supplement treatments (control with straw *vs*. 200 g dry matter/pig/day of oat silage supplement) in a 2 x 2 factorial design until 120 kg of live-weight. There was no significant effect of forage type or sex on growth performance. However, the oat silage had a positive effect on the exploration behaviour on the outdoor run fixtures. The retinol concentration in adipose tissue was higher in the pigs that were given the oat silage than in those receiving the control diet, but the feeding treatment did not affect the concentration of α-, γ- and δ- tocopherols. The oat silage carried out lower cholesterol accretion in adipose tissue than the control diet. The sex did not affect retinol, tocopherols or cholesterol concentration in adipose tissue. Dietary silage supplement may be used by producers to enhance pork vitamin A content.

Highlights

- Oat silage did not affect growth but increased the pen fixture exploration outdoors.

- Entire males and gilts showed similar negative interactions with other pen-mates.
- Oat silage did not affect the concentration of tocopherols in perirenal fat.
- Oat silage increased retinol and decreased cholesterol accretion in perirenal fat.

Keywords: entire males, gilts, carotenoids, retinol, tocopherol, oat silage.

1. Introduction

Organic pigs should have *ad libitum* access to roughage (EU Regulation EC 834/2007 and Commission Regulation 889/2008), with the primary aim to improve the welfare and health of the animals. Access to roughage reduce abnormal (mainly stereotypic) behaviour (De Leeuw *et al.*, 2008). In South European countries this is normally met through bedding pens with cereal straw. According to Wallenbeck *et al.* (2014), roughage (grass/clover silage) can provide some nutrients supply to growing-finishing pigs. Hansen *et al.* (2006) found roughage feeds inclusion without *ad libitum* concentrate may also decrease the growth performance and increase the lean percentage in the carcass. Other studies (Der, 2014; Høøk Presto *et al.*, 2009) showed varying capacity of pigs to consume roughage, according to the type of roughage, nutrient profile and feeding technique (e.g. if fed separately to concentrate, stem length and whether pelleted or not), as these factors affect the ability of pigs to sort out less desirable parts. The intestinal mucosa of the pig lack the enzymes capable of cleaving the dietary fibre, but dietary fibre can have broader effects, such as stimulating gut health (Hovi and Baars, 2001).

The composition of fat in animal tissue results not only from genetic back-ground but also from feed components. Carotenoids and vitamins A and E are fat-soluble micro-nutrients that play an important role as antioxidants in human and livestock health (Chauveau-Duriot, 2010). The α-Tocopherol is the most potent form of vitamin E, accounting for almost all vitamin E activity in living tissues. Carotenes, particularly all-E-β-carotene, are precursors of retinol (vitamin A) (Barker *et al.*, 2011; Eggersdorfer and Wyss, 2018; Weber and Grune, 2012). Animals cannot synthesise carotenoids; thus their carotenoid contents in tissues are

51 derived from diet, ingested with food, selectively or unselectively absorbed, and
52 accumulated unchanged or slightly modified (Eggersdorfer and Wyss, 2018; Rodriguez-
53 Amaya, 2001). Among feeds, the fresh forages contain high contents of α-tocopherol and
54 carotenoids (Chauveau-Duriot, 2010; Nozière e*t al.*, 2006).

55 Cholesterol is found only animals (not in plants or microorganisms) and is the precursor of
56 steroid hormones, vitamin D and the bile acids, moreover is a constituent of cell membranes
57 and bile micelles. From a dietary point of view, it would be interesting to know if an increase
58 in the fiber content in the diet is related to changes in cholesterol in the body tissue of pigs,
59 due to its effect on serum cholesterol (Knud, *et al.* 2001; Kreuzer *et al.*, 2002).

60 Several studies, all using conventional pig production systems, have found higher levels of
61 aggression in entire male pigs compared to female pigs (Rydhmer *et al.*, 2006) irrespective
62 of whether they were housed in single or mixed sex groups. Castration of male pigs is
63 particularly problematic in organic pig production; a production concept that prioritises the
64 natural behaviours (Lund, 2006). Thomsen *et al.* (2012) concluded that is possible to
65 produce entire male pigs within the organic production system without compromising the
66 welfare of the animals. Therefore, during the forthcoming years it will be necessary to gather
67 the growth performance and lipid composition outcomes of entire males compared to their
68 farm sibling gilts, as few references compared them (Pauly et al., 2012). Roughages feeds
69 may play an important role in occupying pigs and therefore contribute to less aggressive
70 behaviours (Høøk Presto *et al.*, 2008) and can influence the pigs' activity pattern and social
71 interactions, reducing stress and aggression between individuals (Høøk Presto *et al.*, 2009).
72 Furthermore, entire males have higher feed efficiency, grow faster and produce leaner meat
73 than gilts and barrows (Babol and Squires, 1995), which would be particularly economically
74 beneficial for farmers. Many fibre rich feedstuffs are relatively inexpensive and may be used
75 to increase the dietary fibre content and gut fill, which may potentially prolong the feeling of
76 satiety and have positive effects on animal welfare.

The present study was carried out to evaluate the effects of supplementing dietary oat silage and sex on the growth performance, behaviour, antioxidants and cholesterol accretion in perirenal adipose tissue (as a carcass lipid proxy) of organic fattening pigs.

2. Materials and Methods

2.1. Animals, experimental design and treatments

A total of ninety-seven growing-finishing pigs (forty-eight females and forty-nine entire males) with an average body weight (BW) of 51.8±7.6 kg (average±SD) were individually ear-tagged and included in this experiment, that was carried out in a commercial organic pig farm in northwest Catalonia ("Granja Casat", Sarroca de Bellera, Lleida, Spain, at 1,030 m above sea level). The experiment was conducted between June and September 2019. The average monthly temperature for June, July, August and September was 17.1, 19.7, 19.2 and 16.7°C, respectively; although, the outside daily maximum temperature reached 30 °C, especially in June (25th-30th) and July (22nd-25th). The pigs were kept in accordance with the EU's standards for organic livestock (space allowance ≥ 1.3 m^2/pig indoor plus 1.0 m^2/pig outdoor in a loose-housed barn) and livestock products (EC-regulation 889/2008 supplementing the EC-regulation 834/2007). The experimental design was factorial, with two feeding strategies (control or silage supplement) and sexes (females or entire males). The pigs belonged to two consecutive 3-week interval batches and they were the progeny of crossbred sows, Large White x Landrace (CG36 Naïma), and Duroc boars (UPB Genetic World, Navàs, Spain). The pigs housed in four pens of 22-24 pigs where entire males were kept in separate pens from gilts. Two pens, one with males and the other with females, were assigned to each treatment. Control (49 pigs) and Silage (48 pigs). The indoor area had a resting area on concrete floor with cereal straw bedding and a longitudinal trough (1.5 m-length) for roughage supply. The supplementary forage trough in the indoor area was filled daily with 200 g dry matter (DM)/pig/day of winter cereal straw in control treatment or with chopped oat silage supplement (5-cm length approximately, pH=5.6±0.07) until the target slaughter weight (120 kg BW, approximately). The outdoor area was a loose-house shed

104 with concrete floor pens without bedding and 20% slatted floor below the concentrate hopper
105 and drinker area. Each pen had two dry single-space self-feeders and two square nipple
106 drinkers in the outdoor area. The barn was naturally ventilated. During the experiment no
107 pig died and one pig was removed because of tail biting. All the pigs were fed *ad libitum* with
108 the same commercial organic pelleted feed, whose ingredients were barley grain (39%), oat
109 (10.0%), wheat (30%), expeller soybean meal (17%), oil sunflower (1.53%), minerals
110 (1.85%) and vitamin-micromineral premix (0.5%). The estimated digestible energy (DE)
111 content of the concentrate based on proximate chemical composition using EvaPig® (2011)
112 was 14.4 MJ DE/kg of feed. The feed provided had a nutrient content shown in Table 1,
113 which approximates the ITAB (2014) nutritional recommendations. The experimental
114 organic concentrate was without retinol acetate supplementation. The proximate chemical
115 composition of the pelleted compound feed and roughages were carried out according the
116 methods of A.O.A.C. (2000) and they are shown in Table 1. Also, pigs received straw bales
117 as bedding material (0.5 kg/day/pig) at weekly intervals, which also served as dietary
118 roughage source *ad libitum.*

119 *2.2. On-farm and abattoir measurements*

120 The pigs were individually weighed three times during the experiment (days 1, 45 and 87)
121 to evaluate the average daily gain. When the average BW was around 120 kg live weight,
122 the pigs were transported to a commercial abattoir, 185 km away (Escorxador Comarcal del
123 Moianès, S.A., Moià, Spain). Pigs were transported in the morning (between 11:00 and
124 12:00) with a small truck provided with a relatively flat loading ramp. At the abattoir, animals
125 were allowed 3-4 hours rest period with full access to water but not to feed. Pigs were
126 stunned by CO_2 using a dip lift system, exsanguinated, scalded, skinned, eviscerated
127 according to standard commercial procedures and carcasses were splitted down the
128 midline. Hot carcass weight was individually recorded after splitting to calculate the dressing
129 out proportion. Within 30 minutes *post-mortem*, a sub-sample of 56 carcasses (control, n=23
130 and silage, n=33), balanced for sex (entire males=36, gilts=20), was weighed (including the

head and tail) and perirenal fat was sampled (100 g approximately). The samples were stored in ice until arrival to laboratory and therefore they were packaged in vacuum-opaque bags and frozen at a temperature of -80ºC until laboratorial analyses.

2.3. Behavioural and Activity observations

Instantaneous scan sampling was used to record behavioural activity budget patterns, social interactions and abnormal behaviours as described by Casal-plana *et al.* (2017) and Fàbrega *et al.* (2019). Once per month, one trained observer carried out direct observations on the outdoor and indoor space areas of the four pens. The four pens were adjacent in the same room, thus allowing direct observation of them by a single technician. The observer was standing outside the pen viewing both indoor and outdoor areas. Each pen was scanned in sessions of four hours, from 9:00 to 13:00, at ten-minute intervals. Thirty minutes before starting the observations, the observer entered the room and walked around to allow the pigs to get used to her presence and to assure that animals paid no attention to the observer. In every scan sampling, the observer counted the number of pigs that were in each location of the pen (indoor and outdoor) and which was their activity and behaviour based on a previously defined ethogram (Table 2) Thus, each observation day provided a total of 24 scans per pen. The pen observations were daily performed in the same recording sequence order.

2.4. Analysis of carotenoids and tocopherols in feedstuffs and tocopherols, retinol and cholesterol in perirenal fat

Carotenoids and tocopherols in feedstuffs were extracted from 200-mg samples according to the methodology described by Blanco *et al*. (2019). The extraction was performed without saponification to avoid degradation of the carotenoids (Fu *et al.*, 2011). The chromatographic and quantification procedure was that used by Chauveau-Duriot (2010), using an ACQUITY UPLC H-Class liquid chromatograph equipped with a silica-based bonded phase column (Acquity UPLC HSS T3, 1.8 µm x 2.1 mm x 150 mm column, Waters), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ Detector; Waters) and a fluorescence

158 detector (2475 Multi λ Fluorescence Detector, Waters). Carotenoids were detected by
159 absorbance at 450 nm, tocopherols by fluorescence emission at λexc = 295 and
160 λemi = 330 nm. β-carotene, lutein and tocopherols were identified by comparison of their
161 retention times and spectral analyses, and quantified by external calibration with those pure
162 standards. Zeaxanthin, neoxanthin, violaxanthin, All-E-β-carotene (13Z+9Z) were identified
163 by comparison of their retention times and spectral analyses previously reported (Chauveau-
164 Duriot, 2010; Rodriguez-amaya, 2001), and quantified (semiquantitative analysis) by
165 calculating response factors to β–carotene calibration based on their molar absorption
166 coefficients (Rodriguez-amaya, 2001).

167 The analysis of tocopherols, retinol and cholesterol in perirenal fat was performed with the
168 method by Bertolín et al. (2018). Briefly, a 100-mg fat sample was submitted to mild
169 overnight saponification, liquid-liquid extraction, evaporation with vacuum evaporator and
170 redissolution. The quantification of the different analytes was performed by Bertolín et al.
171 (2018) the use of the afore-mentioned ultra-high-performance liquid chromatography with
172 diode-array detector for carotenoids, retinol and cholesterol and fluorescence detector for
173 tocopherols. The mobile phase was acetonitrile:methanol:dichlorometane (75:15:10, v:v:v)
174 with a flow rate of 0.3 ml/min. The temperature of the samples and the column were adjusted
175 to 25 °C and 35 °C, respectively. The injection volume was 5 µl and the total run time of the
176 chromatographic procedure was 15 min. Retinol was detected by absorbance at 325 nm and
177 cholesterol at 220 nm. The antioxidants and cholesterol in adipose tissue were expressed
178 as concentration on a fresh weight basis.

179 All-E-β-carotene (97% purity), lutein (97% purity), retinol (97.5% purity) and tocopherols
180 (99% purity α-tocopherol, 97% purity γ-tocopherol, 90% purity δ-tocopherol) were purchased
181 from Sigma-Aldrich. Concentrations of β-carotene, lutein, retinol, α-tocopherol, γ–tocopherol
182 and δ-tocopherol standard solutions were calculated before use by absorbance of each
183 solution using molar absorption coefficients previously reported (Rodriguez-Amaya, 2001;
184 Chauveau-Duriot, 2010; Yagoubi *et al.*, 2018).

185 Average results from the feed analyses of carotenoid and tocopherol contents in organic
186 concentrate and roughages (pooled means of individual pen samples) are shown in Table
187 5.

188 2.5*. Statistical analysis*

189 The data were analysed with the JMP Pro 13 statistical software (SAS Institute, Cary, NC,
190 USA). Before further analyses, the normality of the residuals of all the variables was tested
191 with the Shapiro–Wilk test. The animal performance, lipid antioxidants and cholesterol
192 concentration had normal distribution of the residuals. The experimental unit for the animal
193 performance and antioxidants and cholesterol in lipid tissue was the pig, whereas for the
194 behavioural observations the pen was considered as the experimental unit. The animal
195 performance, lipid antioxidants and cholesterol were analysed through standard least
196 square models that included the dietary treatment and sex as fixed effects. Single
197 interactions between these fixed effects were removed from the final model because they
198 were non-significant ($P > 0.05$). In lipid antioxidants and cholesterol models, the slaughter
199 batch was also considered as fixed effect and the carcass weight as a covariate.

200 The behavioural data from the scan samples was analysed on a pen basis as a mean
201 percentage of the scans in each category (activity or behaviour) in relation to the total
202 number of scans (Fu *et al.,* 2011) per day. The behavioural data were analysed either
203 through a mixed model (variables fitting normal distribution: standing inactive, walking, lying,
204 interaction with enrichment material, interaction with the pen, and positive social interaction)
205 or a non-parametric Wilcoxon test (indoor or outdoor location, sitting inactive, eating
206 concentrate or drinking, eating roughage, and negative social interaction). In all the
207 behavioural variables, the effect of dietary treatment, sex, location within the pen (indoor *vs.*
208 outdoor), month of sampling, and the interaction between dietary treatment and location was
209 considered. In mixed models, the pen was taken into account as a random effect. Values
210 are presented as least square means ± standard error of the mean (SE). The level of

211 significance was set at 0.05. Differences between least square means were assessed with
212 the Tukey test.

213 **3. Results**

214 3.1. Growth and carcass performance

215 During the experiment, three pigs died (2 gilts from straw and 1 entire male from straw) and
216 one pig was removed because of tail biting (one gilt from silage). No leftovers of the oat
217 silage supplement were registered throughout the study whereas the winter cereal straw
218 was offered ad libitum either as a supplement in the trough and as bedding in straw groups
219 or just as bedding in silage groups. The effects of feeding strategy and sex on growth
220 performance of pigs used in this study are shown in Table 3. The BW, at days 45 and 87,
221 did not differ ($P > 0.05$) between feeding strategies (control or supplementary oat silage) or
222 sexes (entire males and gilts) Moreover, there were no differences ($P > 0.05$) between
223 treatments in the average daily gains (ADG) during the growing period (50-85 kg) and
224 finishing period (85-120 kg). The carcass weight was not affected by feeding strategy, but it
225 was lower in entire males than in gilts ($P < 0.01$). The dressing out proportion did not differ
226 between feeding strategies and sexes ($P > 0.05$).

227 3.2. Animal behaviour

228 The effects of location within the pen and dietary roughage supplement are shown in Table
229 4. The month of sampling affected the location outcome of the pigs, that spent more time on
230 the outdoor run in July than in August (37.0 *vs*. 34.2 ± 1.1%, $P < 0.05$), as well as more time
231 standing inactive (9.1% *vs*. 5.2% ± 0.7%, $P < 0.05$), more time eating roughage (4.4 *vs*. 1.3
232 ± 0.8%, $P < 0.05$), and more time having positive social interactions (5.9% *vs*. 4.1 ± 0.5%,
233 $P < 0.05$), respectively.

234 The location of the pigs affected their activities and behaviours. Pigs were more time lying
235 in the indoor than in the outdoor area (73.2 *vs*. 65.6 ± 1.7%, $P < 0.05$) and they were lower
236 time standing inactive indoors (5.4 *vs*. 8.9 ± 0.7%, $P < 0.05$). In addition, the time interacting

237 with enrichment material was higher in the indoor than in the outdoor area (15.1 *vs*. 1.8 ±
238 1.3%, $P < 0.05$), whereas the time interacting with the pen was lower in the indoor than
239 outdoor area (9.1 *vs* 15.6 ± 0.96%, $P < 0.05$).

240 The sex of the pigs did not affect any of the behavioural variables, except the time sitting,
241 that was higher in entire males than in gilts (2.6 *vs*. 0.7 ± 0.86%, $P < 0.05$).

242 The dietary roughage supplement exerted an effect on the overall time that pigs spent
243 interacting with the pen fixtures irrespective of location, that was lower in control than in
244 silage (10.2 *vs.* 14.7 ± 0.09, $P < 0.05$). This difference was less marked in the indoor than in
245 the outdoor area. In fact, the dietary roughage supplement did not affect any other behaviour
246 in the indoor area of the pen.

247 3.3. Carotenoids and tocopherols in feedstuffs

248 The main carotenoids found in the dietary feedstuffs analysed in this study are summarized
249 in Table 5. As shown, carotenoids were lower, or not detected, in the concentrate and straw
250 than in oat silage samples. The two major carotenoids in oat silage were lutein and all-E-β-
251 carotene. The α-tocopherol content was highest in the concentrate, intermediate content in
252 oat silage and lowest in the cereal straw, whereas γ-tocopherol and δ-tocopherol were
253 lowest in the oat silage.

254 3.4. Carotenoids, tocopherols and cholesterol concentrations in perirenal adipose tissue

255 The effects of supplementing oat silage on tocopherols, retinol and cholesterol contents of
256 perirenal adipose tissue is shown in Table 6. The tocopherols content in perirenal adipose
257 tissue were not influenced ($P > 0.05$) by feeding strategy or sex. A high concentration of α-
258 tocopherol was detected in adipose tissue, compared to the γ-tocopherol and δ-tocopherol
259 concentrations, respectively.

260 The retinol in perirenal adipose tissue was affected by feeding strategy ($P < 0.01$), as the
261 pigs that were supplemented with oat silage had higher retinol content than their control

counterparts. In contrast, the cholesterol contents in perirenal adipose tissue lower in the oat silage than in the control group *(P < 0.05*).

4. Discussion

The aim of this study was to determine the response of supplementing dietary oat silage and sex on growth performance, behaviour and vitamins and cholesterol accretion in perirenal adipose tissue of organic fattening pigs. The results showed that despite of numerical differences, there was no significant effect of feeding strategy on growth rate of Duroc-sired pigs, which may be related to high within-pen coefficient of variation of final BW (overall, 14.8%), since the batches were made equal and balanced by initial body weight and sex. The amount of supplementary oat silage did not exert any detrimental effect on performances, as carcass weight and dressing out were also similar between dietary treatments. Unexpectedly, the growth rate did not differ between sexes, maybe because in this case they were not heavy pigs (<120 kg of body-weight at slaughter). Generally, entire males grow faster than gilts (Rhodes *et al.*, 1998), but normally the male pigs raised until heavy weights (>110 kg) are castrates, thus many references compared the growth performance of barrows with gilts (Gispert *et al.*, 2010; Puls *et al.*, 2014). The similar growth performance in both entire males and gilts lead us to hypothesise that the dietary concentrate did not meet the amino acid requirements in entire males, that may have performed below their growth potential. The estimated standardised digestible lysine content of the concentrate was 0.4 g/MJ DE, and according Moore et al. (2013), the lysine requirement for maximum daily gain in entire male pigs was 0.72-0.63 g Lys/MJ DE for 50-95 kg of body-weight interval, whereas the corresponding requirements for female pigs were 0.67-0.58 g Lys/MJ DE, respectively. In the same way, according to NRC (2012), entire male pigs have higher lysine requirements than gilts (0.94% *vs* 0.89%, respectively, from 75 kg to 100 kg of body-weight and 0.86% *vs* 0.74%, respectively, from 100 to 135 kg of BW). Hence, the present organic concentrate feed would have only met the dietary lysine requirements of finishing gilts; thus, entire male pigs of selected Duroc crossbred genotype

289 may have a greater potential to deposit more protein and require greater dietary lysine in
290 the growing and finishing phase. Compared with gilts, entire males in the present study had
291 a numerically lower carcass weight and worse dressing percentage. In general, entire males
292 dressed approximately 2-2.5% less than gilts (Babol and Squires, 1995; Jeremiah *et al.*,
293 1999) and castrates (Friendi *et al.*, 1989), as consequence of heavier kidneys and slightly
294 higher proportions of head, feet and viscera, which may contribute to reduce the dressing
295 percentage (Jeremiah *et al.*, 1999). Even though, the chopped oat silage was intended to
296 supply some micronutrients for pigs as vitamins and fatty acids, but its palatability may be
297 reduced as consequence of the warm weather during the experimental period. Voluntary
298 feed intake is affected by thermal environment factors such as air temperature and humidity
299 (Presto *et al.*, 2019). Moreover, the Neutral-Detergent Fibre and Acid-Detergent Fibre
300 content of the dietary silage supplement could also explain its high fill value (due to non-
301 starch polysaccharides supply) (Knud, 2001; Sarria and Martens, 2013). The capacity of
302 pigs to digest nutrients from roughage depends on the chemical composition, as nutrient
303 digestibility decreases with increasing proportion of fibre fractions (Wallenbeck *et al.*, 2014;
304 Presto *et al.*, 2019). The oat silage used had higher fibre content than some nutritive value
305 recommendations for pigs, that limit the roughage content (ADF to 44% and NDF to 62%)
306 (Sauvant *et al.*, 2002).

307 Additional dietary roughage may enhance pig welfare and reduce pen-mate-directed oral
308 activity and aggression (Lindgren *et al.*, 2014). In this experiment, the additional roughage
309 and the straw were provided in the indoor area, but no differences between dietary
310 treatments were observed therein with regard to the interaction with enrichment materials or
311 interaction with the pen. However, in the outdoor area, silage addition increased the
312 interaction time with the pen (licking, chewing, nosing or sniffing unanimated objects from
313 the pen, other than enrichment material). Furthermore, the increased manipulation of the
314 pen fixture could be a sign of frustration of restriction to the oat silage and conducted to
315 redirected foraging behaviour (Casal-Plana *et al.*, 2017). Hence, compared with straw, better
316 quality supplementary roughage increased the explorative behaviour in the outdoor run

area. This outcome is in agreement with Presto *et al.* (2013), who observed that the time budget was altered when including grass/clover mixture silage (around 16% CP) in pig diets, since the pigs provided chopped silage spent a larger proportion of their time nosing the pen floor than pigs fed the commercial concentrate alone. In addition, lifting of other pigs was instead performed more frequently by the chopped silage treatment, but it reduced the number of wounds on the middle and hind part of the pig body when compared to pigs fed only concentrate. In another study, Presto *et al.* (2019) observed that when pigs were supplemented with intact chicory or red clover silage (both with approximately 16% CP), they were more active and performed more feed directed behaviours and less behaviours directed towards other pigs and pen fittings, i.e. expressed more natural behaviour. The restricted amount supplied and the low nutritive value of the silage used in the present study may have reduced the potential behavioural foraging pattern (rooting bedding material instead of exploring pen fixtures) of the pigs. Accordingly, Studnnitz *et al.* (2007) concluded that in order to be a suitable rooting material the material must stimulate the exploratory behaviour of pigs for an extended length of time; moreover, exploratory behaviour in pigs is more stimulated by materials that are complex, changeable, destructible, manipulable, and contain sparsely distributed edible parts. In the present study, a significant effect of the pen location (indoor *vs* outdoor) on the lying behaviour was found, as a higher percentage of pigs laid on the dark area (indoor), which is in agreement with Opderbeck *et al.* (2020). In July, with hotter temperatures than August, pigs lied down and were less likely to engage in aggressive behaviour, in line with Velarde and Geers (2007). In general, there were no differences in activities between sexes, except that entire male spent more time sitting in a dog position and may be related to apathy and would reflect a behavioural stereotypy (Millet *et al.*, 2005; Teixeira and Boyle, 2014). The sex difference in the time sitting inactive was not observed earlier.

In this experiment, the dietary carotenoids were lower, or not detected, in the concentrate and cereal straw, compared to oat silage samples. Lutein and zeaxanthin were the only carotenoids found in all samples. Lutein and all-E-β-carotene (provitamin A) were the main

carotenoids present in oat silage, which agreed with the results reported by other studies in silages or hays, but their concentrations were lower than in fresh forages (Cardinault *et al.*, 2008; Ellis *et al.*, 2007; Prache *et al.*, 2009). In nature, vitamin A is largely present as lipid esters in animal tissues and as provitamin forms of vitamin A in plants (mainly, β-carotene) (Jaswir *et al.*, 2011). The β-carotene content in oat silage was twice the α-tocopherol content, which agreed with the result reported by Liu *et al.* (2020). The content of α- and δ-tocopherols in concentrate feed were higher than in roughages (straw and silage), whereas the γ-tocopherol content was higher in the cereal straw than in the concentrate and the oat silage. Upon absorption, pro-vitamin A carotenoids (mostly β-carotene) are partially converted into vitamin A (retinol, retinal, retinoic acid, and intermediate compounds) mainly in the gut and liver (Henriquez-Rodriguez *et al.*, 2017). The pigs accrete fat-soluble vitamins, especially retinol (Vitamin A) and tocopherols (Vitamin E) in perirenal adipose tissue, and their contents are affected by their dietary intake (Álvarez *et al.*, 2014). Indeed, these dietary differences may explain the higher retinol in the adipose tissue when supplying additional dietary silage to pigs. A similar result was found by Ayuso *et al.* (2015) when providing a synthetic vitamin A (retinyl acetate) enriched-diet, that permitted higher retinol being accumulated in adipose tissue. Also, an increase in dietary vitamin A could induce lower levels of other vitamins such as α-tocopherol in animal tissues (Olivares *et al.*, 2009), but cannot be concluded from the present results. In this work, the highest vitamin E accretion in perirenal adipose tissue was evidenced by α-tocopherol isomer, which agreed with Aitken *et al.* (1970). This may be explained by the relatively high level of α-tocopherol in the dietary concentrate, which could conceal the additional effect of the silage supplement on α-tocopherol accretion in the adipose tissue.

Cholesterol can be obtained from the diet if it contains animal products or it can be synthesized (Bruss, 1997). As the present dietary concentrate did not contain any animal source ingredient, the differences between treatments may be attributed to divergent biosynthetic pathways that would be partly mediated by the dietary silage supplement. However, Rey *et al.* (2004) concluded that the cholesterol concentration in meat from Iberian

pigs was not significantly affected by the dietary treatments, although others (Barowicz *et al.*, 2000) have reported an hypocholesteronemic influence of dietary n-3 polyunsaturated fatty acids (PUFA) in growing pigs in *Longissimus dorsi* muscle from growing gilts. This would agree with the current study, as the sum of dietary n-3 PUFA content was nearly three-fold in oat silage supplement compared to the concentrate feed (7.5% *vs*. 2.6%, oat silage and concentrate feed, respectively) (Argemi-Armengol *et al.*, 2020). The hypocholesteronemic effect of oat silage in the adipose tissue of pigs might be presumably the result of the combination of soluble dietary fibres as well other bioactive components intake, as phenolic phytochemicals, benzoic acid and cinnamic derivates and lignans (Knud *et al.*, 2017), but this association would require further research.

5. Conclusions

Under organic husbandry, oat silage supplement did not affect growth performance and increased the exploratory behaviour of the pen fixtures in the outdoor run area. It was possible to produce entire male pigs without increasing the negative social interactions with other pen-mate males compared to gilt pens. Retinol concentration in adipose tissue reflected the dietary vitamin A precursor (β-carotene) level of oat silage. However, supplementary oat silage did not exert any influence on vitamin E (α-γ- and δ- tocopherols) and reduced the cholesterol contents in perirenal adipose tissue. Additional dietary silage supply needs to be further explored to enhance pork vitamin content.

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592

593 Table 1 Chemical composition of experimental concentrate and roughages (%, on a fresh weight basis (means
594 ± standard deviation).

	Concentrate	**Winter cereal straw**	**Oat Silage**
Dry matter, %	90.0 ± 0.10	91.5 ± 0.14	36.0 ± 3.18
Starch, %	45.8 ± 0.50	n.a.	n.a.
Crude protein, %[1]	13.5 ± 0.00	3.0 ± 0.14	8.1 ± 0.78
Crude fibre, %	4.6 ± 0.48	53.2 ± 0.18	40.9 ± 1.75
Neutral Detergent Fiber, %	n.a.	83.4 ± 0.57	71.9 ± 3.54
Acid Detergent Fiber, %	n.a.	62.7 ± 0.21	48.4 ± 2.05
Ether extract, %	5.9 ± 0.24	n.a.	n.a.
Ash, %	4.9 ± 0.15	5.2 ± 0.07	8.3 ± 0.92

595 [1] The concentrate provided the following essential aminoacid content (on a fresh weight basis: Lysine 0.78%,
596 Methionine 0.34%, Threonine 0.53%, Isoleucine 0.59%, Valine 0.70%, Leucine 1.05%, Histidine 0.37%,
597 Phenylalanine 0.72%)
598 n.a.: not analysed.

599

600 Table 2 Ethogram used in scan sampling recordings (adapted from Casal-Plana et al. (2017) and Fàbrega et al.
601 (2019))

Category	Definition
Activity	
Lying	Pig is recumbent on its belly or side
Walking	Pig is upright on all four legs, and moves in the pen
Standing inactive	Pig is upright on all four legs, neither moving forward or backward
Sitting inactive	Pig is upright on two front legs, and hindquarter (sitting in a dog position)
Behaviours	
Eat roughage	Pig eats silage or straw from the trough
Eat concentrate or drinking	Head or snout over bowl or feed hopper
Interaction with enrichment material	Chewing or nosing with straw bedding or snout over the trough (silage or straw)
Interaction with the pen	Licking, chewing, nosing or sniffing unanimated objects from the pen, excluding enrichment material
Positive social interactions	Head or snout in mild contact with another pig, positive social behaviour
Negative social interaction	Head or snout in aggressive contact with another pig, negative social behaviour
Inactive	Pig remains immobile, any other behaviour

602

603

604 Table 3 Performance[1] traits according to feeding strategy and sex.

	Feeding strategy (F)		Sex (S)		Level of significance	
Parameter	**Control**	**Silage**	**Entire male**	**Female**		
Number of pigs	46	47	48	45	**F**	**S**
Initial body-weight, kg	51.9 ± 0.97	51.7 ± 0.98	52.2 ± 0.97	51.5 ± 0.98	0.90	0.65
Body-weight at day 45, kg	86.5 ± 1.93	85.9 ± 1.92	85.1 ± 1.90	87.3 ± 1.96	0.44	0.81
Final body-weight at day 87, kg	125.8 ± 3.49	119.5 ± 3.51	120.0 ± 3.72	125.4 ± 3.26	0.27	0.21
ADG day 1-45 (50-85 kg), g	767 ± 26.1	760 ± 25.9	737 ± 25.6	790 ± 26.4	0.85	0.16
ADG day 46-87 (85-120 kg), g	1030 ± 59.6	897 ± 59.8	932 ± 63.5	995 ± 55.7	0.12	0.46
Overall ADG	863 ± 35.2	804 ± 35.3	796 ± 37.5	871 ± 32.8	0.24	0.14
Carcasses number	23	33	36	20		
Carcass weight (kg)	100.4 ± 2.09	97.1 ± 1.32	94.2 ± 1.27	103.2 ± 2.13	0.19	<0.001
Carcass dressing out (%)	77.9 ± 2.46	75.4 ± 1.58	75.03 ± 1.58	78.25 ± 2.34	0.43	0.27

[1]Values are presented as least square means ± standard error of the mean (SEM). The level of significance was set at 0.05. The interaction between F and S was not significant (P > 0.05).

ADG, average daily gain

605

606

607 Table 4 Time budget spent (%, proportion out of the 4-h recording period) in different activities and behaviours
608 by scan sampling as affected by location within the pen and the dietary roughage supplement

	Indoor			Outdoor		
	Control	**Silage**	**SE**	**Control**	**Silage**	**SE**
Activity						
Lying	74.0	72.4	2.36	68.5	62.8	2.36
Walking	18.7	21.9	2.26	22.5	25.1	2.26
Standing inactive	6.2	4.6	1.02	8.4	9.4	1.02
Sitting inactive	1.1	1.1	0.41	0.6	2.7	0.81
Behaviours						
Inactive	64.1	62.4	2.79	61.8	56.2	2.79
Interaction with enrichment material	14.3	15.9	1.80	2.1	1.4	1.80
Interaction with the pen fixtures	8.2	10.2	1.35	12.2^{a}	19.1^{b}	1.35
Eating roughage	5.1	6.1	1.88	0.2	0.1	0.12
Eating concentrate or drinking	1.3	0.7	0.44	18.8	18.3	1.83
Positive social interactions	6.3	4.6	0.73	4.8	4.2	0.73
Negative social interactions	0.5	0.1	0.16	0.1	0.7	0.22

609 The dietary roughage supplement did not affect any behaviour indoors, whereas outdoors, different superscript
610 letters (a,b) denotes statistical differences between treatments ($P < 0.05$).

611

612 Table 5 Carotenoid and tocopherol contents (µg/g dry DM) (means ± SD) of experimental concentrate and
613 roughages.

	Concentrate	Winter cereal straw	Oat Silage
Neoxanthin, µg/g DM	0.1 ± 0.01	n.d.	2.3 ± 0.06
Violaxanthin, µg/g DM	0.1 ± 0.01	n.d.	3.5 ± 0.05
Zeaxanthin, µg/g DM	0.5 ± 0.02	0.1 ± 0.00	0.7 ± 0.05
Lutein, µg/g DM	2.8 ± 0.07	0.5 ± 0.04	40.8 ± 0.49
All-E-β-carotene, µg/g DM	0.20 ± 0.00	n.d.	20.7 ± 0.30
α-tocopherol, µg/g DM	17.2 ± 0.34	2.4 ± 0.09	9.5 ± 0.22
γ-tocopherol, µg/g DM	16.1 ± 0.24	25.1 ± 0.28	6.2 ± 0.24
δ-tocopherol, µg/g DM	6.3 ± 0.18	1.9 ± 0.07	1.0 ± 0.08

614 n.d: carotenoids detected at levels <0.01 ± 0.001 µg/g DM). DM, dry matter.

615

616 Table 6 Tocopherols, retinol and cholesterol contents in perirenal fat according to feeding strategy and sex

	Feeding strategy (F)		Sex (S)		Level of significance	
Parameter	**Control**	**Silage**	**Male**	**Female**	**F**	**S**
n	23	33	36	20		
α-tocopherol, µg/g	10.6±0.58	11.4±0.41	11.0±0.41	11.0±0.64	0.32	0.96
γ-tocopherol, µg/g	1.03±0.054	1.08±0.038	1.10±0.039	1.01±0.060	0.49	0.24
δ-tocopherol, µg/g	0.11±0.006	0.12±0.004	0.12±0.004	0.11±0.006	0.32	0.43
Retinol, ng/g	461.1±12.07	506.4±8.57	475.6±8.65	491.9±13.38	0.004	0.35
Cholesterol, mg/g	0.52±0.018	0.47±0.013	0.50±0.013	0.49±0.020	0.04	0.59

[1]Values are presented as least square means ± standard error of the mean (SEM). The level of significance was set at 0.05. The interaction between F and S was not significant (P > 0.05).

617