Using dried orange pulp in the diet of dairy goats: effects on milk yield and composition and blood parameters of dams and growth performance and carcass quality of kids

J.L. Guzmán[1], A. Pérez-Écija[2], L.A. Zarazaga[1], A.I. Martín-García[3], A. Horcada[4], M. Delgado-Pertíñez[4†]

[1] Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería, Universidad de Huelva, "Campus de Excelencia Internacional Agroalimentario, ceiA3", Campus de la Rábida, 21819 Palos de la Frontera, Huelva, Spain

[2] Departamento de Medicina y Cirugía Animal, Universidad de Córdoba, Campus Rabanales, Córdoba, 14104, Spain

[3] Estación Experimental del Zaidín (CSIC), Profesor Albareda 1, 18008 Granada, Spain

[4] Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, Universidad de Sevilla, Ctra. Utrera km 1, 41013 Sevilla, Spain

[†] E-mail: pertinez@us.es

Short title

Using orange pulp in early lactation diet of goats

Abstract

Although dried orange pulp (DOP) may conveniently replace cereals in ruminant diets, few studies have considered similar diet substitution for goats. We hypothesised that DOP could replace cereal-based concentrate in goat diets without detrimental effects on growth performance and carcass quality of suckling kids and milk performance and blood biochemical parameters of dams in early lactation. We also hypothesised that

DOP substitution may increase the levels of antioxidants, such as phenolic compounds and vitamin E, in milk and improve its overall antioxidant capacity (TAC). Therefore, 44 primiparous Payoya dairy goats were allocated to three experimental groups, each fed a different diet: control (CD, n = 14) based on a commercial concentrate with alfalfa hay as forage; and DOP40 (n = 16) in which 40% and DOP80 (n = 14) in which 80% of the cereal in the concentrate was replaced by DOP. The experiment lasted from the final month of pregnancy to 55 days post-partum. The DOP diets did not affect suckling kids' carcass quality, but at 28 days, led to improvement in live weight (LW) and average daily gain (ADG) from birth, although no differences were found between DOP40 and DOP80 (for CD, DOP40 and DOP80, LW at 28 days was 8.00, 8.58 and 8.34 kg and ADG was 184, 199 and 195 g/d, respectively). Diet had no significant effect on milk yield (average daily milk yield and total yield at 55 days were 1.66 l/d and 90.6 l, respectively) and commercial and fatty acid composition. Nevertheless, α-tocopherol, total phenolic compound and TAC concentration in milk increased with substitution of cereals by DOP (for CD, DOP40 and DOP80, concentration of α-tocopherol was 21.7, 32.8 and 42.3 µg/100 g, total phenolic compounds was 63.5, 84.1 and 102 mg GA equivalents/l, and TAC was 6.63, 11.1 and 12.8 µmol Trolox equivalents/ml, respectively). Every plasma biochemistry parameter considered was within reference values for healthy goats; therefore, no pathological effect was detected for these variables due to dietary treatment. However, DOP diets caused a reduction in plasmatic creatine kinase and aspartate aminotransferase, implying reduced oxidative damage to muscles. In conclusion, DOP may be an interesting alternative to cereals in early-lactation goat diets for increasing farmers' income and the healthy antioxidant capacity of milk.

Keywords: Payoya breed, citrus by-product, fatty acids, phenolic compounds, fat-soluble vitamins.

Implications

Although dried orange pulp (DOP) may conveniently replace conventional cereals in ruminant diets, few studies have considered similar diet substitution for dairy goats. Our results show that DOP could replace cereals in goat diets without detrimental effects on suckling kid growth and dam milk performance and blood parameters. DOP increases phenolic and vitamin E content in milk. These findings may be used to improve goat production with the aim of increasing farmers' income and the healthy antioxidant capacity of milk.

Introduction

In recent decades, goat farms have specialised in milk production for the cheese industry, particularly in economically developed countries in Mediterranean Europe (Castel *et al.,* 2011). In the European Union, Spain has the second highest number of goats (3.1 million head), with Andalusia (in southern Spain) being the region with the largest herd (FAO, 2017). Although most of the goats in Spain are dairy breeds, such as Payoya, goat farms produce suckling kids (the preferred goat carcass) which are exclusively fed with milk from their mothers for a month until they are slaughtered at live weight (LW) of 8–10 kg (Guzmán *et al*., 2019). To achieve greater milk yields, farmers have increased the supply of concentrates fed to goats, thus reducing or even eliminating pasture as a feed source. The demand for cereals and other grains for animal feeding increases the price of concentrate (Castel *et al.,* 2011). This justifies the need to decrease the alimentation cost of livestock by developing strategies, such

77 as greater dependence on local feed resources, which would increase the
78 sustainability of livestock-production systems (López *et al*., 2014).
79 In 2017, Spain was the leading producer of oranges in Europe (3.4 million tonnes;
80 FAO, 2017). The orange juice industry produces organic waste such as dried orange
81 pellets that can be used for animal feeding as a source of dietary energy (Bampidis
82 and Robinson, 2006). Furthermore, citrus pulp can be a source of residual bioactive
83 molecules with antioxidant properties, including phenolic compounds (Santos *et al*.,
84 2014; Luciano *et al*., 2017) and vitamin E (Luciano *et al*., 2017).
85 Replacing cereal grain (Bueno *et al*., 2002; Oni *et al*., 2008) with dehydrated citrus pulp
86 (DCP) improved the post-weaning growth performance of kids. Nevertheless, research
87 on the effects of citrus by-products in the diet on the growth and carcass quality of
88 suckling kids is lacking. Further, studies involving the dietary replacement of starch
89 sources by DCP and its effect on milk yield and composition for dairy goats are scarce.
90 When cereal grain (López *et al*., 2014; Ibáñez *et al*., 2016) was completely replaced
91 by DCP in goat feed, the intake, digestibility of most nutrients, milk yield and
92 commercial composition were not affected. With respect to the milk fatty acid (FA)
93 profile of goat-fed orange pulp, only the study of Ibáñez *et al*. (2016) is known, and
94 their results showed that the FA profile was barely altered by the treatment.
95 Furthermore, the effects of DCP on milk antioxidants and blood metabolites are limited
96 to dairy cows, while no reports are available for dairy goats. In this respect, the addition
97 of DCP to cows' diets increased the total polyphenol and flavonoid concentration in
98 milk (Santos *et al*., 2014) and no significant differences were observed in most blood
99 parameters (Belibasakis and Tsirgogianni, 1996).
100 We hypothesised that the partial replacement of cereals by dehydrated orange pulp
101 (DOP) in the concentrated fraction of the dairy goat diet would not have detrimental

effects on milk production or composition, or the performance of suckling kids, and could even improve the antioxidant status of milk by increasing compounds such as phenolics and vitamin E. Therefore, this study evaluated the effects of replacement of cereal by DOP (as a natural antioxidant source) in the diet of dairy goats in early lactation on suckling kid growth performance and carcass quality and dam milk yield, milk quality and blood metabolites.

Materials and methods

Experimental diets, goats and chemical analyses of feed

This study was carried out at the experimental farm of the University of Huelva (Huelva, Spain). All goats included in the study were of the Payoya breed. All procedures were performed by trained personnel in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013).

Forty-four primiparous goats were assigned to three experimental groups, each with a different diet; the groups were balanced according to the goats' LW and body condition scores (BCS). The animals were housed in a communal pen and each experimental group was separated by metal barriers with free access to a yard. The three experimental diets were as follows: control (CD, n = 14), with a commercial concentrate plus alfalfa hay as forage; DOP40 (n = 16) based on CD, but with 40% of the cereals in the concentrate replaced by DOP; and DOP80 (n = 14), based on CD, with 80% of the cereals in the concentrate replaced by DOP. From the beginning of the third month of gestation, DOP was gradually introduced into the diet of the animals in the DOP40 and DOP80 groups. During the last month of pregnancy, each group of animals received its corresponding experimental prepartum-restricted diet (Supplementary Table S1), with a 60:40 concentrate to forage ratio. After parturition, the animals were

offered the experimental diets adapted to early lactation (Table 1), which consisted of a concentrate to forage ratio of 80:20. The formulation of the rations and the chemical composition of the isoenergetic and isoproteic diets are shown in Supplementary Table S1 and Table 1. The nannies followed these nutrition regimens, which were designed using the Feed Ration Balancer (Format Solutions) software, version 2.0 (2017; Cargill, Inc., USA; www.formatsolutions.com).

Goats were allowed ad libitum access to water and fed once daily in the mornings. After delivery, all goats received 0.4 kg of alfalfa hay daily and the amount of concentrate per animal increased every day, adjusting the amount supplied based on the real capability of consumption (from 0.70 kg/animal/day, after delivery, to 1.88 kg/animal/day at 55 days postpartum). Food intake for each group was calculated daily by subtracting the orts (uneaten food) from the amount of food offered every day. On average, goats were fed 0.4 kg of alfalfa hay and 1.67 kg (during the suckling period) or 1.88 kg (after weaning the kids) of concentrate per animal on a daily basis. After the weaning, the total average intake per goat in the diet groups was determined (DM was 2.04, 2.00 and 1.98 kg/d, crude protein was 0.36, 0.33 and 0.36 kg/d, and gross energy was 9.28, 8.90 and 8.74 Mcal/d in the CD, DOP40 and DOP80 groups, respectively). After weaning, and coinciding with the test-day milk yield recordings (at 45 ±5 days and at 55 ±5 days post-partum), LW and BCS were recorded for all animals. The BCS was always tested by the same handler.

A sample of hay and concentrates were prepared for analysis by mixing equal amounts of five subsamples collected during the trial and storing at −4°C. The samples were dried and ground using a Wiley mill with a 1-mm screen before analysis. AOAC (2005) methods were used to determine dry matter (method 934.01), ash (method 942.05), ether extract (method 920.39), and N (method 984.13) content in the samples of hay

and concentrates. Total N was determined by the Kjeldahl procedure, and converted to crude protein (CP) by multiplying by a factor of 6.25. The analyses of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were carried out according to Van Soest *et al*. (1991). Amylase was not used in the analysis of NDF, and both NDF and ADF were expressed as exclusive of residual ash. Acid detergent lignin (ADL) was determined through solubilisation of cellulose with 72% sulphuric acid. All fibre fractions were analysed with a Fibertec 1030 Hot Extractor (Tecator, Sweden). Fat content was measured by extraction with petroleum ether (boiling point, 40–60°C) on a Soxtec System 1040 Extraction Unit (FOSS Tecator AB, Sweden). Calcium and phosphorus content was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES Jobin Yvon Ultima 2, Longjumeau, France), according to the manufacturer's instructions. The gross energy (GE) content of diets was determined by using an adiabatic calorimeter (model AC600, LECO Corporation, St. Joseph, MI, USA), according to the manufacturer's instructions. Sugar, starch, the forage unit for lactation (UFL) and protein digestible in the small intestine (PDI) were calculated using Feed Ration Balancer software. Total antioxidant capacity (TAC) was analysed by the DPPH assay (2,2-diphenyl-l-picrylhydrazyl) as described by Shin *et al*. (2018). The water-soluble vitamin E analogue Trolox was used as a standard for antioxidant capacity, and TAC was expressed as mmol Trolox equivalents/kg of sample. Total phenolic compound (TPC) content was estimated in methanol-acetone extracts from feedstuffs and a subsequent analysis with Folin–Ciocalteu reagent, according to the procedure described by Seiquer *et al.* (2015), with some modifications. Standard solutions of gallic acid (GA) were used to express the phenolic compounds as g of GA equivalents/kg of DM. The procedures described by Delgado-Pertíñez *et al*. (2013) and Gutiérrez-Peña *et al*. (2018) were used for measurement of FA composition (absolute

177 values expressed as mg/g DM) and vitamin E (α-tocopherol) content in lactation diets
178 (expressed as mg/kg DM). The details for TAC, TPC, FA and vitamin E analyses are
179 given in Supplementary Material S1, S2, S3 and S4, respectively.
180
181 *Growth performance of kids*
182 After parturition, 63 kids of both sexes and goat prolificacy (single or double birth) were
183 individually analysed in the study (n = 20 each for CD and DOP80 groups; n = 23 for
184 DOP40 group). Kids remained with their dams from birth to weaning (at 28 days), with
185 free constant access to mothers' milk. The live weight of kids was recorded at 0, 14
186 and 28 days after birth for all groups, and the average daily gain (ADG) was calculated.
187 .
188 *Slaughter and carcass quality*
189 Thirty male suckling kids were used for the carcass study. Ten male suckling kids from
190 each experimental group were selected and slaughtered at an average weight of 8.90
191 (± 0.17) kg and 32 (± 1.5) days old. All kids were only fed with maternal milk until
192 slaughter. The chemical composition and energy content of mother's milk are shown
193 in Table 2.
194 After the kids reached their target commercial LW, the farm LW (FLW) was recorded
195 on the day prior to slaughter. Suckling kids were sacrificed at the slaughterhouse, after
196 13.5 ± 0.02 h of fasting with free access to water, using standard commercial
197 procedures in accordance with the guidelines of EU Directive 2010/63/EU for the
198 protection of animals used for experimental and other scientific purposes. Slaughter
199 LW (SLW) was recorded immediately prior to slaughter. The tail, kidneys, pelvic fat,
200 and testes were retained in the carcass, and the hot carcass weight (HCW) was
201 recorded immediately after slaughter. The carcasses were kept in a chilling room at

4°C for 24 h. After chilling, the cold carcass weight (CCW) was recorded. The carcass dressing percentage, chilling losses, carcass linear measurements and the indices from these carcass conformation measurements were calculated as described in Guzmán *et al*. (2019). After chilling, the carcasses were split down the dorsal midline, and then the left half of each carcass was removed, weighed and transported under refrigeration to the University of Huelva. The carcass was then physically dissected into five prime cuts (shoulder, flank, neck, ribs and long leg). The prime cuts were weighed and grouped into three categories: extra (long leg and ribs), first (shoulder) and second (neck and flank). Shoulder tissue composition was evaluated according to Guzmán *et al*. (2019). The muscle/bone and muscle/fat indices were calculated. The details of carcass measurements are given in Supplementary Material S5.

Milk sample collection and chemical analyses

After weaning the kids, the dams were milked once a day (at 09:00) in a 12-stall Casse system milking parlour; individual recorder jars were used to measure the volume of milk produced. A repeated measures design was used for recording milk yield from each animal; test-day yields were recorded at 45 ±5 days and 55 ±5 days post-partum, and the total production in early lactation (from birth to 55 days post-partum) was calculated according to the A4 method of the International Committee for Animal Recording (ICAR). The following formula was used to compute milk yield (MY) for the lactation record:

$$MY = I0M1 + I1*[(M1+M2)]/2,$$

where M1 and M2 are the volumes (in litres) of milk yielded in the 24 hours of the recording day, I0 is the interval, in days, between the lactation period start date and the first recording date, and I1 is the interval, in days, between recording dates.

During the suckling period, representative samples (aliquots from each animal for milk traits and TAC analyses) were taken between the 3rd and 4th week post-partum. After 45 days post-partum, representative samples (aliquots from each animal for milk traits, TAC, TPC, FA and fat-soluble vitamin analyses) were taken from the volumetric flask of the parlour during machine milking carried out at the control. The aliquots were placed in 50 ml plastic bottles and wrapped with aluminium foil to protect them from light. Azidiol was used as a preservative in one of the samples used to analyse commercial traits. Samples were deposited in iceboxes, sent to the laboratory and frozen at −20ºC until analysis, except for the samples for vitamin analysis which were frozen at −80ºC.

Milk traits (dry matter, protein, fat and lactose content) were estimated by near-infrared spectroscopy (NIR) according to Delgado-Pertíñez *et al*. (2013). The procedures described by Delgado-Pertíñez *et al*. (2013) and Gutiérrez-Peña *et al*. (2018) were used to measure FA composition (absolute values expressed as mg/g DM); the intra- and inter-assay coefficients of variation (CVs) for this analysis were less than 7% and 10%, respectively. Fat extraction and direct methylation of FAs were performed in a single-step procedure based on the method by Sukhija and Palmquist (1988) and revised by Juárez *et al*. (2008). Fat-soluble vitamin (A and E) analyses (expressed as µg/100 g) were based on the methods by Herrero-Barbudo *et al*. (2005) and Chauveau-Duriot *et al*. (2010) and modified by Gutiérrez-Peña *et al*. (2018); the intra- and inter-assay CVs were less than 3% and 6%, respectively. TAC was analysed by the ABTS method (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) of Pellegrini *et al*. (1999) and modified by Delgado-Pertíñez *et al*. (2013). TAC was expressed as mmol vitamin E analogue Trolox equivalents/ml of sample; the intra- and inter-assay CVs were less than 4% and 6%, respectively. TPC were quantified

252 according to the procedure described by Vázquez *et al* (2015) using the Folin-
253 Ciocalteu method, with some modifications. The values were reported in mg of GA
254 equivalents/l; the intra- and inter-assay CVs were less than 4% and 7%, respectively.
255 The details for the analyses of NIR, FA, fat-soluble vitamins, TAC and TPC are given
256 in Supplementary Material S6, S3, S4, S7 and S8, respectively.
257
258 *Blood analyses*
259 Biochemical analysis was performed during the last month of pregnancy and at 45 ± 5
260 days post-partum. Blood was collected aseptically from the left jugular vein in fasting
261 animals in the morning and poured into tubes containing lithium heparin. After
262 centrifugation at 3500 rpm for 30 minutes, the plasma was kept frozen at −20ºC until
263 analysis. Every parameter was measured in an automated spectrophotometer
264 (Biosystems A25, Biosystems, Barcelona, Spain) using specific kits previously
265 validated for small ruminants (Biosystems, Barcelona, Spain), according to the
266 manufacturer's instructions. The intra- and inter-assay CVs for all blood parameters
267 were less than 3.5% and 6%, respectively.
268
269 *Data treatment and statistical analysis*
270 The data for the kids' growth were analysed using factorial ANOVA and a general linear
271 model (GLM), with birth weight as a covariate and dietary treatment (CD, DOP40 or
272 DOP80), sex and the interactions between these factors as fixed effects. The carcass
273 parameters were analysed using a one-way ANOVA model to test the effect of dietary
274 treatment. The data for milk composition during the suckling period were analysed
275 using a factorial model, with dietary treatment and goat prolificacy (single or double
276 birth) and the interactions between these factors as fixed effects. The goats' weight,

277 BCSs, commercial milk traits and daily milk yield recorded at 45 ± 5 days and at 55 ±
278 5 days post-partum were analysed with the repeated measures procedure. The model
279 included the fixed between-subjects factors dietary treatment and prolificacy, the fixed
280 within-subjects factor week of lactation (repeated measures), and the interactions
281 between these factors. For simplification, the results for the factor week of lactation in
282 both repeated measures analyses have not been presented in this paper. The data for
283 total milk yield from birth to 55 days post-partum and composition parameters of milk
284 samples taken at 55 ± 5 days post-partum (FA, vitamins, TAC and TPC) were analysed
285 using a factorial model which included dietary treatment and prolificacy and the
286 interactions between these factors as fixed effects. Plasma biochemical parameters at
287 pregnancy and at 45 ± 5 days post-partum were analysed using a factorial model which
288 included dietary treatment and prolificacy and the interactions between these factors
289 as fixed effects.
290 In all the statistical analyses, the individual kids or goats were considered experimental
291 units and the significance level was set at $P \leq 0.05$. Tukey's honestly significant
292 difference (HSD) test was used where appropriate for pairwise comparisons of means.
293 The statistical analyses were performed using IBM SPSS Statistics for Windows
294 (version 25.0; IBM Corp., Armonk, NY, USA).
295
296 **Results**
297 *Growth performance and carcass quality of kids*
298 No significant effects of diet treatment on milk components and metabolisable energy
299 during the suckling period were observed, except for CP ($P < 0.01$) and TAC ($P <$
300 0.001; Table 2). CP content was significantly higher in the milk of the DOP40 group
301 (4.84%) than in the CD (4.02%) and DOP80 (3.94%) groups. Furthermore, TAC in the

302 milk increased as the percentage substitution of cereals by DOP increased (7.25, 9.38
303 and 13.1 ± 0.49 µmol Trolox® equivalents/ml in CD, DOP40 and DOP80 treatments,
304 respectively).
305 Diet treatment had a significant effect on the LW of kids at 28 days and on the postnatal
306 growth rate from birth to 28 days (Table 3). The CD treatment showed lower values for
307 both LW at 28 days and ADG from birth to 28 days ($P < 0.05$), but no differences were
308 found between DOP40 and DOP80 treatments (LW at 28 days was 8.00, 8.58 and
309 8.34 ± 0.20 kg and ADG from birth to 28 days was 184, 199 and 195 ± 4.3 g in CD,
310 DOP40 and DOP80, respectively). Finally, replacement of cereals by DOP did not
311 influence carcass weight and quality ($P > 0.05$; Supplementary Tables S2 and S3).
312
313 *Yield and composition of milk in early lactation*
314 The milk production and composition and the LW and BCS of goats are presented in
315 Table 4. LW and BCS were similar for all three diets (38.9 kg and 2.67 on average,
316 respectively). Although milk yield was numerically higher in the DOP diet groups than
317 in CD, this difference was not statistically significant (average daily milk yield and total
318 yield at 55 days were 1.66 l/d and 90.6 l, respectively). Similar results were obtained
319 for commercial composition, with the exception of DM ($P < 0.05$). Milk DM percentage
320 differed between DOP40 (12.4%) and DOP80 (11.9%), while it was an intermediate
321 value for CD (12.2%) which did not differ significantly from the values for the DOP
322 diets. The level of α-tocopherol ($P < 0.01$), TPC ($P < 0.001$) and TAC ($P < 0.001$) in
323 the milk increased as the rate of substitution of cereals by DOP increased (the
324 concentration of α-tocopherol was 21.7, 32.8 and 42.3 ± 2.99 µg/100 g, TPC was 63.5,
325 84.1 and 102.4 ± 2.94 mg GA equivalents/l, and TAC was 6.63, 11.1 and 12.8 ± 0.53
326 µmol Trolox® equivalents/ml in CD, DOP40 and DOP80 treatments, respectively).

The individual FA concentrations and the sum of FAs (mg/g DM) are shown in Supplementary Table S4. The FA profile was not changed by different diets for any of the variables examined ($P > 0.05$).

Clinical blood parameters

Biochemistry results showed no significant differences in most blood parameters between the diet groups (Table 5). However, both DOP40 and DOP80 showed significantly lower ($P < 0.05$) plasma glucose compared to CD (a 21% and 19% reduction, respectively, at early lactation, and a 16% reduction for both groups at the end of gestation). Creatine kinase (CK) and aspartate aminotransferase (AST) values were significantly lower ($P < 0.05$) in the DOP40 and DOP80 groups compared to those in CD at the end of pregnancy (a 50% and 31% reduction for CK and a 31% and 24% reduction for AST, respectively). The total calcium concentration during early lactation also showed significant differences ($P < 0.05$) between groups, with higher values in DOP80 compared to CD and DOP40. Magnesium levels were significantly different ($P < 0.05$) between groups at the end of pregnancy, with the highest level in CD and the lowest in DOP80.

Discussion

Growth performance and carcass traits of kids

DOP can replace cereals in the diet of nannies, without depressing the growth performance and carcass quality of their suckling kids, as well as improving ADG and LW at 28 days of life. Growth essentially depends on ingested energy; nevertheless, in this study was not evaluated. Although no significant effects of diet treatment on milk components and metabolisable energy were observed, the incorporation of DOP into

the diet could be expected to influence energy intake. Indeed, milk production in DOP diets is about 10% higher (although not significant; see Table 4) than in CD goats, which is the same range as the difference in kids' ADG, and could explain our results, at least partially. Furthermore, and in light of the results for the antioxidant status parameters measured in milk during the suckling period (Table 2) and after weaning (Table 4), the kids' growth might also be attributable to antioxidants and redox homeostasis (McGrath *et al*., 2018). Antioxidant supplementation may help to counter negative effects of oxidative stress associated with growth in young ruminants, reducing the incidence of morbidity and mortality, and ultimately improving their performance (McGrath *et al*., 2018). In this study, partial replacement of cereals by DOP in the goats' feed increased TAC, TPC and α-tocopherol in the diet (see Table 1) and, consequently, in milk (see Table 4). To our knowledge, no previous studies have reported the effect of feeding DOP to small ruminants on the concentration of these antioxidants in their milk. However, the addition of 9% and 18% of pelleted citrus pulp to normal diets increases the total polyphenol and flavonoid concentration, and ferric reducing antioxidant power in cows' milk (Santos *et al*., 2014). Vitamin E has been extensively demonstrated to play a major role in protecting animal tissue against lipid peroxidation. Citrus phenolic compounds, mainly flavonoids, possess antioxidant activity, and their intake with the diet has been suggested to increase the antioxidant capacity of animal tissue. However, the possible mechanisms for the antioxidant effects are not clear (Luciano *et al*., 2017). Indeed, Luciano *et al*. (2017) reported that despite the fact that flavonoids are generally considered the main antioxidants in citrus fruits, α-tocopherol plays the major role in improving antioxidant capacity of muscle of lambs fed diets supplemented with DCP. Further research should undoubtedly clarify the antioxidant effect of dietary orange pulp in ruminants.

377

378 *Milk yield and composition*

379 In this study, individual intake was not measured, but the average intake per animal
380 obtained indirectly through the consumption of the group shows similar values in the
381 different dietary treatments. Similarly, in López *et al*. (2014), when total cereal (corn)
382 was replaced by DCP for lactating Murciano-Granadina goats, the intake and apparent
383 total tract digestibility of most nutrients were not affected. Incorporation of DCP (about
384 20% DM) in diets of dairy cows often depresses dry matter intake (DMI; Solomon *et*
385 *al*., 2000; Santos *et al*., 2014), probably due to the increased rumen fill by hydration of
386 DCP and the slightly lower rumen degradation rate of pectin than that of starch (Santos
387 *et al*., 2014). Studies on inclusion of DCP to replace cereals in lactating dairy ewe diets
388 did not report a reduction in DMI (Fegeros *et al*., 1995; Santos-Silva *et al*., 2016),
389 suggesting that the response of ewes and goats may be different from that of dairy
390 cows. The lack of an effect of diet on LW and BCS and the similar intake values
391 observed in the dietary treatments may be consistent with the lack of change observed
392 in milk yield and commercial traits in the present study, as was also reported previously
393 for DCP (López *et al*., 2014; Ibáñez *et al*., 2016).

394 With respect to the FA profile, Ibáñez *et al. (*2016) found that when barley was fully
395 replaced by DCP for goats in mid-lactation, there was no effect on FAs with 4 to 15
396 carbon atoms, but there were significant effects on other FAs. Ibáñez *et al.* (2016)
397 specifically highlighted the lower C15:0 and higher C17:0 FA content in the milk of
398 goats fed DCP, suggesting a negative impact on rumen bacterial fermentation activity
399 and fat mobilisation. However, in the present study, the FA composition of milk was
400 not affected by replacement of cereals by DOP in the diet; these results are similar to

401 those obtained by Fegeros *et al*. (1995) for lactating sheep fed concentrate rations with
402 30% DCP.
403 As discussed above, DOP antioxidants in the present study were transferred from the
404 diet to the milk, as previously reported by Santos *et al*. (2014) for cows fed citrus pulp.
405 The result for α-tocopherol is rather unexpected, as there are no data currently for the
406 effects of feeding DOP to ruminants on the concentration of α-tocopherol in milk and
407 other dairy products. From a global perspective, our results are very promising. In
408 recent years, food containing natural antioxidants have become popular all over the
409 world as antioxidants can neutralise and scavenge free radicals and reduce their
410 harmful effects, which are continuously produced in the body (Khan *et al*., 2019).

411

412 *Clinical blood parameters*

413 All the values determined for plasma biochemistry were within reported reference
414 values for healthy goats (Tschuor *et al*., 2008). Contrary to previous reports on dairy
415 cows (Belibasakis and Tsirgogianni, 1996), dietary citrus pulp supplementation did not
416 cause changes in the plasma levels of cholesterol in goats. Both DOP diets caused a
417 significant decrease in plasma glucose compared to the CD group, as previously
418 observed for calves fed with citrus by-products (Alnaimy *et al*., 2017). Although
419 improved insulin sensitivity has been reported as a general benefit of diets containing
420 *Citrus* spp. (Flores-Fernández *et al*., 2017), our lack of data on insulin concentrations
421 makes further studies necessary to elucidate the importance of this finding.
422 Interestingly, both CK and AST were significantly lower in supplemented groups at the
423 end of pregnancy. Both enzymes are related to muscular damage and oxidative
424 muscular stress (Brancaccio *et al*., 2010), which implies a beneficial impact of DOP
425 supplementation under those conditions. The higher total calcium found during early

lactation in the DOP80 group may be related to the effect of calcium oxide addition during citrus pellet preparation. Finally, magnesium levels also differed between groups at the end of pregnancy, although a clear relationship between DOP content in the diet and the plasma levels of magnesium ions does not seem straightforward.

Conclusions

Our data suggest that DOP can be an interesting alternative to cereals in the concentrate fraction of diets for dairy goats in early lactation. Replacement of 40% and 80% of cereals by DOP in the diet of Payoya dairy goats modifies growth performance of suckling kids, leading to an improvement in LW at 28 days and ADG from birth to 28 days, although no differences were found between the DOP40 and DOP80 treatments. The levels of α-tocopherol, TPC and TAC in milk increased as the percentage of pelleted orange pulp replacing cereals increased. DOP also caused lower plasmatic levels of CK and AST, which implies reduced muscular oxidative damage. Furthermore, DOP can be used as a cereal grain substitute without negatively affecting the carcass quality of suckling kids and the milk performance and health status of dairy goats.

Acknowledgments

The authors express special thanks to the Excma. Diputación Provincial de Huelva for financial support through the Integrated Project 'Aprovechamiento de Subproductos de las Empresas Agroalimentarias para Alimentación del Ganado'. The authors also thank Cítricos del Andévalo, S.A. (J. García Carrión) for supplying the orange pellets, OVIPOR, Soc. Coop. And. for their contribution to the preparation of diets, and the Servicio General de Investigación Agraria (Universidad de Sevilla) and the Laboratorio

Agroalimentario de Sevilla (Junta de Andalucía) for technical assistance with laboratory analysis.

Declaration of interest

The authors declare no conflict of interest.

Ethics statement

All experiments performed followed Spanish standards for the protection of animals used for scientific purposes. The procedures applied were supervised by the Bioethics Committee of the University of Huelva (Spain).

Software and data repository resources

None of the data were deposited in an official repository.

References

AOAC 2005. Official methods of analysis of AOAC International, 18th ed. AOAC International Gaithersburg, MD, USA, Official Method 2005.08.

Alnaimy A, Gad AE, Mustafa MM, Atta MAA and Basuony HAM 2017. Using of citrus by-products in farm animals feeding. Open Access Journal of Science 1, 58–67.

Bampidis VA and Robinson PH 2006. Citrus by-products as ruminant feeds: a review. Animal Feed Science and Technology 128, 175–217.

Belibasakis NG and Tsirgogianni D 1996. Effects of dried citrus pulp on milk yield, milk composition and blood components of dairy cows. Animal Feed Science and Technology 60, 87–92.

Brancaccio P, Lippi G and Maffulli N 2010. Biochemical markers of muscular damage. Clinical Chemistry and Laboratory Medicine 48, 757–67.

Bueno MS, Ferrari E, Bianchini D, Leinz FF and Rodrigues CFC 2002. Effect of replacing corn with dehydrated citrus pulp in diets of growing kids. Small Ruminant Research 46, 179–185.

Castel JM, Mena Y, Ruiz FA, Camuñez-Ruiz J and Sánchez-Rodríguez M 2011. Changes occurring in dairy goat production systems in less favoured areas of Spain. Small Ruminant Research 96, 83–92.

Chauveau-Duriot B, Doreau M, Nozière P and Graulet B 2010. Simultaneous quantification of carotenoids, retinol, and tocopherols in forages, bovine plasma, and milk: validation of a novel UPLC method. Analytical and Bioanalytical Chemistry 397, 777–790.

Delgado-Pertíñez M, Gutiérrez-Peña R, Mena Y, Fernández-Cabanás VM and Laberye D 2013. Milk production, fatty acid composition and vitamin E content of Payoya goats according to grazing level in summer on Mediterranean shrublands. Small Ruminant Research 114, 167–175.

FAO 2017. FAOSTAT Data. http://www.fao.org/faostat/en/#data. Retrieved on 4 September 2019.

Fegeros K, Zervas G, Stamouli S and Apostolaki E 1995. Nutritive value of dried citrus pulp and its effect on milk yield and milk composition of lactating ewes. Journal of Dairy Science 78, 1116–1121.

Flores-Fernández JM, Barragán-Álvarez CP, Díaz-Martínez NE, Villanueva-Rodríguez S and Padilla-Camberos E 2017. *In vitro* and *in vivo* postprandial glycemic activity of *Citrus limetta* peel flour. Pharmacognosy Magazine 13, 613–616.

Gutiérrez-Peña R, Fernández-Cabanás VM, Mena Y and Delgado-Pertíñez M 2018. Fatty acid profile and vitamins A and E contents of milk in goat farms under Mediterranean wood pastures as affected by grazing conditions and seasons. Journal of Food Composition and Analysis 72, 122–131.

Guzmán JL, de la Vega F, Zarazaga LA, Argüello A and Delgado-Pertíñez M 2019. Carcass Characteristics and Meat Quality of Conventionally and Organically Reared Suckling Dairy Goat Kids of the Payoya Breed. Annals of Animal Science 19, 1143–1159.

505 Herrero-Barbudo MC, Granado-Lorencio F, Blanco-Navarro I and Olmedilla-Alonso B. 2005.
506 Retinol, α-and γ-tocopherol and carotenoids in natural and vitamin A- and E-fortified
507 dairy products commercialized in Spain. International Dairy Journal 15, 521–526.
508 Ibáñez C, Criscioni P, Arriaga H, Merino P, Espinós FJ, Fernández C 2016. Murciano-
509 Granadina goat performance and methane emission after replacing barley grain with
510 fibrous by-products. PLoS ONE 11, 1–21.
511 Juárez M, Polvillo O, Contò M, Ficco A, Ballico S and Failla S 2008. Comparison of four
512 extraction/methylation analytical methods to measure fatty acid composition by gas
513 chromatography in meat. Journal of Chromatography A, 1190, 327–332.
514 Khan IT, Nadeem M, Imran M, Ullah R, Ajmal M and Jaspal MH 2019. Antioxidant properties
515 of Milk and dairy products: a comprehensive review of the current knowledge. Lipids in
516 Health and Disease 18, 41–53.
517 López MC, Estellés F, Moya VJ and Fernández C 2014. Use of dry citrus pulp or soybean hulls
518 as a replacement for corn grain in energy and nitrogen partitioning, methane emissions,
519 and milk performance in lactating Murciano-Granadina goats. Journal of Dairy Science
520 97, 7821–7832.
521 Luciano G, Roscini V, Mattioli S, Ruggeri S, Gravador RS, Natalello A, Lanza M, De Angelis A
522 and Priolo A 2017. Vitamin E is the major contributor to the antioxidant capacity in
523 lambs fed whole dried citrus pulp. Animal 11, 411–417.
524 McGrath J, Duval SM, Tamassia LFM, Kindermann M, Stemmler RT, de Gouvea VN, Acedo
525 TS, Immig I, Williams SN and Celi P 2018. Nutritional strategies in ruminants: A lifetime
526 approach. Research in Veterinary Science 116, 28–39.
527 Nsahlai IV, Goetsch AL, Luo J, Johnson ZB, Moore JE, Sahlu T, Ferrell CL, Galyean ML and
528 Owens FN 2004. Metabolizable energy requirements of lactating goats. Small
529 Ruminant Research 53, 253–273.
530 Oni AO, Onwuka CFI, Oduguwa OO, Onifade OS and Arigbede OM 2008. Utilization of citrus
531 pulp based diets and *Enterolobium cyclocarpum* (JACQ. GRISEB) foliage by West
532 African dwarf goats. Livestock Science 117, 184–191.

533 Pellegrini N, Ke R, Yang M and Rice-Evans C 1999. Screening of dietary carotenoids and
534 carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-azinobis(3-
535 ethylenebenzothiazoline-6-sulfonic acid) radical cation decolourisation assay. Methods
536 in Enzymolology 299, 379–389.
537 Santos GT, Lima LS, Schogor ALB, Romero JV, De Marchi FE, Grande PA, Santos NW,
538 Santos FS and Kazama R 2014. Citrus pulp as a dietary source of antioxidants for
539 lactating Holstein cows fed highly polyunsaturated fatty acid diets. Asian-Australasian
540 Journal of Animal Sciences 27, 1104–1113.
541 Santos-Silva J, Dentinho MT, Francisco A, Portugal AP, Belo AT, Martins APL, Alves SP and
542 Bessa RJB 2016. Replacing cereals with dehydrated citrus pulp in a soybean oil
543 supplemented diet increases vaccenic and rumenic acids in ewe milk. Journal of Dairy
544 Science 99, 1173–1182.
545 Seiquer I, Rueda A, Olalla M and Cabrera-Vique C 2015. Assessing the bioavailability of
546 polyphenols and antioxidant properties of extra virgin argan oil by simulated digestion
547 and Caco-2 cell assays. Comparative study with extra virgin olive oil. Food Chemistry
548 188, 496–503.
549 Shin EC, Shurson GC and Gallaher DD 2018. Antioxidant capacity and phytochemical content
550 of 16 sources of corn distillers dried grains with solubles (DDGS). Animal Nutrition 4,
551 435–441.
552 Solomon R, Chase LE, Ben-Ghedalia D and Bauman DE 2000. The effect of nonstructural
553 carbohydrate and addition of full fat extruded soybeans on the concentration of
554 conjugated linoleic acid in the milk fat of dairy cows. Journal of Dairy Science 83,1322–
555 1329.
556 Sukhija PS and Palmquist DL 1988. Rapid method for determination of total fatty acid content
557 and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry
558 36, 1202–1206.

559 Tschuor AC, Riond B, Braun U and Lutz H 2008. Hämatologische und klinisch-chemische
560 Referenzwerte für adulte Ziegen und Schafe. Schweizer Archiv für Tierheilkunde, 150,
561 287–295.
562 Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent
563 fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy
564 Science 74, 3583–3597.
565 Vázquez CV, Rojas MG, Ramirez CA, Chávez-Servin JL, Garcia-Gasca T, Ferriz-Martinez RA,
566 Garcia OP, Rosado JL, Lopez-Sabater CM, Castellote AI, Montemayor HM and de la
567 Torre CK 2015. Total phenolic compounds in milk from different species. Design of an
568 extraction technique for quantification using the Folin-Ciocalteu method. Food
569 Chemistry 176, 480–486.
570
571

Table 1. Ration ingredients and proximate and fatty acid (FA) composition of the experimental diets used to feed goats during early lactation.

Items	Early lactation experimental diets[1]		
	Control	DOP40	DOP80
Ration ingredients, % DM basis			
Alfalfa hay	17.4	17.5	17.6
Concentrate			
Dehydrated orange pulp (pellets)	0	20.0	40.0
Grain oats	22.1	13.2	4.38
Grain barley	8.53	5.11	1.70
Grain corn	19.3	11.6	3.89
Soy flour, 44%	7.31	10.2	13.0
Sunflower pellets, 28%	12.8	12.5	13.8
Grain peas	10.3	8.12	4.05
Salt	0.41	0.41	0.41
Stabilised lard	0.41	0.00	0.00
Vitamins and minerals[2]	1.31	1.31	1.32
Proximate composition and nutritive value, % DM			
DM, %	90.5	89.8	90.0
Crude protein	17.8	16.6	18.4
Neutral detergent fibre	24.3	22.6	25.4
Acid detergent fibre	12.3	15.9	19.0
Acid detergent lignin	4.14	4.19	5.45
Sugar and starch	39.3	31.4	22.4
Ether extract	3.42	2.31	1.71
Ash	4.82	6.44	7.50
Calcium	0.42	0.80	1.02

Phosphorus	0.39	0.34	0.37
Gross energy, kcal/g DM	4.56	4.44	4.42
Forage unit for lactation, UFL/kg	0.98	0.98	0.97
Protein digestible in the intestine (PDI)	10.5	11.0	11.5
Total phenolic compounds, g/kg DM	5.29	6.74	8.57
TAC[3], mmol Trolox® equivalents/kg DM	14.9	20.4	26.3
α-tocopherol, mg/kg DM	3.18	26.5	70.1
FA composition[4], mg/g DM			
C8:0–C14	1.54	3.12	4.54
C16:0	31.4	23.6	26.3
C16:1	1.04	0.63	0.62
C18:0	11.8	9.70	10.7
C18:1 n-9 *cis*	34.1	23.3	19.3
C18:2 n-6 *cis*	48.3	34.8	28.2
C18:3 n-6	0.18	0.18	0.44
C18:3 n-3	2.92	3.81	4.12
∑SFA	44.7	36.4	41.5
∑MUFA	35.1	24.0	19.9
∑PUFA	51.4	38.8	32.8
∑n-6	48.5	35.0	28.7
∑n-3	2.92	3.81	4.12
n6/n3	16.6	9.17	6.97

[1]Control, diet based on commercial concentrates plus alfalfa hay; DOP40, diet based on concentrate with 40% of cereals replaced by dehydrated orange pulp (DOP) plus alfalfa hay; DOP80, diet based on concentrate with 80% of cereals replaced by DOP plus alfalfa hay.

[2]Nutral cabras LD granulado, Cargill®, Spain.

[3]TAC, Total antioxidant capacity by DPPH (2,2-diphenyl-l-picrylhydrazyl) assay.

[4]SFA: saturated FAs; MUFA: monounsaturated FAs; PUFA: polyunsaturated FAs.

Table 2. Effects of experimental diets[1] and goat prolificacy on the chemical composition of milk during the natural suckling period.

Item	Diet (D)[1]			Prolificacy (PR)		SEM	*P*[2]	
	Control	DOP40	DOP80	Single	Twins		D	PR
No. of goats	12	12	12	17	19			
Dry matter, %	12.4	12.2	12.2	12.1	12.3	0.32	ns	ns
Crude protein, %	4.02[b]	4.84[a]	3.94[b]	4.58	3.99	0.14	**	*
Fat, %	4.92	5.09	4.29	4.77	4.76	0.21	ns	ns
Lactose, %	5.63	5.54	5.35	5.50	5.51	0.16	ns	ns
TAC[3], µmol Trolox® equivalents/ml	7.25[c]	9.38[b]	13.1[a]	9.25	10.5	0.49	***	ns
ME[4], MJ/kg	3.45	3.52	3.20	3.39	3.39	0.08	ns	ns

Means with different letters (a, b, c) within each row differ significantly ($P \leq 0.05$).

[1]Control, diet based on commercial concentrates plus alfalfa hay; DOP40, diet based on concentrate with 40% of cereals replaced by dehydrated orange pulp (DOP) plus alfalfa hay; DOP80, diet based on concentrate with 80% of cereals replaced by DOP plus alfalfa hay.

[2]Statistical probability for comparisons: ns, not significant ($P > 0.05$); *, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$. No significant interactions between these factors were noted ($P > 0.05$).

[3]TAC, total antioxidant capacity by ABTS (2,2′-azinobis[3-ethylenbenzothiazoline-6-sulphonic acid]) assay.

[4]The metabolisable energy (ME) concentration was estimated according to Nsahlai *et al*. (2004).

Table 3. Effects of the experimental diet[1] of goats and kids' sex on the growth performance of naturally suckling kids.

Item	Diet (D)[1]			Sex (S)		SEM	*P*[2]	
	Control	DOP40	DOP80	Female	Male		D	S
No. of kids	20	23	20	33	30			
Live weight, kg								
Birth	2.84	3.06	2.95	2.92	2.99	0.11	ns	ns
14 d	5.56	5.80	5.72	5.45	5.97	0.14	ns	***
28 d	8.00^{b}	8.58^{a}	8.34^{a}	7.93	8.76	0.20	*	***
Average daily gain, g/d								
Birth–14 d	194	196	198	181	213	4.8	ns	***
14–28 d	174^{b}	206^{a}	194^{ab}	177	209	5.5	**	***
Birth–28 d	184^{b}	199^{a}	195^{a}	179	209	4.3	*	***

Means with different letters (a, b) within each row differ significantly (P ≤ 0.05).

[1]Control, diet based on commercial concentrates plus alfalfa hay; DOP40, diet based on concentrate with 40% of cereals replaced by dehydrated orange pulp (DOP) plus alfalfa hay; DOP80, diet based on concentrate with 80% of cereals replaced by DOP plus alfalfa hay.

[2]Statistical probability for comparisons: ns, not significant ($P > 0.05$); *, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$. No significant interactions between these factors were noted ($P > 0.05$).

Table 4. Effects of experimental diets[1] and prolificacy on the early (0–55 days) lactation performance of goats.

Item[3]	Diet (D)[1]			Prolificacy (PR)		SEM	*P*[2]		
	Control	DOP40	DOP80	Single	Twins		D	PR	D × PR
No. of goats[3]	14	16	14	23	21				
Live weight, kg	38.0	39.3	39.3	38.9	38.9	0.65	ns	ns	ns
Body condition score	2.74	2.63	2.64	2.73	2.60	0.03	ns	ns	ns
Daily milk yield, l/d	1.62	1.68	1.67	1.47^{b}	1.85^{a}	0.06	ns	**	ns
Milk yield (55 d), l	84.7	92.7	93.7	80.9^{b}	100.7^{a}	4.1	ns	*	ns
DM, %	12.2^{ab}	12.4^{a}	11.9^{b}	12.2	12.2	0.09	*	ns	ns
Crude protein, %	2.94	3.07	2.94	2.96	3.01	0.03	ns	ns	ns
Crude protein yield, g/d	50.0	53.1	53.7	49.5^{b}	57.5^{a}	3.10	ns	*	ns
Fat, %	3.78	3.84	3.53	3.66	3.77	0.06	ns	ns	ns
Fat yield, g/d	64.1	66.5	65.0	59.5^{b}	72.0^{a}	4.26	ns	*	ns
Lactose, %	4.64	4.72	4.64	4.71	4.63	0.03	ns	ns	ns
Lactose yield, g/d	78.4	82.0	81.5	75.5	88.3	4.74	ns	*	ns
Retinol, µg/100 g	2.46	1.92	2.56	2.43	2.15	0.28	ns	ns	ns

α-Tocopherol, µg/100 g	21.7[b]	32.8[ab]	42.3[a]	35.4	29.7	2.92	**	ns	ns
Total phenolic compounds, mg GA[4] equivalents/l	63.5[c]	84.0[b]	102.3[a]	80.6	86.2	2.94	***	ns	ns
TAC[5], µmol Trolox® equivalents/ml	6.63[c]	11.1[b]	12.8[a]	9.62	10.7	0.53	***	ns	*

Means with different letters (a, b, c) within each row differ significantly ($P \leq 0.05$).

[1]Control, diet based on commercial concentrates plus alfalfa hay; DOP40, diet based on concentrate with 40% of cereals replaced by dehydrated orange pulp (DOP) plus alfalfa hay; DOP80, diet based on concentrate with 80% of cereals replaced by DOP plus alfalfa hay.

[2]Statistical probability for comparisons: ns, not significant ($P > 0.05$); *, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[3]For vitamins, TAC and total phenolic compound analyses, n = 12 in each group.

[4]GA, gallic acid.

[5]TAC, total antioxidant capacity by ABTS (2,2′-azinobis[3-ethylenbenzothiazoline-6-sulphonic acid]) assay.

Table 5. Effects of experimental diets[1] on blood biochemical parameters of goats in late (120–150 days) gestation and early (0–55 days) lactation.

Parameter (units)[3]	Early lactation[1,2]			SEM	Late gestation[1,2]			SEM
	Control	DOP40	DOP80		Control	DOP40	DOP80	
Total proteins, g/l	76.4	78.3	75.6	1.2	69.1	68.1	67.6	1.4
Albumin, g/l	30.1	32.1	31.4	1.0	30.4	31.2	30.3	1.0
Triglycerides, mmol/l	0.26	0.32	0.29	0.02	0.42	0.34	0.33	0.02
Cholesterol, mmol/l	2.03	2.07	1.76	0.09	1.90	1.75	1.83	0.10
Glucose, mmol/l	3.95[a]	3.15[b]	3.23[b]	0.12	3.54[a]	3.00[b]	3.00[b]	0.15
Urea nitrogen, mmol/l	6.07	5.14	4.53	0.28	4.57	4.58	4.67	0.22
Creatinine, µmol/l	57.4	54.8	54.8	2.5	58.3	60.1	61.8	2.4
Total bilirubin, µmol/l	3.77	4.95	4.44	0.35	4.19	5.39	5.30	0.39
Alanine aminotransferase, IU/l	12.9	12.1	14.6	1.5	13.1	9.6	13.6	1.2
Aspartate aminotransferase, IU/l	99.3	93.3	87.8	4.1	98.6[a]	68.5[b]	75.2[b]	3.0
Alkaline phosphatase, IU/l	80.4	231.5	78.6	30.1	172.8	216.4	127.2	33.3
Creatine kinase, IU/l	178.9	212.2	149.2	21.0	273.4[a]	137.0[c]	191.3[b]	18.7
Amylase, IU/l	53.6	62.9	35.6	11.1	40.4	32.5	36.0	3.0

Phosphorus, mmol/l	2.70	2.49	2.03	0.19	2.22	2.48	2.23	0.13
Total calcium, mmol/l	2.17[b]	1.82[b]	2.75[a]	0.09	2.59	2.24	2.42	0.11
Magnesium, mmol/l	1.22	1.18	1.44	0.09	1.43[a]	1.04[c]	1.26[b]	0.06
Sodium, mmol/l	147.3	149.1	150.9	1.5	149.7	144.9	145.7	0.6
Potassium, mmol/l	4.59	4.59	4.96	0.10	5.40	5.52	5.30	0.15
Chloride, mmol/l	108.6	107.4	109.6	0.9	111.1	109.0	108.9	0.6
Iron, µmol/l	23.1	23.2	24.5	1.6	22.9	25.2	22.15	2.0

Means with different letters (a, b, c) within each row differ significantly ($P \leq 0.05$).

[1]Control, diet based on commercial concentrates plus alfalfa hay; DOP40, diet based on concentrate with 40% of cereals replaced by dehydrated orange pulp (DOP) plus alfalfa hay; DOP80, diet based on concentrate with 80% of cereals replaced by DOP plus alfalfa hay.

[2]No effect of prolificacy or diet × prolificacy interaction was observed for any variable examined ($P > 0.05$).

[3]For every blood parameter analysis, n = 10 in each group.