



## Is the inclusion of carob (*Ceratonia siliqua* L.) pulp in the concentrate of weaned light lambs worth it?

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### ABSTRACT

This study aimed at evaluating the effects of dietary carob (*Ceratonia siliqua* L.) pulp, a by-product of the fruit processing industry, on light lamb performances, time budget behaviour, coefficients of total tract apparent digestibility (CTTAD) and blood metabolites. A total of 144 crossbred lambs with  $41 \pm 5.5$  days of age were monitored in two consecutive batches. They were housed in groups of 6 lambs (3 females and 3 males) balanced according to weaning body-weight and assigned to one of three isoenergetic and isoproteic concentrate-based diets: C0 (without carob pulp), C15 (150 g/kg of carob pulp) and C30 (300 g/kg of carob pulp). At 50, 65 and 80 days of age, feed, faeces and blood samples were collected, and behaviour was recorded. The type of concentrate did not have effect on lamb growth and concentrate intake ( $P > 0.05$ ). However, the concentrate feed conversion ratio (FCR) was higher for C15 and C30 compared to C0 ( $P < 0.05$ ). The time budget behaviours were not affected by diets ( $P > 0.05$ ). The time spent lying resting and standing static increased throughout the fattening period ( $P < 0.05$ ), but self-grooming, feeding, exploration, movement and positive social interactions decreased with age ( $P < 0.05$ ). Regarding the CTTAD of dry matter (DM), organic matter (OM) and crude protein (CP), it was noted that C30 had the lowest digestibility of these nutrients with respect to C0 and C15 ( $P < 0.05$ ). Furthermore, the total condensed tannins (CT) content in faeces was higher in C30 compared to C0 and C15 ( $P < 0.05$ ). The age of lambs affected the CTTAD of DM, OM, CP and phosphorus, which were lower at 50 days than in the rest of control days ( $P < 0.05$ ). Blood metabolites were not affected by experimental diets ( $P > 0.05$ ). However, urea concentrations increased significantly from 50 to 65 days of age, while glucose decreased concomitantly ( $P < 0.05$ ). In conclusion, dietary carob pulp inclusion up to 300 g/kg does not negatively affect animal daily gains, daily activity budgets and metabolic nutritional status, but reduces diet digestibility and increases the concentrate FCR. Although a carob pulp inclusion of up to 150 g/kg in the energy-rich concentrate-based diet for weaned light lambs increases the concentrate FCR

**Abbreviations:** DM, Dry Matter; BW, Body-Weight; CP, Crude Protein; CT, Condensed Tannins; ADG, Average Daily Gain; FCR, Feed Conversion Rate; AIA, Acid-Insoluble Ash; CTTAD, Coefficients of Total Tract Apparent Digestibility; OM, Organic Matter; EE, Ether Extract; aNDFom, Neutral-Detergent Fibre assayed with a heat stable amylase and expressed exclusive of residual ash; NDFom, Neutral-Detergent Fibre expressed exclusive of residual ash.

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while keeping the daily gain, this dietary strategy would be a good trade-off between animal performances and circular economy principles.

## 1. Introduction

To address the environmental impact of sheep production, the formulation of diets combining circular economy principles and key nutrients to mitigate climate warming would be desired (Guyomard et al., 2020). Carob (*Ceratonia siliqua* L.) is evergreen legume tree that has been cropped traditionally in the Mediterranean area because of its drought resistance, adaptation to soil salinity and low fertilization requirements, which may represent a carbon sink in arid environments. Spain is the top producer of carobs around the globe, with about 60,000 Tm of carob pods produced per year. Carob pod fruits are long, indehiscent, straight or curved, thickened at the sutures, and composed by two major parts, pulp (90 %) and seeds (10 %) (Malagón, 2020).

Carob products are purposed for several foods, pharmaceutical and cosmetic industry (Zhu et al., 2019). Carob pulp by-products are yielded during this processing applications, which are rich in sugar (260–550 g/kg dry matter, DM) and lignin (100–430 g/kg DM) contents, whilst are low in hemicellulose (6–33 g/kg DM), crude protein (20–70 g/kg DM) and lipid (1–15 g/kg DM) contents, which make them a potential source of fodder, mainly for ruminants (Albanell et al., 1991). Carob pulp is also deficient in phosphorus (Heuzé et al., 2016). In contrast, carob pods are considered an exceptionally rich source of non-extractable polyphenols (Silanikove et al., 2006). The main phenolic compounds in carob pulp (in decreasing importance) are gallic acid, myricetin- and quercetin- derivatives, and procyanidins galloyl esters (Stavrou et al., 2018; Rico et al., 2019).

In this framework, the most proven dietary strategies to reduce enteric methane emission are the supply of tannins and/or lipids (INRA, 2018). The dietary inclusion of carob pulp has been previously evaluated in heavy lambs (from 20 to 25–30–40 kg of slaughter body-weight, BW) fed with total mixed rations with forage sources (Obeidat et al., 2011; Gobindram et al., 2015; Benbati et al., 2021) or concentrate pellets with moderate energy content (Priolo et al., 1998; Lanza et al., 2001). In other studies, it has been supplied with polyethylene glycol to bind their proanthocyanidins (or condensed tannins, CT) and thereby disentangle their role in animal performance and digestion (Priolo et al., 2000, 2002). Despite extensive descriptions of the detrimental effects of tannins on feed conversion (Priolo et al., 2000, 2002; Silanikove et al., 2006), the ratio whereby carob pulp inclusion affects feed digestibility and productive performance in high energy concentrate diets remains to be determined.

Some Mediterranean countries, as Spain, produce early weaned lambs fed on energy-rich and concentrate-based pellet diets and slaughtered at light BW. In this production system, graded inclusion of dietary carob pulp has not been evaluated. Therefore, the goal of this study was to assess the effects of carob pulp level in the concentrate pellet on animal performances and budget behaviours, total tract digestibility of nutrients and blood metabolic indicators in light lambs from 15 to 25 kg of BW.

## 2. Materials and methods

The animals were handled and slaughtered in accordance with the Spanish Animal Protection Regulations RD 53/2013, which complies with European Union Directive 2010/63 with regard to the protection of animals used for experimental and other scientific purposes. The lambs were raised in commercial conditions following the Council Directive 98/58/EC concerning the protection of animals kept for farming purposes.

### 2.1. Animals, diets and experimental design

The experiment was conducted in the experimental facilities Nial of BonÀrea Agrupa, located in Guissona (Lleida, Catalonia, Spain,

**Table 1**  
Ingredients of the concentrate pellets (g/kg).

| Ingredients                         | C0  | C15 | C30 |
|-------------------------------------|-----|-----|-----|
| Maize                               | 280 | 280 | 227 |
| Barley                              | 289 | 150 | 50  |
| Wheat                               | 80  | 80  | 80  |
| Wheat bran                          | 40  | 20  | 10  |
| Gluten maize feed                   | 120 | 71  | 30  |
| Soybean meal (470 g CP/kg)          | 144 | 188 | 225 |
| Carob pulp                          | 0   | 150 | 300 |
| Palm oil                            | 9   | 30  | 54  |
| CaCO <sub>3</sub>                   | 25  | 18  | 11  |
| Salt                                | 10  | 10  | 10  |
| Vitamin-mineral premix <sup>a</sup> | 3   | 3   | 3   |

<sup>a</sup> The vitamin-mineral premix contained in each kilogram of feed: Vitamin A, 10620 UI; Vitamin D3, 2550 UI; All-rac-tocopheryl-acetate (vitamin E), 300 UI; Vitamin B1, 6.7 mg; Zn, 61.1 mg; Mn, 46.6 mg; Fe, 21.4 mg; Cu, 7.5 mg; I, 0.77 mg; Co, 0.72 mg; Se (Sodium selenite), 0.45 mg; Butylated hydroxytoluene (E321), 112.5 mg and Propyl gallate (E310), 13.5 mg.

41°46'32.2" N, 1°16'33.2" E; 484 m above sea level). A total of 144 weaned male and female crossbred lambs ( $14.7 \pm 1.5$  kg of BW;  $41 \pm 5.5$  days of age) from two consecutive batches were allotted into 12 pens each batch (3 male and 3 female lambs per pen) in loose-housed sheds ( $0.86 \text{ m}^2/\text{lamb}$ ) until slaughter at a target BW of 25 kg. The study was replicated in two equal batches of 72 lambs each: batch 1 in winter, from January to February (6 weeks) and batch 2 in summer, from June to July (7 weeks). The lambs of both batches were distributed in homogeneous light and heavy pen groups according to their initial BW to minimize the within-pen BW variation. The average monthly temperature for January and February was 4.8 and 9.1 °C, and for June and July it was 19.4 and 24.6 °C, respectively.

The lambs were submitted to one of three isocaloric (1760 kcal of Net Energy for Ruminants/kg of concentrate) and isoproteic concentrates (175 g/kg of crude protein, CP) (Table 1), which had been formulated with graded carob pulp inclusion: C0 (without carob pulp), C15 (150 g/kg of carob pulp) or C30 (300 g/kg of carob pulp) with a total of 8 pen replicates per treatment (4 replicates/treatment x 2 batches).

The nutritional value and technological properties of the pellet concentrates is shown in Table 2. As a first mechanical process, the pods are crushed to separate the seeds from the pulp, and then the carob pulp is ground into simple pieces, resulting in a final product of tiny kibbles (Rodríguez-Solana et al., 2021). The carob pulp had 902 g DM/kg, 33 g ash/kg DM, 51 g CP/kg DM, 333 g of neutral-detergent fibre (aNDFom)/kg DM, 399 g of acid-detergent fibre (ADFom)/kg DM, 191 g lignin/kg DM, and 543 g total sugars/kg DM. The closeness between the aNDFom and ADFom values in carob pulp has been reported elsewhere (Albanell et al., 1991), and it may be because the method of ADFom determination leads to some polymerisation of the sugars with the fibre fraction, resulting in an artificial increase of the apparent ADFom content (Milad et al., 2010). These differences and the large variability in ADFom and lignin content could be also explained by the presence of tannins, as tannin-protein complexes may be detected as artificial fibre (Silanikove et al., 2006). Total polyphenol content was 21.5 eq-g tannic acid/kg and its total CT content was 263.4 eq-g CT carob pulp/kg DM.

Before the experiment started, the lambs were fed a standard commercial concentrate without coccidiostatic for a 5-day adaptation period. The experimental concentrates were formulated with the same ingredients and additives and they were manufactured in the same batch. The feed presentation was granulated with a pellet diameter of 3.5 mm (Fig. 1) and the granulation temperature was 60 °C.

The lambs had free access to concentrate pellets and, wheat straw through separate feeders, and they were supplied water ad libitum in circular drinking bowls. The nutritional value of the used wheat straw was: 51 g ash/kg DM, 21 g CP/kg DM, 5 g phosphorus/kg DM. Total polyphenol content of wheat straw was 13.5 eq-g tannic acid/kg DM, and its total CT content was 5.7 eq-g CT carob pulp/kg DM.

## 2.2. Animal performance

Lambs were weighed individually once weekly to calculate their average daily gain (ADG, g/day). At the target slaughter BW, the lambs were transported to the commercial abattoir (3 km away from the farm), where they had water ad libitum. The fasting period lasted 3–4 h. Pre-slaughter BW and hot carcass weight were recorded to calculate the carcass dressing percentage.

The supplied dietary concentrate and straw was recorded daily, their orts were measured once weekly on a pen basis. The feed conversion rate (FCR, g/g) of concentrate was calculated as the ratio between average daily concentrate intake (as-fed basis) and ADG

**Table 2**  
Chemical composition, colour attributes and physical pellet quality of the concentrates.

| Analysed composition                                                | C0   | C15  | C30  |
|---------------------------------------------------------------------|------|------|------|
| Gross energy (MJ/kg)                                                | 18.1 | 18.6 | 19.3 |
| Dry matter (g/kg, DM basis)                                         | 884  | 882  | 880  |
| Ash (g/kg, DM basis)                                                | 62   | 67   | 60   |
| Crude protein (CP) (g/kg, DM basis)                                 | 173  | 180  | 176  |
| Ether extract (g/kg, DM basis)                                      | 38   | 50   | 72   |
| Starch (g/kg, DM basis)                                             | 467  | 415  | 294  |
| Total sugars (g/kg, DM basis)                                       | 60   | 98   | 150  |
| Neutral-detergent fibre (aNDFom) (g/kg, DM basis)                   | 211  | 229  | 235  |
| Acid-detergent fibre (ADFom) (g/kg, DM basis)                       | 64   | 101  | 150  |
| Lignin (sa) (g/kg, DM basis)                                        | 6.4  | 28.6 | 63.6 |
| Phosphorus (g/kg, DM basis)                                         | 3.9  | 3.5  | 3.0  |
| Total polyphenols (g tannic acid eq./kg freeze-dried)               | 8.0  | 9.9  | 12.1 |
| Total condensed tannins (g carob pulp total CT-eq./kg freeze-dried) | 4.1  | 20.6 | 54.0 |
| Extractable (% out of total condensed tannins)                      | 23.4 | 4.5  | 3.6  |
| Protein-bounded (% out of total condensed tannins)                  | 54.2 | 67.9 | 79.3 |
| Fibre-bounded (% out of total condensed tannins)                    | 22.4 | 27.6 | 17.1 |
| <b>CIELAB colour attributes</b>                                     |      |      |      |
| L* (Lightness)                                                      | 72.9 | 67.9 | 63.1 |
| a* (Redness)                                                        | 7.2  | 7.3  | 8.4  |
| b* (Yellowness)                                                     | 21.9 | 26.0 | 29.7 |
| <b>Physical pellet quality</b>                                      |      |      |      |
| Fine particles (%)                                                  | 1.0  | 0.8  | 5.6  |
| Durability (%)                                                      | 97.1 | 96.7 | 96.2 |

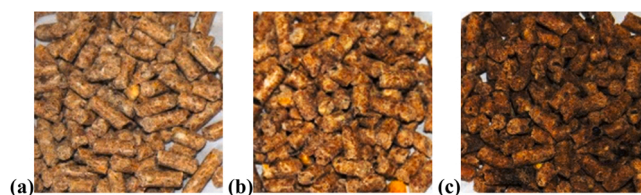


**Fig. 1.** Concentrate pellet views: C0 (0 g of carob pulp/kg of concentrate) (a), C15 (150 g of carob pulp/kg of concentrate) (b) and C30 (300 g of carob pulp/kg of concentrate) (c).

of lambs on a pen basis. In addition, total FCR was also calculated by including straw intake in the previously used formula.

### 2.3. Faecal sampling and calculation of apparent nutrient digestibility

Individual faeces samples were collected by rectal stimulation in all lambs of each pen at 50, 65 and 80 days of age between 8:00 and 9:00 a.m. in each batch, pooled (50–70 g approximately per pen) and stored in plastic food bags following the method described in Pelegrin-Valls et al. (2021a). Samples of concentrate and straw were also collected on a pen basis at the same time for technological assessment and chemical analyses. All feedstuffs and faecal samples that were submitted to subsequent chemical determinations were kept at  $-20^{\circ}\text{C}$  until analysis. Acid-insoluble ash (AIA) was analysed in them to estimate the coefficients of total tract apparent digestibility (CTTAD) of DM and selected nutrients by the nutrient-marker ratio method, as follows:

$$\text{CTTAD} = 1 - [(\text{Marker}_{\text{diet}}/\text{Marker}_{\text{faeces}}) \times (\text{Z}_{\text{faeces}}/\text{Z}_{\text{diet}})]$$

where  $\text{Z}_{\text{faeces}}$  and  $\text{Z}_{\text{diet}}$  are the nutrient concentrations in faeces and diet (both concentrate and straw), respectively. The  $\text{Marker}_{\text{faeces}}$  and  $\text{Marker}_{\text{diet}}$  are the concentrations of AIA in faeces and diet, respectively. The  $\text{Z}_{\text{diet}}$  and  $\text{Marker}_{\text{diet}}$  were calculated by considering the amount of concentrate and straw consumed per pen. The recovery rate of AIA in faeces was assumed to be complete (Sales and Janssens, 2003).

### 2.4. Behaviour recording

At the age of 50, 65 and 80 days, behaviour recordings were performed by a single observer by scan sampling of experimental pens between 9:00 a.m. and 2:00 p.m. at 10 min intervals, following the procedure of Averós et al. (2014). Briefly, for each observation day, the experimental pens were observed in a random order, starting at 9:00 a.m. Each experimental pen was observed twice (10 min x 2 replicates) through a series of continuous scan samplings. During each 10 min scan sampling, the behaviour of all lambs within the pen were sequentially collected. An average of 10 scan samplings was collected per 10 min observation. The ethogram used for scan sampling comprised: drinking, exploring the pen fixtures, eating concentrate, eating straw on the feeder or bedding, moving around the pen, negative social interactions, positive social interactions, lying resting, self-grooming, and standing static.

### 2.5. Blood sampling

Blood samples were collected from two lambs per pen (one female and one male) the day after animal behaviour recordings (days 50, 65 and 80 of age). The same lambs, that were randomly selected at the start of the experiment, were sampled the same three times. Vacuum tubes with EDTA were used to collect 4 mL of blood from the jugular vein (8 pen replicates/dietary treatment) and were centrifuged in situ at 3000g for 10 min to obtain the plasma, which was stored in identified aliquots for each sampled lamb at  $-20^{\circ}\text{C}$  until metabolites analysis.

### 2.6. Analyses

Selected technological characteristics, as the colour and the pellet quality of the concentrates, were assessed in triplicate. The colour attributes were determined after chopping the pellets by the CiELab colour coordinates ( $L^*$ , lightness;  $a^*$ , redness; and  $b^*$ , yellowness) with spectrophotometer (CM-2600d Konica Minolta Sensing Inc., Osaka, Japan). A screen sieve of 2 mm was used to determine the percentage of feed non-pelletized dust particles after a 30-second shaking of a 300 g-sample. The pellet durability was evaluated subsequently with a 150 g-sample in a Pfast durability tester (weight of pellets after 10 min of tumbling 5 min x 50 rpm/weight of pellets before tumbling x 100).

The feedstuffs and faecal samples that had been frozen were thawed, weighed again and dried in a forced air stove at  $60^{\circ}\text{C}$  for 72 h. Their moisture was determined by the weight difference between the fresh and DM. After drying, samples were ground in a knife mill to pass a 1 mm sieve to analyse AIA, organic matter (OM), CP, ether extract (EE), and phosphorus to calculate the CTTAD.

The gross energy of concentrates was analysed with a calorimeter bomb (Gallenkamp auto bomb CBA-305–010 M; Sanyo Gallenkamp PLC, Sussex, UK). Furthermore, the ash content to calculate OM was determined with 2 g of sample in a muffle furnace at  $550^{\circ}\text{C}$  for 3 h. The AIA of concentrates and straw was analysed following a standard procedure (BOE, 1995). CP content ( $\text{N} \times 6.25$ ) was determined following the Dumas procedure, using a nitrogen and protein analyser (Model NA 2100, CE Instruments, Thermoquest SA,

Barcelona, Spain). EE was determined with an XT10 Ankom extractor (Ankom Technology Corporation, Fairport, NY, USA). Phosphorus was determined by ultraviolet-visible spectroscopy (ICP-OES, HORIBA Jobin Yvon, Activa family, with AS-500 Autosampler, HORIBA Scientific, Madrid, Spain). In addition, concentrates and straw were sequentially analysed for ADFom and lignin (sa), whereas the concentrate was analysed for aNDFom and the straw was alternatively analysed for NDFom, using the Ankom 200/220 fibre analyser (Ankom Technology Corporation, Fairport, NY, USA). The aNDFom was assayed with a heat-stable amylase and expressed exclusive of residual ash, whereas ADFom was expressed exclusive of residual ash and lignin was determined by solubilization of cellulose with sulphuric acid. Total starch and total sugars in the concentrates were determined by the approved polarimetry (EWERS) and Luff-Schoorl methods, respectively (BOE, 2000).

In addition, total polyphenols and CT composition were analysed in the feedstuffs and faeces. The total polyphenols and CT fractions were extracted in dried feedstuffs and faeces samples according to Rufino-Moya et al. (2019). Samples and standard calibration were measured with a He $\lambda$ ios  $\beta$  spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) at 725 nm, and polyphenol contents were expressed as tannic acid equivalents. The extractable CT, protein-bound CT and fibre-bound CT were fractioned following the method of Terrill et al. (1992), and quantified by the colorimetric HCl-butanol method described by Grabber et al. (2013). The standard used for the quantification of the samples was extracted and purified from freeze-dried carob pulp using the method described by Wolfe et al. (2008). Finally, samples and standard calibration were measured with the He $\lambda$ ios  $\beta$  spectrophotometer at 550 nm and CT concentrations were expressed as carob pulp CT-equivalents. Feedstuffs and faeces chemical determinations were analysed in duplicate.

Plasma urea, creatinine, glucose, triglycerides and cholesterol were analysed by an automatic analyser (GernonStar, RAL S.A., Barcelona, Spain). Protocols and reagents were provided by the analyser manufacturer (RAL, Barcelona, Spain). The mean intra- and inter-assay coefficients of variation for these metabolites were < 4.4 % and < 7.7 %, respectively.

## 2.7. Statistical analyses

The productive performance from 11 lambs (2 lambs from C0, 5 lambs from C15, and 4 lambs from C30) were removed from the statistical dataset due to death ( $n = 3$ ) or wasting syndrome ( $n = 8$ , reduction of BW from the start to the end of the experiment). These lambs belonged to the winter ( $n = 2$ ) and summer batch ( $n = 9$ ), and they had not been involved in blood sampling. The FCR variable considered the pen average concentrate or total feed intake and the mean ADG of the remaining lambs in the pen.

The data were analysed with the statistical software JMP Pro15 (SAS Institute Inc., Cary, NC, USA). Concentrate and total feed intake and FCR were analysed with least square models including the fixed effects of dietary treatment (C0, C15, or C30) and the batch (1 vs. 2). Growth performance, behavioural parameters, plasma metabolites and nutrient digestibility variables were analysed through mixed models with repeated measurements that included the dietary treatment (C0, C15, or C30), the lamb age (50, 65 or 80 days), and the batch (1 vs. 2) as fixed effects, as well as the animal (for plasma metabolites) or pen (for behaviour and nutrient digestibility) nested within dietary treatments and batch as a random effect. The single interactions between fixed effects were also included in the models but they are only reported in the results section if they resulted significant ( $P < 0.05$ ). The lamb sex effect and its interactions with diet, age and batch were also considered in growth performance and plasma metabolic variables, that were recorded in individual replicates. Least square means and their standard errors are presented. The separation of means was carried out with the Tukey's test. The level of significance was set at 5 %. The association between dietary treatments and wasting syndrome or mortality of lambs was evaluated through Pearson's contingency coefficients.

**Table 3**

Effect of dietary carob pulp inclusion on lamb performances during the fattening period.

| Items                                                     | Carob pulp treatment <sup>a</sup> |                   |                   |       |         |
|-----------------------------------------------------------|-----------------------------------|-------------------|-------------------|-------|---------|
|                                                           | C0                                | C15               | C30               | SEM   | P-value |
| Age at start (days of age)                                | 41.6                              | 41.0              | 41.2              | 0.82  | 0.89    |
| Initial BW (kg)                                           | 14.7                              | 14.8              | 14.7              | 0.23  | 0.97    |
| Within-pen coefficient of variation of initial BW (%)     | 5.54                              | 5.95              | 6.35              | 0.675 | 0.70    |
| ADG (g)                                                   | 230                               | 232               | 220               | 11.6  | 0.76    |
| Slaughter BW (kg)                                         | 25.4                              | 25.6              | 25.0              | 0.61  | 0.76    |
| Within-pen coefficient of variation of slaughter BW (%)   | 15.6                              | 11.6              | 12.6              | 1.80  | 0.28    |
| Carcass weight (kg)                                       | 12.2                              | 12.2              | 11.6              | 0.35  | 0.39    |
| Carcass dressing (%)                                      | 46.4                              | 47.6              | 46.3              | 0.72  | 0.37    |
| Concentrate intake (g/day, as-fed basis)                  | 730                               | 783               | 793               | 42.6  | 0.54    |
| Straw supply (g/day)                                      | 184                               | 186               | 184               | 4.1   | 0.94    |
| Concentrate FCR (g/g)                                     | 3.44 <sup>b</sup>                 | 3.99 <sup>a</sup> | 3.95 <sup>a</sup> | 0.150 | 0.03    |
| Total FCR (g/g)                                           | 4.09                              | 4.33              | 4.55              | 0.205 | 0.31    |
| Wasting syndrome (%)                                      | 4.17                              | 6.25              | 6.25              | –     | 0.87    |
| Mortality (%)                                             | 0.00                              | 4.17              | 2.08              | –     | 0.36    |
| Female/male ratio of lambs reaching target slaughter date | 24/24                             | 24/22             | 23/24             | –     | 0.95    |

<sup>a</sup> C0: concentrate without carob pulp, C15: concentrate with 150 g carob pulp/kg and C30: concentrate with 300 g carob pulp/kg. SEM = Standard error of the mean. The concentrate feed conversion ratio (FCR) was calculated as the average daily concentrate intake divided by the average daily gain (ADG). The total FCR was calculated as the average daily concentrate and straw intake divided by the ADG. Different letter denotes statistical differences between dietary treatments in feed conversion ratio ( $P < 0.05$ ).

### 3. Results

#### 3.1. Animal performances and behaviour

The inclusion of dietary carob pulp did not affect most of the lamb performances (Table 3), except the concentrate FCR, that was impaired in C15 and C30 compared to control (C0) ( $P < 0.05$ ). The straw to concentrate ratio decreased linearly throughout the fattening period ( $226, 192$  and  $133 \pm 5.5$  g of straw/kg of diet in C0, C15 and C30, respectively;  $P < 0.05$ ). On the other hand, female lambs had lower ADG than male lambs ( $211$  vs.  $244 \pm 9.5$  g/day,  $P < 0.01$ ), but the carcass weight did not differ across sexes ( $11.7$  vs.  $12.3 \pm 0.29$  kg, respectively,  $P > 0.05$ ).

The dietary treatment did not affect the activity budget or behaviour of lambs (Table 4), that was mainly affected by the lamb age. The time spent lying resting and the time standing static increased throughout the fattening period ( $P < 0.05$ ). In contrast, the time spent eating straw and concentrate, as well as the time exploring the pen fixtures and moving around the pen, decreased concomitantly ( $P < 0.05$ ). Finally, the time spent in self-grooming and the positive social behaviours decreased throughout the fattening period ( $P < 0.05$ ).

#### 3.2. Nutrient digestibility and faecal condensed tannins composition

The interaction between dietary carob inclusion and lamb age on the CTTAD of OM, CP, EE and phosphorus is shown in Fig. 2. The CTTAD of OM and CP did not differ among treatments at 50 days of age, but they were higher in C0 than in C30, while C15 was similar between diets at day 65 of age (Fig. 2 A and B, respectively,  $P < 0.05$ ). However, at day 80 of age both C0 and C15 were higher than C30 ( $P < 0.05$ ). Moreover, the CTTAD of EE did not change between dietary treatments at 50 and 65 days of age ( $P > 0.05$ ), but it was higher in C0 than in C30 at 80 days of age (Fig. 2 C,  $P < 0.05$ ). Finally, the CTTAD of phosphorus was lower in C15 than C0 and C30 at day 50 of age ( $P < 0.05$ ) whereas no differences were observed at the subsequent days of age (Fig. 2 D,  $P > 0.05$ ).

The independent effects of dietary carob pulp level and lamb age on the CTTAD of DM and CT composition are shown in Table 5. The following variables were not affected by the interaction between treatment and age period, thereby their main significant effects are exposed separately. Concerning the main effect of dietary carob, the CTTAD of DM were lower in C30 compared to C0 and C15 ( $P < 0.05$ ). The total CT in faeces was higher in C30 compared to C0 and C15 ( $P < 0.05$ ). The extractable and protein-bound CT fraction decreased linearly while the fibre-bound CT fraction increased linearly in the faeces with increasing the dietary carob pulp in the concentrate pellet ( $P < 0.05$ ).

With regard to the effect of lamb age, the CTTAD of DM increased from 50 days of age onwards ( $P < 0.05$ ). However, the total CT in faeces was not steady throughout the fattening period. Whilst the extractable CT fraction did not differ with lamb ages ( $P > 0.05$ ), protein-bound CT fraction decreased at 80 days of age ( $P < 0.05$ ) while fibre-bound CT fraction was lowest at 65 days of age ( $P < 0.05$ ).

#### 3.3. Blood metabolites

The dietary treatments did not affect plasma urea, creatinine, glucose, triglycerides and cholesterol concentrations (Table 6,  $P > 0.05$ ), whereas the lamb age influenced plasma urea, which increased from 50 to 65 days of age in the fattening period ( $P < 0.05$ ) and glucose, that decreased concomitantly ( $P < 0.05$ ). The lamb sex only affected plasma urea levels, that were higher in female than male lambs ( $34.0$  vs.  $30.9 \pm 1.22$  mg/dL, respectively,  $P < 0.05$ ).

**Table 4**  
Effects of dietary carob pulp level and lamb age on the activity budget and behaviours (% proportion of time, 9:00 a.m. to 2:00p.m.).

| Items <sup>b</sup>                        | Carob pulp treatment <sup>a</sup> |      |      |       |         | Days of age       |                    |                    |       |         |
|-------------------------------------------|-----------------------------------|------|------|-------|---------|-------------------|--------------------|--------------------|-------|---------|
|                                           | C0                                | C15  | C30  | SEM   | P-value | 50                | 65                 | 80                 | SEM   | P-value |
| Lying resting                             | 49.2                              | 52.8 | 44.4 | 6.72  | 0.67    | 30.5 <sup>b</sup> | 51.2 <sup>a</sup>  | 64.7 <sup>a</sup>  | 6.04  | <0.001  |
| Eating straw on the feeder or the bedding | 21.4                              | 17.5 | 22.2 | 3.56  | 0.60    | 27.8 <sup>a</sup> | 22.7 <sup>a</sup>  | 10.5 <sup>b</sup>  | 3.56  | 0.003   |
| Eating concentrate                        | 10.2                              | 12.1 | 15.6 | 2.23  | 0.24    | 15.9 <sup>a</sup> | 12.5 <sup>ab</sup> | 9.5 <sup>b</sup>   | 1.94  | 0.05    |
| Standing static                           | 8.70                              | 5.60 | 5.81 | 1.780 | 0.41    | 6.80 <sup>b</sup> | 4.10 <sup>b</sup>  | 9.14 <sup>a</sup>  | 1.510 | 0.04    |
| Exploring the pen fixtures                | 6.73                              | 6.30 | 7.30 | 1.371 | 0.87    | 11.1 <sup>a</sup> | 5.80 <sup>b</sup>  | 3.31 <sup>b</sup>  | 1.371 | <0.001  |
| Drinking water                            | 1.10                              | 0.62 | 1.13 | 0.267 | 0.34    | 1.41 <sup>a</sup> | 0.93 <sup>ab</sup> | 0.53 <sup>b</sup>  | 0.267 | 0.06    |
| Moving around the pen                     | 1.10                              | 2.74 | 1.53 | 0.588 | 0.12    | 3.33 <sup>a</sup> | 0.94 <sup>b</sup>  | 1.10 <sup>b</sup>  | 0.518 | <0.001  |
| Self-grooming                             | 0.90                              | 1.00 | 1.00 | 0.216 | 0.94    | 1.30 <sup>a</sup> | 1.00 <sup>ab</sup> | 0.60 <sup>b</sup>  | 0.216 | 0.08    |
| Negative social interactions              | 0.65                              | 0.90 | 0.40 | 0.196 | 0.24    | 1.05 <sup>a</sup> | 0.24 <sup>b</sup>  | 0.62 <sup>ab</sup> | 0.196 | 0.02    |
| Positive social interactions              | 0.24                              | 0.45 | 0.70 | 0.170 | 0.21    | 0.70 <sup>a</sup> | 0.60 <sup>ab</sup> | 0.13 <sup>b</sup>  | 0.170 | 0.07    |

<sup>a</sup> C0: concentrate without carob pulp, C15: concentrate with 150 g carob pulp/kg and C30: concentrate with 300 g carob pulp/kg. SEM = Standard error of the mean. Within each row and effect, different letter denotes statistical differences between dietary treatments or lamb ages ( $P < 0.05$ ).

<sup>b</sup> Negative social interactions were considered as sudden, strong head contact with other lambs or kick another lamb to leave the resting place, while positive social interactions were considered as sniffing, nosing, grooming, nudging or licking other lambs.

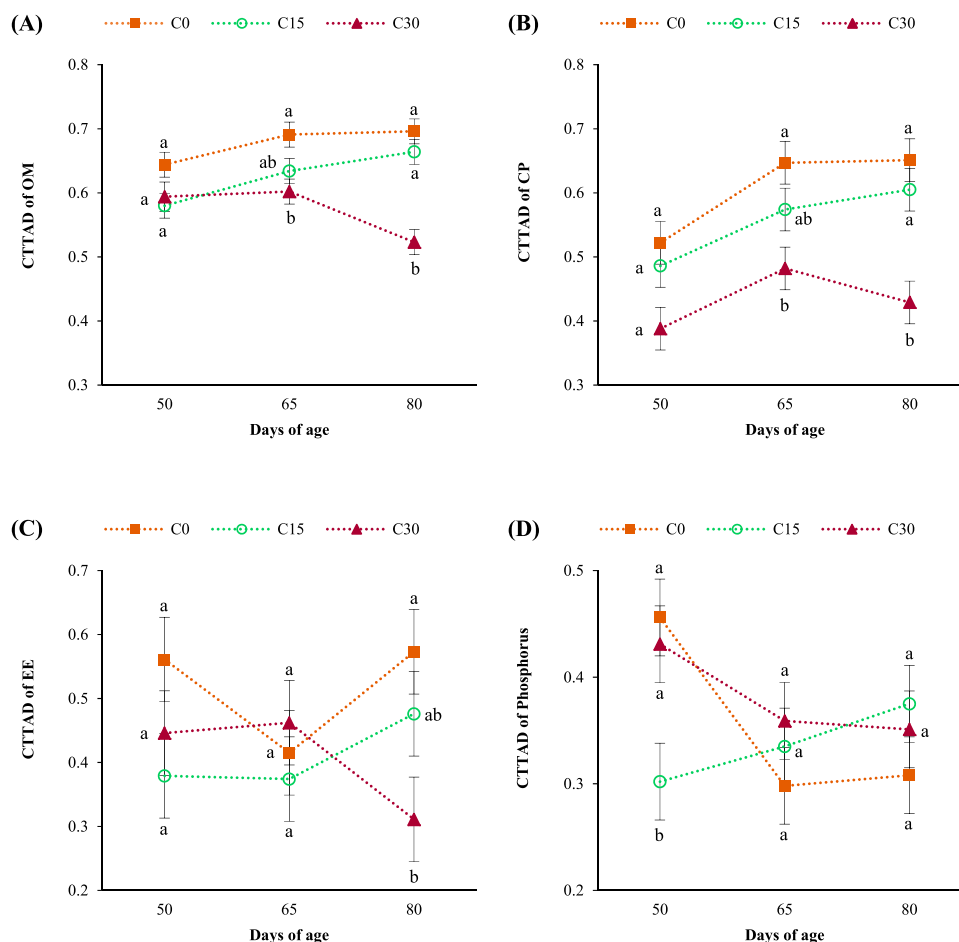


**Fig. 2.** Interactions between dietary carob pulp inclusion (C0 = 0 g of carob pulp/kg of concentrate, C15 = 150 g of carob pulp/kg of concentrate and C30 = 300 g carob pulp/kg of concentrate) and lamb age on the coefficients of total tract apparent digestibility (CTTAD) of organic matter (OM) (A), crude protein (CP) (B), ether extract (C) and phosphorus (D) (least square mean values  $\pm$  standard error). Within each sampling day, different letters (a, b) denote statistical differences ( $P < 0.05$ ) between dietary treatments.

**Table 5**

Effects of dietary carob pulp level and lamb age on the faecal coefficient of total tract apparent digestibility (CTTAD) of dry matter and condensed tannins (CT) composition.

| Items                                | Carob pulp treatment <sup>a</sup> |                   |                   | SEM   | P-value | Days of age        |                   |                   | SEM   | P-value |
|--------------------------------------|-----------------------------------|-------------------|-------------------|-------|---------|--------------------|-------------------|-------------------|-------|---------|
|                                      | C0                                | C15               | C30               |       |         | 50                 | 65                | 80                |       |         |
| CTTAD of faecal DM                   | 0.86 <sup>a</sup>                 | 0.83 <sup>a</sup> | 0.77 <sup>b</sup> | 0.013 | <0.001  | 0.79 <sup>b</sup>  | 0.83 <sup>a</sup> | 0.83 <sup>a</sup> | 0.008 | <0.001  |
| <b>Faecal CT composition</b>         |                                   |                   |                   |       |         |                    |                   |                   |       |         |
| Total CT (g CT-eq/kg DM)             | 13.6 <sup>c</sup>                 | 31.9 <sup>b</sup> | 83.2 <sup>a</sup> | 4.16  | <0.001  | 43.2 <sup>b</sup>  | 36.8 <sup>c</sup> | 48.7 <sup>a</sup> | 2.70  | <0.001  |
| Extractable CT (% out of total CT)   | 11.8 <sup>a</sup>                 | 5.30 <sup>b</sup> | 1.80 <sup>c</sup> | 0.511 | <0.001  | 6.05               | 6.40              | 6.41              | 0.380 | 0.64    |
| Protein-bound CT (% out of total CT) | 48.8 <sup>a</sup>                 | 28.4 <sup>b</sup> | 21.5 <sup>c</sup> | 1.60  | <0.001  | 33.5 <sup>a</sup>  | 34.6 <sup>a</sup> | 30.6 <sup>b</sup> | 1.24  | 0.01    |
| Fibre-bound CT (% out of total CT)   | 39.4 <sup>c</sup>                 | 66.3 <sup>b</sup> | 76.7 <sup>a</sup> | 1.92  | <0.001  | 60.4 <sup>ab</sup> | 59.0 <sup>b</sup> | 63.0 <sup>a</sup> | 1.42  | 0.03    |

<sup>a</sup> C0: concentrate without carob pulp, C15: concentrate with 150 g carob pulp/kg and C30: concentrate with 300 g carob pulp/kg. SEM = Standard error of the mean. Within each row and effect, different letter denotes statistical differences between dietary treatments or lamb ages ( $P < 0.05$ ).

#### 4. Discussion

Currently, there is no specific recommendation about the optimum dietary carob pulp that may be added to lamb diets in Mediterranean environments. The presence of CT in carob pulp may have adverse effects on digestion when it is included in high proportion in the diets of ruminants (Richane et al., 2022). The present study aimed at establishing the effects of dietary carob inclusion in concentrate-based diets on the growth performance, behaviour and metabolic markers of nutritional status in weaned light lambs.

The main constraint when including carob pulp is the low CP and high fibre content, that urges to balance the feed mixture with

**Table 6**

Effects of dietary carob pulp level and lamb age on plasma metabolites.

| Items                 | Carob pulp treatment <sup>a</sup> |      |      |       |         | Days of age       |                   |                   |       |         |
|-----------------------|-----------------------------------|------|------|-------|---------|-------------------|-------------------|-------------------|-------|---------|
|                       | C0                                | C15  | C30  | SEM   | P-value | 50                | 65                | 80                | SEM   | P-value |
| Urea (mg/dL)          | 32.9                              | 33.9 | 30.5 | 1.53  | 0.27    | 29.3 <sup>b</sup> | 33.1 <sup>a</sup> | 34.9 <sup>a</sup> | 1.26  | 0.002   |
| Creatinine (mg/dL)    | 0.81                              | 0.76 | 0.78 | 0.028 | 0.37    | 0.81              | 0.78              | 0.76              | 0.025 | 0.45    |
| Glucose (mg/dL)       | 78.6                              | 82.4 | 78.5 | 2.00  | 0.29    | 82.3 <sup>a</sup> | 78.6 <sup>b</sup> | 78.6 <sup>b</sup> | 1.42  | 0.01    |
| Triglycerides (mg/dL) | 4.25                              | 3.68 | 4.38 | 1.055 | 0.88    | 5.03              | 4.47              | 2.81              | 0.916 | 0.15    |
| Cholesterol (mg/dL)   | 29.6                              | 32.4 | 25.8 | 2.97  | 0.30    | 29.1              | 27.8              | 30.8              | 2.14  | 0.39    |

<sup>a</sup> C0: concentrate without carob pulp, C15: concentrate with 150 g carob pulp/kg and C30: concentrate with 300 g carob pulp/kg. SEM = Standard error of the mean. Within each row and effect, different letter denotes statistical differences between dietary treatments or lamb ages ( $P < 0.05$ ).

protein concentrates and oil sources to assure similar nitrogen and net energy supply to the animals. To overcome this drawback, the use of concentrate compound pellets may be an optimum feed presentation to improve the ADG and FCR compared to forage-based rations (Blanco et al., 2015). The study proved that daily growth rates of lambs were similar among dietary treatments and this led to similar final BW and carcass performance. This is in agreement with earlier studies using concentrate pellets (Priolo et al., 1998; Lanza et al., 2001) or total mixed rations for heavy lambs (Obeidat et al., 2011; Gobindram et al., 2015; Benbati et al., 2021). In this study, the concentrate pellets were formulated following the commercial nutrient recommendations for finishing light lambs in Spain (Ferret et al., 2008), which imply high energy and protein contents. However, the experimental diets of most of the afore-mentioned references included between 10 % and 35 % of carob pulp and were compared with control diets which had lower energy content than in the current work. When calculating the total CT contents of concentrates on the basis of carob pulp analysis and level of inclusion, their values were lower than expected (ranging from -31.6 % to -47.8 %). The reduction of CT content after pelleting may be explained by CT oxidation due to high temperature. In this process, plant cells would be damaged, allowing the release of previously sequestered soluble CT from the vacuole into the cytosol and forming complexes with proteins and fibres that may increase the protein-bound and fibre-bound CT fractions (Girard et al., 2018).

In the present study, the concentrate pellet intake was numerically greater when carob pulp was included in the diet, leading to a negative effect on concentrate FCR that was significantly increased in C15 and C30 compared to control (C0) treatment. In this regard, the dietary inclusion of carob pulp may have implications on feeding costs depending on certain feed price scenarios, as more protein and oil sources have to be added when a high proportion of carob pulp (C30) is included in the concentrate. An economic analysis was conducted by taking into account the highest, average and lowest price scenarios of feed ingredients and carcasses in 2020. The inclusion of carob pulp up to 300 g/kg did not affect the incomes and gross margin per lamb. In the lowest feed ingredients price scenario, the feeding costs per lamb did not differ across diets. However, in the average and highest feed ingredient price scenario, the feeding costs per lamb in C30 (11.1€) were around 1€ to 2.5€ higher than in C15 and C0, respectively. Therefore, the inclusion of carob pulp above 150 g/kg would affect the feed cost, but depending on the income per carcass it may have no impact on the gross margin, even with an inclusion of 300 g/kg (Pelegrin-Valls et al., 2021b). Likewise, the proportion of lambs showing wasting syndrome or mortality were kept similar across diets.

Apparently, dietary treatment did not have effect on the activity budget and behaviours. In this regard, Gobindram et al. (2015) observed that including up to 35 % of carob in heavy lamb diets did not affect the overall feed intake throughout the day but modified the feeding behaviour by modulating the rate of ingestion (delaying the number of visits to the feeder, not attending soon after daily supply). As the overall concentrate and straw intake did not differ among dietary treatments, it would be feasible that carob-fed lambs reduced their eating rate compared to control diet in ad libitum feeding conditions, but no differences were detected in the time spent consuming concentrate or straw. The current results would support the hypothesis of temporal distribution of feeding activity according the avoidance-tolerance continuum mechanism (Iason and Villalba, 2006). Hence, behavioural strategies appear to be self-organized in ad libitum feeding in order to overcome the toxic effects of plant secondary metabolites, as could be CT.

Although, digestibility procedures fit the analytical standards, the effect of sampling time was evaluated within one day (Pelegrin-Valls et al., 2021a), but the variability between days could affect digestibility estimation (Thonney et al., 1985; Morris et al., 2018). For this reason, the results must be interpreted with caution as in some cases it would be interesting to obtain grab samples over several days when using low AIA diets. This study revealed a linear decrease in total tract digestibility of OM, CP and faecal CT content in lambs with increasing carob supply in the concentrate. However, the EE and phosphorus total tract digestibility were less affected by diet, which would imply the contribution of both nutrients to keep similar growth rates in lambs, regardless of carob inclusion. Priolo et al. (2002) found lower total tract digestibility of nutrients when including 56 % of carob pulp in ground mixed diets including 26 % of alfalfa hay for light lambs, which was attributed to the carob CT contents. This outcome was less marked in Awassi heavy lambs during fattening from 18 to 32 kg with an inclusion rate of 25 % of dietary carob pods replacing barley grain, as they could stand with total tract digestibility of nutrients (Obeidat et al., 2011). Reduced straw to concentrate ratio may also have contributed to the differences in the CTTAD of nutrients as the lambs grew. However, the inclusion of 300 g/kg of carob pulp in the diet of lambs would worsen nutrient digestibility (especially CP) probably as a result of the presence of high CT level, which may reduce proteolysis rate and inhibit the growth of proteolytic rumen microbes (Obeidat et al., 2012).

If the FCR were expressed as Digestible DM intake/kg ADG, the values would have been numerically similar across dietary treatments (3.0, 3.1 and 3.0 for C0, C15 and C30, respectively), suggesting that differences in animal performance would be related with the differences in nutrient digestibility.

The higher faecal CT content in carob-fed lambs (C15 and C30) may be beneficial to the environment when using this manure as fertilizer, as there is a delayed OM breakdown in soils and long-term build-up carbon sequestration and slow organic nitrogen release (Fagundes et al., 2021) that may mitigate nitrogen loss in crop or pasture systems by slowing nitrogen mineralization (Clemensen et al., 2020). Moreover, Gobindram et al. (2015) observed that although lambs were able to cope with a diet including up to 35 % of carob pulp without hindrance to animal performance, they were probably under some form of metabolic stress, as they increased linearly the peripheral urea and reduced their triglycerides and cholesterol concentrations. However, the present study did not reveal any sign of undernutrition in the dietary treatments, which supports the coping abilities of feedlot light lambs to boost resilience and proves the diversification of feed ingredients in the concentrates as a viable strategy.

Finally, concerning the lamb age effect on animal behaviours, CTTAD of nutrients and metabolic status, this experiment observed an overall increase in passive activities that was concomitant to less time eating and drinking, and less time for social interactions, which proved that animal welfare was not compromised with advancing lamb fattening, since reduced lying and increased standing have been considered as indicators of discomfort in feedlot environments (Commun et al., 2012). In addition, CTTAD of nutrients (except phosphorus) increased throughout the fattening period, which may be linked to improved digestive function and metabolic adaptation to energy-rich concentrate feeding, as glucose oxidation by rumen epithelial cells isolated from lambs is normally low at birth, peaks at 14 days of age, and then decreases to adult levels between 42 and 56 days of age (Baldwin and Jesse, 1992). The reduction of blood glucose throughout the fattening period may reflect a gradual rumen adaptation to digest more carbohydrates which produce more volatile fatty acids as a source of energy to the detriment of their intestinal hydrolysis. Even though blood glucose decreases, the apparent DM digestibility of growing lambs can be kept steady (Giráldez et al., 2021), which is in line with the present study. This may suggest that other gastrointestinal nutrients uptake or their liver synthesis sustains the energy balance in fattening lambs.

## 5. Conclusions

The results of this study indicate that dietary carob pulp inclusion up to 300 g/kg does not negatively affect animal daily gains, behaviour daily activity budgets and metabolic nutritional status, but reduces diet digestibility. However, the concentrate feed conversion ratio increases above 150 g/kg of carob pulp inclusion. Thereby, the dietary inclusion of up to 150 g/kg of carob pulp in the energy-rich concentrate-based diet for weaned light lambs would be a good trade-off to keep the digestibility of nutrients, without any detrimental effect on behaviour time budgets or peripheral markers of energy and protein metabolism. This feeding strategy would have positive implications to boost circular economy principles within the Mediterranean sheep production systems.

## CRedit authorship contribution statement

Javier Álvarez-Rodríguez, Beatriz Serrano-Pérez, Margalida Joy: Conceptualization. Daniel Villalba: Methodology. Jonathan Pelegrin-Valls, Daniel Villalba, Margalida Joy: Formal analysis. Jonathan Pelegrin-Valls, Beatriz Serrano-Pérez, Daniel Villalba, Ester Molina, Javier Álvarez-Rodríguez: Investigation. Jordi Espinal: Resources. Jonathan Pelegrin-Valls: Data curation, Visualization. Javier Álvarez-Rodríguez: Writing- Original draft. Jonathan Pelegrin-Valls, Beatriz Serrano-Pérez, Daniel Villalba, Ester Molina, Margalida Joy, Javier Álvarez-Rodríguez: Writing- Review and Editing. Daniel Villalba: Supervision. Margalida Joy, Javier Álvarez-Rodríguez: Funding acquisition.

## Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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