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Artificial milk preference of newborn lambs is prenatally influenced by transfer of the flavor from the maternal diet to the amniotic fluid

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Highlights

Cumin flavor is partially transferred from the pregnant ewe‘s diet to amniotic fluid

Prenatal cumin flavor experience drives lambs’ acceptance of flavored artificial milk

Abstract (204 words)

The present study examined in lambs whether exposure to flavors derived from pregnant mother‘s diet and transferred to amniotic fluid (AF) could induce a preference for artificial milk containing one of these flavors. To test this hypothesis, cumin was added to the maternal diet in the last month of gestation. Preference for artificial milk containing p-cymene, one of the chemosensory compounds of cumin, was tested within the first two days after birth in maternally deprived lambs born from mothers fed a cumin-flavored diet (Cumin group), or an unflavored diet (Control group). Aromatic profile of AF from cumin-fed mothers was analyzed by GC-MS/MS to determine whether p-cymene could be detected. While the control group avoided the flavored artificial milk on day 1, the Cumin group did not and showed a preference for the cumin-scented formula on day 2. GC-MS/MS profile of AF revealed that four of the main volatile cumin compounds, p-cymene, p-cymenene, β-pinene and γ-terpinene were present in variable amounts in all samples, p-cymene being the most frequently detected. These findings indicate that newborn lambs can memorize flavors from the mother’s diet present in AF and that prenatal experience influences their preference for an artificial milk containing one specific flavor.

Keywords: Prenatal environment, Olfaction, Flavor, Sheep, Artificial rearing, GC-MS analysis

1. Introduction

In vertebrates, the capacity of fetuses to memorize sensory information from the prenatal environment is widespread and thought to pre-adapt the future neonate to its postnatal environment [1-6]. Particularly, maternally-derived flavor components promote locomotion, suckling, and dietary preferences at birth or later. Commonly, neonates show a positive attraction towards chemosensory (olfactory/gustatory) compounds present in the maternal environment and this adaptive mechanism drives neonates towards presupposed safe chemosensory cues.

In mammals, amniotic fluid (AF) plays an important role in the development of chemosensory preferences. For instance, mouse pups [7, 8], rat pups [9, 10], piglets [11] and lambs [4] show an attraction to the odor of AF at birth. In rodents, attraction to the nest area, the mother and initiation of suckling can be driven by volatile olfactory cues released from the AF [7]. The odor of milk also has highly powerful effects on the newborn of rodents, lagomorphs and humans, inducing searching and grasping the nipples [for review: 12]. These two perinatal substrates share some identical olfactory information with similar motivational characteristics leading to the notion of transnatal chemosensory continuity that appears evolutionary conserved in various mammals [5, 13, 14]. As a result, rabbit [5], porcine [15] and human infants [16] fail to show a preference between odors of AF and colostrum at birth. Further evidence of transnatal chemosensory continuity is supported by the ability of newborns to prefer milk containing odorants that resemble those transferred from the maternal diet to the AF during gestation. Addition of artificial odors to the maternal diet could alter the odor of AF and milk in various species, eliciting subtle modifications of the maternal chemical signature that are perceived by newborns. These initially irrelevant odors once experienced in utero initiate suckling at birth [7] and trigger behavioral attraction [2, 17-

20]. For instance, newborn rabbits born to females fed with a cumin-added diet during pregnancy are attracted to colostrum from cumin-fed females [5].

In sheep, data on the early postnatal period are scarce. It has been shown that newborns display preference for AF paired with citral after prenatal exposure to this odorant, but that the behavioral response induced in lambs by maternal aromas appears to be weak [4] relatively to the behavioral effect of prenatal odorant exposure in the rat [21]. Some studies investigating the role of flavor prenatal exposure in feeding preferences after weaning reported a decreased [22] or an increased preference [23] for that flavor in solid feeds. However, no data is available concerning the impact of prenatal flavor exposure on the short-term preference for milk containing that flavor or some of its components, as it was reported for rabbit pups [5]. Because lambs belong to precocial species with fully functional sensory systems, it could be thought that they are less sensitive to prenatal chemosensory learning compared to altricial neonates (most rodents, lagomorphs, carnivores) which only rely on somesthesis and chemoreception. In addition, in dairy-farm systems, or when ewe's milk is not available in sufficient amount or of poor sanitary conditions, newborns are separated from their mothers after birth and fed a milk replacer from a feeder. However, they are not able to suck on their own and training is time-consuming [24]. One explanation for the unwillingness to drink artificial milk could be found in the chemosensory characteristics of the substitute as it is not made of ewe's milk, breaking the transnatal continuity. A more rapid acceptance of the artificial teat is reported when lambs were fed ewes milk instead of milk replacer [25]. Thus, testing the existence of prenatal chemosensory learning through a flavored diet given to the pregnant mothers may facilitate adaptation of lambs to artificial feeding, should the same flavors be added to the artificial milk. If this is the case, such methodology could then be adapted to suit farming conditions.

To this end, cumin was added as the source of flavors to the maternal diet in the last month of gestation. Cumin was chosen as a source of flavors because a previous report, as well as our pilot study, showed that it was easily and readily accepted by ewes [26]. Some of the odorant compounds, p-cymene, β-pinene, γ-terpinene and cuminaldehyde are transferred to milk [26]. It is also devoid of any fetal toxicity in sheep [27] as reported in humans [28] and rabbits [5]. At birth, preference of lambs born to odorized and non-odorized mothers was tested using a p-cymene flavored milk. Indeed, p-cymene is one of the two chemosensory compounds of cumin transferred into milk in quantifiable amounts, and the only one to be detected in both maternal plasma and milk [26]. However, as the presence of this compound in AF is uncertain [27], AF was also collected from cumin-fed and control mothers and its aromatic profile was analyzed by gas chromatography/tandem mass spectrometry (GC-MS/MS), which provides a high separation capability, sensitivity and specificity. According to the literature, our hypothesis was that the p-cymene compound would be transferred from the maternal diet to AF and, that lambs born from the cumin-fed ewes would exhibit an attraction towards p-cymene-flavored artificial milk, compared to an unflavored milk.

2. Material and methods

2.1. Animals, housing and flavor exposure

Experiments were conducted on 22 multiparous Ile de France ewes (3 years-old) from the INRAE experimental unit UEPAO Val de Loire (Indre et Loire, France). Animals were permanently housed indoors, with free access to water and were fed daily 600 g of concentrated pelleted feed mainly composed of wheat (60%), sunflower meal, dehydrated alfalfa granules, maize, molasses, 1% mineral-vitamin complement (Axereal Elevage, St Germain de Salles, France) and straw. Reproduction was fully

synchronized by using vaginal sponges impregnated with fluorogestone acetate for 14 days followed by an intra-muscular injection of pregnant-mare-stimulating gonadotropins to induce ovulation.

Thirty-seven days before parturition, all ewes were acclimated to their housing conditions (4x2 m individual pens) and fed with similar troughs so that ingestion of the dietary rations could be measured. During the last 30 days of gestation, 9 out of the 22 ewes randomly chosen, received a similar daily diet added with 250 g of cumin seeds (African ecotype, purchased at La Bovida, Paris, France). Each of the 9 ewes consumed 80% of their food during the first 4 days and 100% of the ration the following 26 days. The cumin-fed ewes were housed in a different room from that of the control ewes (n=13) to avoid any odorant cross-contamination.

Parturition was induced by an injection of 16 mg of dexamethasone (Dexadreson, Intervet France) on day 144 of gestation. Thus, lambing occurred within a period of 24-36 h, starting 36h after dexamethasone injection. Fifteen lambs from the 9 cumin-fed ewes composed the Cumin group (4 single, 8 twin and 3 triplet lambs). Fifteen lambs from the 13 ewes fed with the unflavored diet composed the Control group (11 single and 4 twin lambs). Each group consisted of 7 females and 8 males. No difference in body weight at birth was detected between groups (Control group: 4.11±0.7 kg; Cumin group: 3.69±0.9 kg, $p=0.2$, t test).

Immediately after expulsion, lambs were separated from their mother to avoid any postnatal contact with some cumin compounds via suckling. They were dried, ear-tagged, weighed and assigned to a straw-bedded pen (1x2m), 3 meters apart so that any visual or direct contact between the two groups was possible. Two hours after birth, lambs were bottle-fed twice with 100 ml of bovine colostrum (from our local

experimental farm) on their first day of life to ensure passive immunological protection. Bovine colostrum might contain p-cymene [29, 30] and one cannot exclude that lambs of both groups have been exposed to this compound 2 hours after birth. However, p-cymene is specifically found in milk of cows grazing natural diversified upland pasture, which was not the case for the cows reared in our research center.

2.2. Behavioral tests

Eight hours after birth, testing for milk preference started. Each lamb was transferred to an adjacent pen in which 2 rubber teats, 10 cm apart, fitted on a partition of the pen were connected to two graduated cylinders. One experimenter filled both cylinders with 300 ml of milk replacer (23% protein, 25% fat, 8% ash, 3.5% water, 0.15% cellulose, 2.1% minerals, 200 g/L freshly prepared, 30°C; Agnodor Tradition plus, Soreal Nutrition Animale, Vonnas, France), one being flavored with 10^{-6}M p-cymene. This concentration was chosen from a GC-MS/MS pilot analysis of AF from five cumin-fed ewes (not shown). P-cymene at 10^{-6}M could not be detected by an experimenter (not shown). At that age, lambs were not yet able to feed on their own and milk preference was determined as follows. Another experimenter, different from the one preparing the flavored or unflavored artificial milk, gently introduced one teat into the lamb's mouth, let it suck for 20 s and proceeded similarly with the other teat for 20 s. If the lamb rejected the teat, the experimenter reintroduced the teat in the lamb's mouth. The experimenter was not aware of which teat delivered the flavored or unflavored artificial milk and could not detected any odor difference. This procedure was performed 3 times so that a maximum of 60 s of suckling per teat was achieved. The experimenter who had prepared the unflavored and flavored milk recorded the volumes consumed during the three repetitions of the procedure by measuring the leftovers in each cylinder. He was different from the experimenter holding animals, but

was the same who recorded the volume of unflavored and flavored milk consumed during the three repetitions of the procedure. A session consisted of the three repetitions for each teat that delivered the flavored or unflavored milk. This session was repeated 4 times at 6-hour intervals, at 8, 14, 20, 26 hours of age. To control for side biases, the position of the teats was randomly assigned for the first session, then switched between two sessions. At the start of each session, milk replacement was freshly prepared at constant concentration (200 g/L) and temperature (30°C). To avoid cross-contamination, a plastic cylinder was designated to be filled with the flavored milk or with the unflavored milk. In addition, before each session both cylinders were washed with water.

Preference for the flavored milk on day 1 was calculated within each group as the total volume of flavored milk consumed during the four sessions divided by the total volume of both flavored and unflavored milk consumed.

At 36 hours of age (day 2), because lambs were able to feed on their own, they were allowed to choose to suck freely between the two rubber teats for 3 min (choice test). Before the start of the choice test, the experimenter held the lamb and made it sniff both teats for 5 s and then positioned the lamb on the midline between the teats. The volume of unflavored and flavored milk consumed was recorded at the end of the test. This choice test was performed 4 times at 6-hr intervals, at 36, 42, 48, 54 hours of age. Localization of the flavored milk was randomly assigned at the first choice test, and then systematically switched between two subsequent tests by the experimenter who had prepared both liquid feeds. Preference for the flavored milk on day 2 was calculated within each experimental group as the total volume of that milk consumed during the four choice tests divided by the total volume of both flavored and unflavored milk consumed. During the whole testing procedure lambs did not show any sign of

fear toward the experimenter and they were highly motivated to suck. To avoid any odor contamination, Control and Cumin groups were tested in different rooms. Consequently, the experimenter who handled the lambs was aware of the allocation of the lambs to a group.

2.3. Sampling procedure and GC-MS/MS analyses

To evaluate the presence of volatile compounds at birth, AF samples from Control group (n= 3) and Cumin group (n=11) lambs were collected from their coat at birth in glass vials and stored at -20°C until analysis.

For calibration purpose and identification of the main volatile components of cumin, a standard made of cumin essential oil (5 µl of a 10^{-5} diluted essential oil of *Cuminum cyminum*; AROMA-ZONE, France) was extracted by HeadSpace Solid-Phase Microextraction (HS-SPME) using a 2 cm DVB/CAR/PDMS fiber. It was incubated at 60°C for 30 min (agitation cycle 10 sec on /10 sec off) and the fiber (Triplus with SPME module; Thermo Scientific) was introduced after 15 min of incubation. The fiber was then placed into the injector port of the GC (trace 1300, Thermo Scientific) for 1 min at 250°C in splitless mode (held 0.5 min; 1.2 ml/min). Separation of the volatile components was performed on a DB-FFAP column (60 mx0.32 mm i.d., 1 mm film thickness; Agilent Technology). Helium was used as the carrier gas at a constant flow of 1.2 ml/min. The volatiles components were desorbed using a GC oven temperature program starting at 40°C, ramping to 240°C at a rate of 10°C x min^{-1}, with a final step at 240°C for 15 min. The mass spectrometer (TSQ 8000, Thermo Scientific) was operated in the electron impact (EI) ionization positive mode (70 eV). The temperatures of the transfer line and the ion source were set at 250°C and 260°C, respectively. Argon was used as collision gas. The parent ion characteristic (mass to charge ratio, m/z) of

each of the eight prominent volatile compounds was split into three specific product ions peaks as given (Table S1).

Then, GC-MS/MS analyses were carried out on crude extracts of AF in the same conditions. The abundance of each of the eight main compounds was calculated by summing areas under the curves (AUC) of the three product ions peaks in each AF sample. The relative abundance of each compound in individual AF for each lamb numbered from 1 to 2 (twins) or 3 (triplets) born to each mother (M) was calculated as the percentage of each AUC relative to the sum of AUC of all compounds in the same AF sample.

2.4. Statistical analysis

Due to lack of normality, preference for the flavored milk was analyzed with non-parametric tests. Inter-group comparisons were analyzed using two-tailed Mann-Whitney tests, and intra-group comparisons were analyzed using Wilcoxon tests. The preference was also compared to chance (0.5) using Wilcoxon tests. Statistical analyses were performed using the statistical package *Statistica 10* and the level of statistical significance was set at $p \leq 0.05$. All behavioral data are represented as median and interquartile ranges.

2.5. Ethics statement

Animal care and experimental treatments complied with the guidelines of the French Ministry of Agriculture for animal experimentation and European regulations on animal experimentation (86/609/EEC). They were performed in accordance with the local animal regulation (authorization N° 006352 of the French Ministry of Agriculture in accordance with EEC directive) and with the ethical committee Val de Loire N°19

(N° CE19 – 2015-01). All efforts were made to minimize the number of animals (15 animals per group).

3. Results

3.1. GC-MS/MS identified p-cymene as one of the major compounds detectable in AF from flavored ewes

The GC-MS/MS analyses of AF of randomly selected Cumin lambs (n=11) and Control lambs (n=3) at birth was focused on the eight prominent volatile compounds of *Cuminum Cyminum* (Fig. 1). Individual variations were noticed in AF of Cumin lambs, but none of these compounds was found in detectable levels in AF of Control lambs. While α-pinene, limonene, cuminaldehyde and 2-caren-10-al were almost undetectable in all AF samples, β-pinene, γ-terpinene, p-cymene and p-cymenene were detected above the basal noise in AF of Cumin lambs. β–pinene, p-cymene and γ-terpinene were the most abundant compounds, although their abundance displays large variations among AF samples. When expressed as a percentage of total compounds, the relative proportion of seven of the cumin compounds in AF was roughly similar to that we found in *Cuminum cyminum* essential oil (Table 1). Only the cuminaldehyde that represents 24% of the eight selected volatile compounds in cumin essential oil was almost undetectable in all AF. Large qualitative variations were observed in the aromatic profile between AF of lambs from siblings (Table 1).

3.2. Newborn cumin-enriched lambs exhibit preference for flavored milk

Analysis of the preference for the flavored milk, test by test, did not reveal any significant difference between both groups or within a group across time either on day 1 or on day 2 (data not shown, $p>0.05$ for the eight comparisons). However, when

pooling the four sessions on day 1, preference for the flavored milk was significantly higher in the Cumin than in the Control group (Z=2.53, p=0.009, Fig. 2). The total volume of flavored and unflavored milk ingested during the four sessions did not significantly differ between both groups (Control *vs* Cumin: 345 ml [285-415] vs 300 ml [220-370], Z=1.36, p=0.17). Intra-group comparisons revealed that the preference for the flavored milk in the Control group was significantly lower than by chance (Z=2.83, p=0.004), whereas it did not differ from chance in the Cumin group (Z=0.9, p=0.36).

On day 2, preference for the flavored milk was significantly higher in the Cumin than in the Control group (Z=2.32, p=0.018, Fig. 2). The total quantity of flavored and unflavored milk ingested during the four choice tests did not significantly differ between both groups (Control *vs* Cumin: 500 ml [320-820] vs 425 ml [235-560], Z=1.24, p=0.21). Intra-group comparisons revealed that the preference for the flavored milk in the Cumin group was significantly higher than by chance (Z=2.83, p=0.004) whereas it did not differ from chance in the Control group (Z=0.11, p=0.9).

No statistical difference in the preference for the flavored milk was observed between day 1 and day 2 in the Control group (Z=1.36, p=0.17), while the preference increased on day 2 in the Cumin group (Z=1.98, p=0.04). The total volume of flavored and unflavored milk ingested during the two days of testing did not significantly differ between both groups (Control *vs* Cumin: 980 ml [605-1260] vs 755 ml [620-900], Z=1.24, p=0.21). There were no significant differences in the preference for the flavored milk between males and females either at day 1 (Z=1.22, p=0.2) or at day 2 (Z=0.5, p=0.5) or between single lambs and twin/triplet lambs either at day 1 (Z=1.41, p=0.16) or at day 2 (Z=0.5, p=0.5).

4. Discussion

The study shows that prenatal experience with complex flavors influences behavioral feeding-related responses to one of its components when added to artificial milk. Specifically, this is the first evidence in lambs that a chemosensory compound, p-cymene, is transmitted from the mother's diet to AF and then drives a preference for it when added in artificial milk within 2 days after birth.

Cumin was chosen as a source of flavors because it was easily and readily accepted by ewes [26] and devoid of any fetal toxicity [27]. We extend here the observation that a longer period of cumin consumption affected neither diet consumption of mothers nor fetal growth of lambs. Another characteristic of cumin is that, some of its flavor components were already shown to be transferred into milk and plasma in ewes after a single intake of cumin seeds [26]. Mainly p-cymene, β-pinene, and to a lesser extent γ-terpinene and cuminaldehyde, were identified in milk and, only p-cymene was detected in plasma. The present study clearly shows that p-cymene is also transferred into AF. This compound, together with p-cymenene, β-pinene and γ-terpinene was found in almost all samples of AF of cumin-fed lambs in quantifiable amounts, albeit remaining low for γ-terpinene. The fact that these compounds were not detectable in AF in the study by Schaal et al. [27] can be explained by a difference in sample preparation and extraction, in the use of a tandem mass spectrometry procedure, 10 times more sensitive than a single MS, or in a longer time exposure window to cumin diet during gestation. However, in both studies, limonene and cuminaldehyde remained undetectable, indicating that some components of cumin aromas cannot be transferred into the fetal compartment and are probably metabolized. Such qualitative variations in the efficiency of transfer of individual aromatic compounds, in or between samples have been described in AF [31] and lacteal matrix for other compounds [32-35]. A chemosensory qualitative and/or

quantitative difference between the maternal diet flavor and the conveyed compounds in AF could participate to equivocal postnatal behavioral responses to compounds that are partly transferred in maternal fluids [4, 21, 36].

The postnatal behavioral response associated with the presence of p-cymene in AF indicates that lambs from cumin-fed mothers developed a clear preference for the artificial milk containing this compound within 48 hr. On day 1, the proportion of flavored milk ingested by Control lambs was less than that what was expected by chance, indicating a slight aversion to p-cymene. Cumin lambs, on the other hand, did not show such aversion since the proportion of flavored milk they ingested did not differ from random. This indicates that the two milks were discriminated based on olfactory/gustatory cues by both groups and that the behavioral differences relied on prenatal experience. Behavior of lambs on day 1 is unlikely to result from a difference in the handling procedure during the test. Helping lambs to feed is a mandatory step, as lambs must learn how to suck artificial teats. Since the experimenters were not aware of which teat delivered the flavored or unflavored artificial milk they could not bias the lamb's choice. On day 2, while aversion weakened in Control lambs, the preference for the flavored milk emerged in Cumin lambs, the proportion of flavored milk ingested being higher in comparison to chance. In addition, on both days, this proportion was significantly higher in the Cumin group. It is unlikely that differences in food motivation accounts for these behavioral effects since the total amount of milk ingested during the tests did not differ between both groups. Among other parameters that may have contributed to the observed effect, we cannot exclude that the supplementation in cumin seed has provided a caloric increase beneficial to the mothers. However, such enrichment did not modify lamb's weight by the time of birth nor the total amount of milk ingested.

Whether the development of a preference for the flavored artificial milk is specific to p-cymene or could expand to other flavors was not the aim of the present study but it would have been interesting to test the preference of Cumin lambs for other odorants or to add another experimental group fed another flavored mother's diet.

Hesitancy to consume unfamiliar foods usually referred as 'food neophobia' is widespread throughout the animal kingdom including sheep [1, 37-40] and p-cymene avoidance in control lambs on day 1 could be explained as such. The lack of avoidance expressed by Cumin lambs indicates that the chemosensory cues had been memorized and were perceived as familiar. Similar attenuation of food neophobia was reported in newborn mice exposed to an aversive flavor during their fetal life [41].

On day 1, the behavioral effect induced in Cumin lambs by the presence of p-cymene in the artificial milk was unnoticeable since no clear preference was observed, even though the GC-MS/MS analysis indicated that p-cymene is found in quantifiable amount in AF. Likewise, newborn lambs exposed to citral in utero do not express a preference for that odorant relatively to AF odor [4]. It may be possible that the concentration of p-cymene added to the artificial milk was inadequate to induce a preference. The weak effect observed in Cumin lambs on day 1 could also be due to the fact that p-cymene on its own, like citral, is not an appropriate stimulus and that using a mix of compounds might have induced an immediate postnatal preference. Indeed, a blend of compounds representative of the cumin aromas transferable to AF, including β-pinene, γ-terpinene, and p-cymenene in addition to p-cymene, as our GC-MS/MS analysis suggested, would have been more efficient to induce a behavioral response.

The slight qualitative variations observed in the aromatic profile between AF of lambs suggest a possible differential impregnation of animals through maternal diet

that could participate in the diversity of behavioral postnatal responses to p-cymene. However, in absence of internal standards or calibration curve, the GC-MS/MS prevents us to calculate the abundance of each compound. Whether the relative p-cymene abundance as measured by GC-MS/MS at birth is directly correlated to the in utero exposure remains to be demonstrated. Indeed, several parameters could have participated in both intra- and inter-variability. Physiologically, the olfactory impregnation depends on the elapsed time between maternal food consumption and AF sampling, on the heterogeneity of transfer between compounds and on the metabolic kinetics. Methodologically, conditions of sample collection (volume, viscosity), elapsed time between collection and analyses, might have also interfered with the GC-MS/MS.

Because lambs were submitted to repetitive sessions on day 1, it is conceivable that the gustatory and post-ingestive effects of the flavored milk could have become more prevalent to establish a preference than its sole chemosensory properties. However, avoidance of p–cymene in Control lambs could not be due to negative post-ingestive consequences since it vanished on day 2 and was never seen in Cumin lambs.

Our findings that p-cymene induced a clear preference on day 2 in Cumin lambs points out the remarkable ability of newborn lambs to detect and memorize a single flavor from the composite aroma of AF, as shown in rabbit pups [5], in rat pups [6, 17] and in babies [42]. The present study supports earlier research reporting that newborn lambs display preference for their own AF compared to AF of unfamiliar ewes [4] despite very similar chromatographic profiles [43]. Thus, it appears that precocial neonates possess selective perception and learning of the chemosensory properties of their fetal environment, similarly to altricial newborns. This prenatal chemosensory

experience may play a key role in shaping the development of postnatal feeding behaviors by orienting the newborn towards familiar chemosensory compounds.

The current study supports the hypothesis that flavor preference displayed at birth by lambs can be shaped by prenatal chemosensory experience through maternal diet. In sheep dairy farming systems, or when mother's milk is not sufficient to feed the whole litter, newborns are separated soon after birth and fed a milk replacer. Training lamb to suck is time-consuming and lamb mortality can be high [44]. Flavor exposure during the last month of gestation could be a beneficial method to adapt artificially reared lambs to their new diet by adding the same flavor to milk replacement. Thus, both the welfare and performance of artificially reared lambs may be improved, but some precautions should be required. In young mammals, graded concentrations of odors can elicit graded responses ranging from attraction to avoidance [45]. Because both trigeminal and olfactory receptors are present within the nasal cavity, adding flavors in the maternal diet may also have negative perceptual consequences. Installing olfactory continuity between the prenatal and post-natal environment – with a moderate intensity of chemosensory signals – may be the best option to help young lambs to orient positively towards artificial milks. Further studies are required to better characterize the most efficient blend of cumin aroma and concentration to facilitate suckling at an automatic feeder.

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Declaration of Competing Interest

The authors have no financial or non-financial competing interests to declare.

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Figure captions

Figure 1: Abundance of the eight main *Cuminum cyminum* volatile compounds in amniotic fluid collected on the lamb's coat and born to ewes that had received cumin seeds or not in their diet during the last month of gestation.

The abundance of each compound, expressed as the areas under the curve of their three product ions from MS-MS, is represented as a scatter boxplot with dots representing individual amniotic fluid. C: Control lambs born to non-cumin fed mothers, n=3; Cum: lambs born to cumin-fed mothers, n=11.

Figure 2. Preference for p-cymene flavored artificial milk in lambs born to ewes that had received cumin seeds or not in their diet during the last month of gestation.

Preference for the flavored milk was expressed within each experimental group as the total volume of that milk consumed on day 1 or day 2 divided by the total volume of both flavored and unflavored milk consumed). Medians, interquartile ranges and individual data are represented. # Different from chance, $p<0.01$.

Table 1: Relative abundance of each compound in individual amniotic fluid of lambs born to cumin-fed mothers.

Heat map showing the relative abundance of each compound in individual amniotic fluid expressed as a percentage of total aromatic compounds. M: mother; Cumin Oil: *Cuminum cyminum* essential oil, dilution factor 10^{-5}.

Table S1: MS parameters and energy transitions monitored for the eight main *Cuminum cyminum* volatile compounds analysed by GC-MS/MS. m/z: mass to charge ratio.

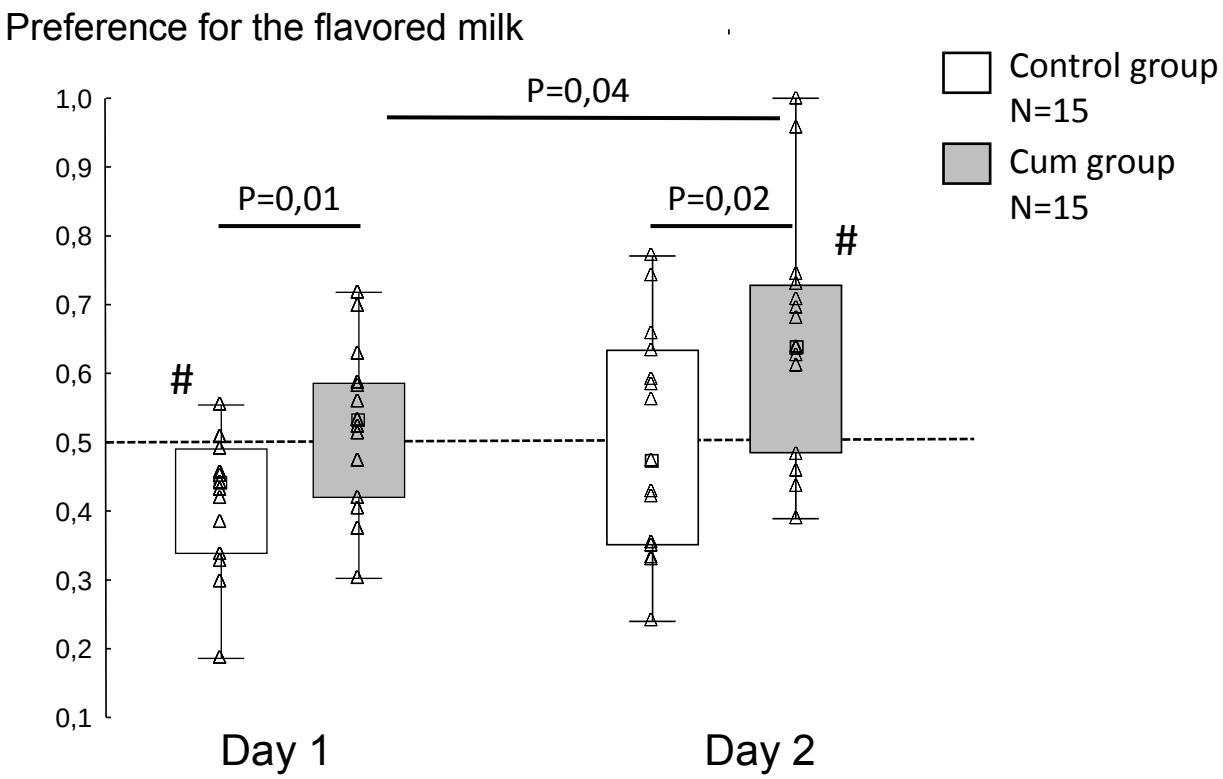

Preference for the flavored milk
Control group
N=15
Cum group
N=15
P=0,04
P=0,01
P=0,02
#
#
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
Day 1
Day 2

Figure 1

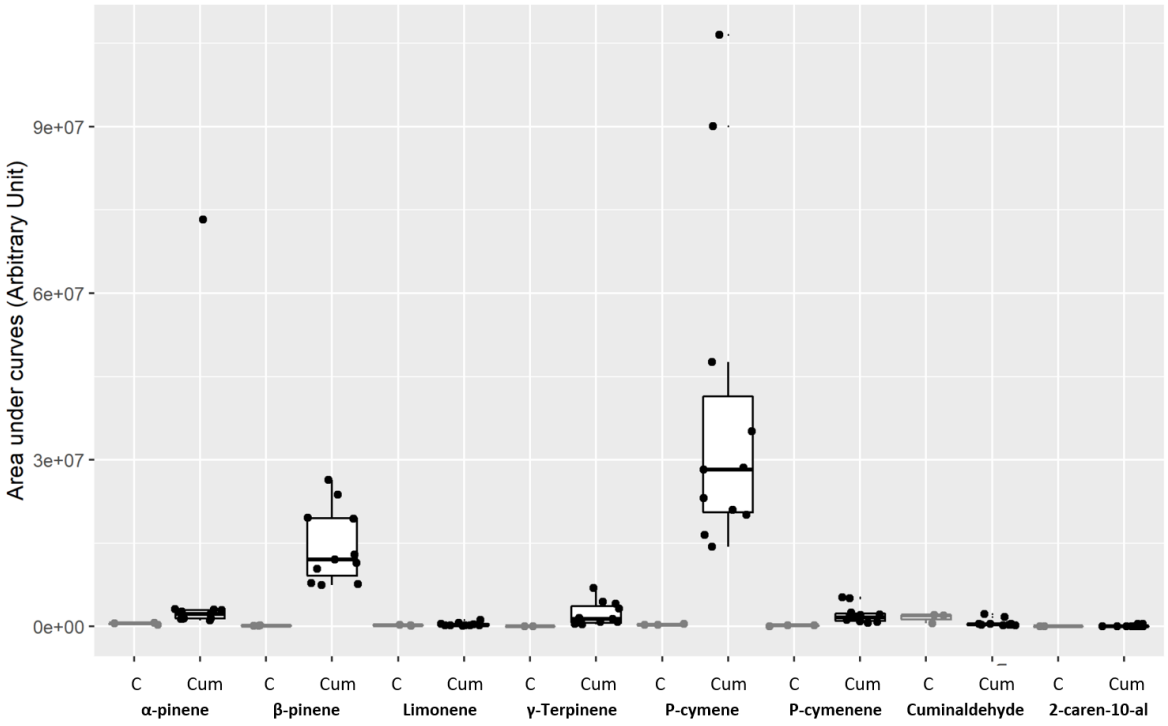

Area under curves (Arbitrary Unit)
9e+07
6e+07
3e+07
0e+00
C
Cum
α-pinene
β-pinene
Limonene
γ-Terpinene
P-cymene
P-cymenene
Cuminaldehyde
2-caren-10-al

Figure 2

Compound	retention time (min)	parent ion (m/z)	product ion (m/z)	collision energy (eV)
α-pinene	8.68	92.8	50.7	22.00
		92.8	77.1	12.00
		92.8	91.1	6.00
β-pinene	10.22	93	51	22.00
		93	77.1	10.00
		93	91.1	6.00
Limonene	11.58	68.2	39.5	18.00
		68.2	53.1	10.00
		68.2	67.1	4.00
γ-terpinene	12.33	136.2	77.1	26.00
		136.2	93.1	10.00
		136.2	121	8.00
p-cymene	12.79	119.1	77.2	20.00
		119.1	91	12.00
		119.1	116.8	8.00
p-cymenene	15.25	132.2	91.1	24.00
		132.2	115.1	20.00
		132.2	117.1	12.00
Cuminaldehyde	20.05	148	105.1	16.00
		148	119.1	6.00
		148	133.1	8.00
2-caren-10-al	20.32	106.9	51	32.00
		106.9	77	18.00
		106.9	79.1	8.00

Table 1 suppl

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	α-pinene	β-pinene	Limonene	γ-terpinene	p-Cymene	p-Cymenene	Cuminaldehyde	2-Canen-10-al
RT	8.68	10.22	11.58	12.33	12.79	15.25	20.12	20.32
M1-Lamb	2	13	0	2	78	4	1	0
M2-Lamb 1	4	22	1	1	68	3	1	0
M2-Lamb 2	72	12	1	0	14	1	0	0
M2-Lamb 3	8	26	1	3	58	3	1	0
M3-Lamb 1	2	18	0	5	71.5	3.5	0	0
M3-Lamb 2	3	16	0	4	73	4	0	0
M4-Lamb	4	34	1	5	50	3	3	0
M5-Lamb 1	4	32	0	2	56	5	1	0
M5-Lamb 2	4	28	1	2	58	6	1	0
M6-Lamb 1	5	33	1	7	48	2	3	1
M6-Lamb 2	3	28	0	3	62	3	1	0
Cum Oil	1	20	0.5	7	45	0.5	24	2

Table 1

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