

Exposure to a novel feedstuff by goat dams during pregnancy and lactation versus pregnancy alone does not further improve post-weaning acceptance of this feedstuff by their kids

Phan Vu Hai,^{a*} J Thomas Schonewille,^b Tien Dam Van,^a Henk Everts^b and Wouter H Hendriks^b

Abstract

BACKGROUND: Previous experiments demonstrated the existence of *in utero* learning in goats. However, in contrast to other animal species, in goats there is no information about the potential of flavour transmission from maternal feed to goat kids during lactation. The aim of the current study was to assess the role of post-natal exposure of *Chromonaela odorata* leaf meal (COLM) in relation to the preferences to this feedstuff by goat kids after weaning. It was hypothesised that exposure of COLM to the dams during both pregnancy and lactation *versus* pregnancy alone, additionally affects post-weaning intake of COLM by their offspring.

RESULTS: Consumption of COLM by the goat kids was similar during the first week post-weaning for all treatments. However, after 4 weeks the intake of COLM was at least 1.8 times greater when kids were exposed to COLM during pregnancy whereas it remained virtually unchanged when kids were exposed to COLM during lactation only. The increase in COLM consumption was in line with the observations on latency to eat and meal size.

CONCLUSION: Transmission of feeding behaviour from goat dams to offspring does not occur during lactation. However, the concept of *in utero* learning in goats was confirmed.

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Keywords: feeding behaviour; *Chromonaela odorata*; pregnancy; goat kids

INTRODUCTION

Nowadays, socio-economic and environmental issues such as bio-fuel production, methane mitigating and development of new human foods, enforces the innovation of processing technologies of raw biomaterials, thereby yielding a variety of novel by-products which are of potential interest in animal feeding, including goats. However, the successful feeding of novel feedstuffs to goats is generally frustrated by neophobia.^{1,2}

It is well known that flavours from the diet consumed by pregnant animals can reach the foetus,^{3,4} thereby influencing elements of the chemosensory system of the foetus and consequently increase the saliency of the stimulus when experienced after birth.^{5,6} As such, introduction of a novel feedstuff during pregnancy, may improve the intake of this feedstuff by the offspring after weaning. This mechanism in goats as shown by our earlier work^{7–9} clearly demonstrates an improved acceptance of *Chromoneala odorata* by weaned goat kids after prenatal exposure to this feedstuff. In these studies,^{7–9} *C. odorata* was used as a test feedstuff because goats are reluctant to consume it voluntarily.

Next to *in utero* learning, flavours can also be experienced post-natally through the consumption of maternal milk in rabbits,¹⁰ dogs,⁵ and humans.¹¹ However, to the authors' knowledge, there is no information available regarding the vertical transmission of flavour during the suckling period in goats. The latter could provide another mechanism to overcome neophobia in goats and increase feed intake of ingredients avoided by goats. This reasoning is corroborated by the observations in rabbits, pigs and humans indicating that both pre- and post-natal learning impact feed preferences. In the present study we therefore tested whether the ingestion of *C. odorata* by dams during lactation

* Correspondence to: Phan Vu Hai, Faculty of Animal Science and Veterinary Medicine, Hue University of Agriculture and Forestry, Hue University, 102 Phung Hung St, Hue, Vietnam. E-mail: hai.phanvu@huaf.edu.vn

^a Faculty of Animal Science and Veterinary Medicine, Hue University of Agriculture and Forestry, Hue University, 102 Phung Hung St, Hue, Vietnam

^b Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.151, 3508 TD, Utrecht, the Netherlands

improves the consumption of *C. odorata* by their kids after weaning. It was hypothesised that exposure of *C. odorata* to the dams during both pregnancy and lactation versus pregnancy alone, additionally increases post-weaning intake of *C. odorata* by their offspring.

MATERIAL AND METHODS

Animals, feeds and management

This study was conducted in accordance with the recommendations in the European Community directive 86/609/EEC for accommodation and care of animals. The experiment was carried out in the Experimental Farm of Hue University of Agriculture and Forestry (HUAF), Hue, Vietnam.

Forty-nine, 20–24-month-old, healthy local female goats (Co breed) that had lambed once, with an initial mean (\pm SEM) body weight of 39 ± 1.8 kg were used. Goat dams were individually housed in wooden pens (1.2×2 m). The goats had not had any previous exposure to *C. odorata* before the experiment. The goats were vaccinated against foot-and-mouth disease (Aftovax, 3 type, O, A, Asia1; Gaborone, Botswana) and were de-wormed (Ivermectin; Hanvet Co., Hanoi, Vietnam), 1 month before the experiment. Goats were synchronised using intravaginal sponges, which were impregnated with 60 mg medroxy-progesterone acetate (Veramix; Pharmacia & Upjohn, Orangeville, Canada) and inserted for 14 days at an unknown stage of the oestrous cycle. At the time of sponge removal, the females received an intramuscular injection of 400 IU PMSG (Folligon; Intervet, Boxmeer, the Netherlands). Goats were inseminated with fresh diluted pooled semen (0.25 mL containing 300 to 400 million sperm diluted in homogenised–pasteurised skim milk) collected from four fertile Co bucks by an artificial vagina. Pregnancy was confirmed by not returning to oestrus and by the use of a Preg-Tone detector (Renco Corp., Minneapolis, MN, USA) at day 30 to 40 after insemination. At 40 days after insemination, 32 pregnant goats were randomly allocated to four groups (eight animals per group). Selected goat kids remained with their mothers till weaning. Testing the feed intake of the kids started after weaning.

Selected kids were individually housed after weaning. The experimental barn was divided into four areas separated by a 2.5 m wide aisle. Each area consisted of a row of eight metal pens (1.2×2.0 m) which were separated by a solid dark plastic sheet, thereby avoiding visual or physical contact between neighbouring goats. During the time of lactation, the pens were enlarged to nearly double their size. The feeds used in the experiment were elephant grass and an agro-industrial by-product mixture (AIBM), which included (g kg^{-1} as fed) rice bran (500 g kg^{-1}), cassava leaves and stems (200 g kg^{-1}), beer residue (200 g kg^{-1}) and cassava root residue (100 g kg^{-1}). Grass was collected from existing pastures of the Livestock Research Centre (HUAF) twice a day, at 07:00 hours and 14:00 hours, and subsequently chopped to a length of 10 to 20 cm, directly before feeding. All animals had free access to mineral blocks (630 g kg^{-1} NaCl, 90 g kg^{-1} Ca, 110 g kg^{-1} P, 12.6 g kg^{-1} Mg, 10 g kg^{-1} Fe, 1.5 g kg^{-1} Cu, 1.2 g kg^{-1} Mn, 0.5 g kg^{-1} I, and 0.1 g kg^{-1} Co) and fresh water.

Experimental treatments

C. odorata leaves were harvested at 120 days after germination, between 10 and 40 cm from the top of the plant and sun dried. Then, the leaves were separated and crumbled by hand to produce *C. odorata* leaf meal (COLM). Only *C. odorata* leaves at the top of

Table 1. Chemical composition of *Chromolaena odorata* leaf meal

Parameter	Mean \pm SD
Dry matter (g kg^{-1})	853 ± 1.6
Crude protein (g kg^{-1} DM)	190 ± 0.8
Ether extract (g kg^{-1} DM)	21 ± 0.3
Ash (g kg^{-1} DM)	56 ± 0.5
Crude fibre (g kg^{-1} DM)	113 ± 1.1
Nitrogen free extract (g kg^{-1} DM)	621 ± 3.7

the plant were used because N-oxide of pyrrolizidine alkaloids – a harmful chemical to goats – is reported to be low compared to other parts of the plant.¹² Young leaves (10 cm from the top) were deliberately avoided due to the possibility of high concentrations of nitrate.¹³ Proximate analysis of the COLM (Table 1) was performed in triplicate at the Central Laboratory of HUAF, Hue, Vietnam.

Each goat was fed 100 g of AIBM (as fed) either without (control) or with 50 g of COLM (as fed) at 10:00 hours during 30 min. The AIBM containing COLM was fed either between the 50th and the 145th day of pregnancy (PREG group) or during 3 months of the lactation (day 3 to 90 after parturition) (LAC group) or during both pregnancy and lactation (PREG + LAC group). COLM treatment was terminated on day 141 of gestation, about 1 week before farrowing, to prevent flavour exposure through the mother's skin, faeces, fur, or breath before the post-natal COLM treatment started. Kids were separated from their mothers by a dark plastic fence during the time of feeding COLM and 1 h after, to avoid physical contact.

All dam goats consumed fresh elephant grass (DM of 3% of body weight) twice a day at 11:00 and 16:00 hours and received an additional 200 g (as fed) of AIBM at 20:00 hours per head per day. During the 3 month lactation period, AIBM was increased to 500 g (as fed) per head per day for dams with one kid or 600 g (as fed) for dams with two kids. The kids were weaned at 3 months and one kid from each goat dam was selected to measure voluntary intake of COLM. The selected kids were individually housed. The distribution of kids of different sexes was balanced for treatments (the ratio of male and female kids was 4:4 for all groups). Kids were fed *ad libitum* hay and up to 500 g (as fed) per day of a mixture containing locally purchased soybean meal (250 g as fed) and rice bran (250 g as fed). At 23:00 hours, all feed provided to the kids was withdrawn and the kids had no access to feed before measurement of voluntary COLM intake at 08:30 hours the next morning. At the start of a 30 min COLM intake measurement, 50 g of COLM (as fed) was provided to each kid. At the end of the measuring period, COLM were removed and weighed. The total experimental period lasted 4 weeks during which time COLM intake and feeding behaviour of the kids was recorded daily (a total of 28 measurements for each kid).

Measurements

Feed intake was measured as the difference between the quantities offered and refused as measured by a digital balance (iBalance 201 ($200 \text{ g } 0.01 \text{ g}^{-1}$; My weigh Co., West Yorkshire, England). Feeding behaviour of the kids was monitored by a time-lapse video recording system.¹⁴ Computer connected webcams (Logitech® Webcam Pro 9000; Lausanne, Switzerland) were mounted at 1.5 m height above each individual pen floor (32 webcams) and controlled by i-Catcher Sentry (ver.2) software (iCode Systems Ltd,

Southampton, England). The feeding behaviour of all animals was continuously recorded for 30 min when COLM was offered. The calculated variables from records were based on the video motion analysis software: Motionpro (CyberAccess123 Inc., OH, USA), in which specific functions such as 'stopwatch' and 'movement' are available. The lip, jaw and forehead of the kids were identified, within the software package, by means of different colour markers to monitor the movement of different parts of the goat's head. The movement of the markers was used to calculate the duration of each movement. Based on the recorded jaw movement,¹⁵ eating was defined as the total time when the goat was eating from the feed bunk with its muzzle in the feed bunk or chewing or swallowing feed with its head above the feed bunk. Latency to eat (min) was defined as the time between the goat standing at the trough and taking the first bite.¹⁶ If a goat kept its head in the trough for more than 15 s, this was considered a visit to the trough. A visit with COLM intake was recorded as a meal, otherwise it was recorded as a visit without intake. Meal frequency was the total number of visits to the trough with COLM being consumed during testing. Eating bout length was calculated as the total eating time divided by meal frequency. Total COLM intake was calculated as the difference between the amount of COLM offered and that left at the end of the 30 min testing. Intake rate was calculated as the intake of COLM divided by the eating time and meal size was intake of COLM divided by the number of meals.

Data analyses

All experimental data were analysed using IBM SPSS Statistics 20.0 for Windows (NY, USA). Prior to statistical analysis, the daily feed intake of the kids was averaged per week. Data within each of the experimental treatments was normally distributed (Kolmogorov–Smirnov test). Data were analysed using repeated measures ANOVA with treatment as factor. Post-hoc tests with Bonferroni correction were used to identify groups with different effects on the variable involved. Data were subjected to a two-sided *t*-test to separate treatment effects in each week. Differences within a treatment between weeks were also tested using a two-sided *t*-test. Throughout, the level of statistical significance was pre-set at $P < 0.05$.

RESULTS

Reproductive performance of the dams and body weight of the selected kids

The dams and kids used for experiments remained healthy during the experiment. All goats farrowed within a 6-day period (gestation length of 148–154 d). On average, 29 males and 24 female kids were born to the dams in the four groups (1.66 kids per dam).

Mean body weight (\pm SE) of the selected kids at birth was 2.7 kg (± 0.22) and increased to 17.1 kg (± 0.72) at weaning (3 months of age). For all groups combined, the mean body weight (\pm SE) of the selected goat kids at the end of the experiment (4 months of age) was 22.6 kg (± 1.09). The mean body weight of the goat kids did not differ significantly among the treatments at any point in time. Goat kids were apparently healthy during the experiment.

Intake of *C. odorata* by the dams and goat kids

The dams completely consumed the offered amounts of AIBM mixture with or without COLM during pregnancy and lactation. During the post-weaning period, kids consumed, on average, less than 10 g per day of COLM during the first week of the experiment without significant differences between the four treatment groups (Table 2). In the subsequent 3 weeks, COLM intake of the kids gradually increased in the PREG and PREG + LAC groups (interaction between treatment and time $P < 0.01$) and mean daily COLM intakes per week were found to be 1.8 and 1.9 times greater in the respective groups during the last week of the experiment (Table 2). In contrast, mean daily intake per week of COLM remained constant for the goat kids in the control and the LAC group (Table 2). Finally, the difference in COLM intake between the control and the LAC group on the one hand, and the PREG and PREG + LAC group on the other hand, became apparent (interaction between treatment and time $P < 0.01$) 10 days after the start of the experimental period (Fig. 1). During the first 9 days, responses in COLM intake were similar between the treatment groups.

Feeding behaviours related to the acceptance of COLM

Besides the higher consumption of COLM by kids from the dams fed COLM during pregnancy (Table 2 and Fig. 1), the goat kids displayed their preference for the COLM by a significantly ($P < 0.05$) shorter latency to eat and a larger meal size (Table 3). The consumption of larger meals was associated with a longer chewing time but the difference between the experimental groups was found to be non-significant. The number of visits without COLM intake, meal frequency, eating rate and eating bout length did not differ between the treatments.

DISCUSSION

The outcome of the current experiment clearly shows that the consumption of COLM by dams solely during lactation alone did not improve COLM intake nor changed feeding behaviour of the goat kids. This result is in contrast to studies in humans, rats, and rabbits, in which exposure to the mother's milk alone led to a shift in the preference of the offspring.^{10,11,17} These observations are in line with the notion that flavours of the maternal diet

Table 2. Average post-weaning intake (g DM) of *Chromonaela odorata* leaf meal (COLM) during 30 min by goat kids ($n = 8$) born to dams not fed COLM (Control) or fed COLM during pregnancy (PREG), lactation (LAC) or PREG + LAC

Week post-weaning	Control	PREG	LAC	PREG-LAC	Pooled SEM	<i>P</i> -value (treatment)
1	7.9	9.6 ^a	8.3	9.9 ^a	2.25	0.149
2	9.3 ^A	12.5 ^{bB}	9.0 ^A	14.1 ^{bB}	2.68	0.027
3	7.6 ^A	16.3 ^{cB}	10.1 ^A	14.5 ^{bB}	2.85	0.007
4	7.8 ^A	17.2 ^{cB}	9.1 ^A	19.1 ^{cB}	3.25	0.010
<i>P</i> -value (week)	0.105	0.003	0.399	0.001	–	–

Means with different superscripts within columns (a,b,c) or rows (A,B) differ significantly ($P < 0.05$), *P* treatment \times week < 0.01 .

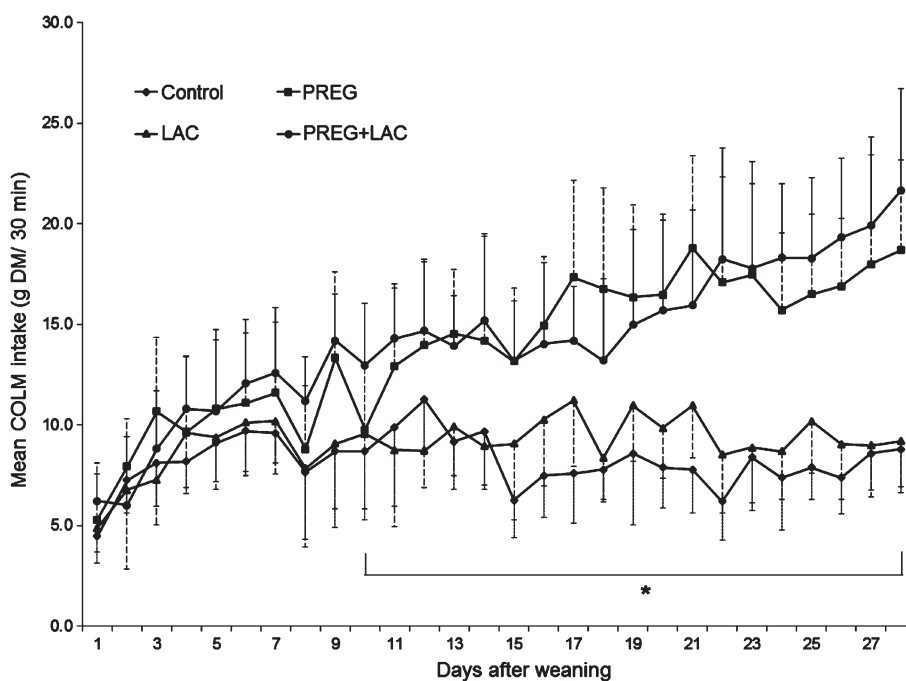


Figure 1. Daily mean (\pm SEM), post-weaning intake of *Chromonaela odorata* leaf meal (COLM) during 30 min by goat kids born to does fed COLM during pregnancy (PREG), lactation (LAC), PREG + LAC or not fed COLM (Control). *Indicates differences ($P < 0.05$) among groups for individual days (P for treatment \times days, < 0.01).

Table 3. Mean values of selected indices of feed acceptance of *Chromonaela odorata* leaf meal (COLM) by goat kids ($n = 8$) born to dams fed no COLM (Control) or fed COLM during pregnancy (PREG), lactation (LAC) or PREG + LAC

Parameter	Control	PREG	LAC	PREG + LAC	Pooled SEM	P-value
Latency to eat (min)	2.56 ^a	1.72 ^b	1.89 ^b	1.56 ^b	0.563	0.031
Meal frequency (times)	4.31	3.25	3.87	4.11	1.838	0.638
Eating bout length (min meal ⁻¹)	1.29	1.35	1.88	1.49	0.753	0.841
Number of visits without intake (times)	1.69	2.55	2.15	3.16	1.025	0.377
Intake rate (g DM min ⁻¹)	2.29	2.54	2.46	3.05	0.538	0.495
Meal size (g DM meal ⁻¹)	1.83 ^a	4.28 ^b	2.45 ^a	3.50 ^b	1.095	0.011
Chewing time (min)	6.01	9.11	6.15	8.12	1.355	0.722

Values represent 30-min daily observations over a 4 week period. Means within row with different superscripts differ significantly ($P < 0.05$). DM, dry matter.

are transmitted to the offspring through milk, and the offspring more readily accept flavours which they have already experienced through milk when fed as solid foods/feeds at weaning.

However, in other species, post-natal exposure alone may be an unimportant mechanism to modify offspring's preference. For example, post-natal exposure to anise did not change the preference for this flavour in dogs⁵ and pigs.¹⁸ According to our observations, goats belong to the group of animal species in which the transfer of flavour preferences from the mother to the kids does not occur via milk. Obviously, the current study does not provide data to explain the lack of response to maternal milk in goat kids, but it can be speculated that the relative maturity of goat kids at birth plays a role. The goat is considered to be a precocial animal and in such animals the brain undergoes a prenatal growth spurt.¹⁹ Such brains are probably less plastic in the period after birth compared to altricial species such as humans, rats, and rabbits,²⁰ and therefore less sensitive to post-natal modification of flavour preference through milk. Furthermore, precocial animals

generally start exploring and consuming solid feed relatively soon after birth.²⁰ Therefore, brain related programming of feed preference after birth,²¹ may be less beneficial. The exception for dogs may be related to the fact that wolves do not need to experience differences in feed materials as they are carnivores.

The higher acceptance to COLM after weaning of goat kids with *in utero* exposure of COLM is consistent with the outcome of our previous studies.⁷⁻⁹ It is believed that flavours from the mother's diet are transmitted to the amniotic fluid and swallowed by the foetus.^{11,22} Exposure to dietary flavours in amniotic fluid may be one of the ways for the dam to teach her offspring during gestation about feeds which are 'safe' to consume. The low palatability of *C. odorata*, which leads to a low feed intake, is presumably due to its strong smell. In goats, odour-feedback interactions decrease the probability of plant ingestion when post-ingestive consequences are aversive and increases the probability of plant ingestion when the consequences are positive.¹ Here, low palatability was overcome by '*in utero* learning'.

The present result is in line with the findings in a cross-fostering experiment,⁸ showing that the consumption of milk without exposure during pregnancy does not improve the intake of COLM by the kids after weaning. This observation proved that there is no indirect transfer of the flavour preferences via body reserves of the dam.⁸ The present experiment shows that there is also no direct transfer via the milk and corroborated earlier finding.

The kids exposed to COLM during pregnancy had a significantly lower latency to eat. This observation suggests that these animals overcame feed neophobia, because the latency to eat can be considered as a specific indicator of feed neophobia.²³ Interestingly, the kids that experienced only post-natal exposure of COLM (LAC group) also showed a significantly lower latency to eat. This observation can probably be explained by the fact that there is some flavour learning associated with milk feeding or the presence of COLM in the neighbourhood, which reduces the latency to eat. This reasoning is in line with the observation that perinatal flavour learning in pigs can reduce the reluctance to eat feeds with low palatability.²⁴

The differences in intake, intake rate and meal size compared to the values observed in the previous study⁹ cannot be unequivocally explained but they are probably related to the different breed used in the current study. The goats used in the present experiment were pure bred Co with lower body weights than the cross-breed (Bachthao × Co) goats that were used in the previous study.⁹

CONCLUSIONS

The outcome of the current study does not support the hypothesis that the exposure of *C. odorata* to the dams during both pregnancy and lactation versus pregnancy alone, additionally affects post-weaning intake of *C. odorata* by their offspring. It was found that the transmission of information regarding feed ingredient choice by goat dams to offspring does not occur during lactation. However, it is confirmed that *in utero* learning can reduce the resistance to eat novel feeds by goat kids. The outcome of the current experiment implies that the introduction of new feedstuffs for goats should occur during pregnancy and not during lactation.

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REFERENCES

- 1 Provenza FD, Lynch JJ, Burritt EA and Scott CB, How goats learn to distinguish between novel foods that differ in postingestive consequences. *J Chem Ecol* **20**:609–624 (1994).
- 2 Tien DV, Modifying ingestive behaviour to raise animal production in central Vietnam. PhD thesis, Utrecht University, Utrecht (2002).
- 3 El-Haddad MA, Chao CR and Ross MG, N-Methyl-D-aspartate glutamate receptor mediates spontaneous and angiotensin II-stimulated ovine fetal swallowing. *J Soc Gynecol Invest* **12**:504–509 (2005).
- 4 Schaal B, Orgeur P and Arnould C, Olfactory preferences in newborn lambs: Possible influence of prenatal experience. *Behaviour* **132**:351–365 (1995).
- 5 Hepper PG and Wells DL, Perinatal olfactory learning in the domestic dog. *Chem Senses* **31**:207–212 (2006).
- 6 Semke E, Distel H and Hudson R, Specific enhancement of olfactory receptor sensitivity associated with foetal learning of food odors in the rabbit. *Naturwissenschaften* **82**:148–149 (1995).
- 7 Hai PV, Everts H, Van Tien D, Schonewille JT and Hendriks WH, Feeding *Chromolaena odorata* during pregnancy to goat dams affects acceptance of this feedstuff by their offspring. *Appl Anim Behav Sci* **137**:30–35 (2012).
- 8 Hai PV, Schonewille JT, Van Tien D, Everts H and Hendriks WH, Improved acceptance of *Chromolaena odorata* by goat kids after weaning is triggered by *in utero* exposure during late but not consumption of milk. *Appl Anim Behav Sci* **146**:66–71 (2013).
- 9 Hai PV, Schonewille JT, Van Tien D, Everts H and Hendriks WH, Improved acceptance of *Chromolaena odorata* by goat kids after weaning is caused by *in utero* exposure during late but not early pregnancy. *Appl Anim Behav Sci* **159**:50–54 (2014).
- 10 Bilko A, Altbacker V and Hudson R, Transmission of food preference in the rabbit: the means of information transfer. *Physiol Behav* **56**:907–912 (1994).
- 11 Mennella JA, Jagnow CP and Beauchamp GK, Prenatal and postnatal flavor learning by human infants. *J Pediatr* **107**:1–6 (2001).
- 12 Biller A, Boppré M, Witte L and Hartmann T, Pyrrolizidine alkaloids in *Chromolaena odorata*. Chemical and chemoeological aspects. *Phytochemistry* **35**:615–619 (1994).
- 13 Wollenweber E, Dörr M and Muniappan R, Exudate flavonoids in a tropical weed, *Chromolaena odorata* (L.). *Biochem System Ecol* **23**:873–874 (1995).
- 14 Vasilatos R and Wangsness PJ, Feeding behavior of lactating dairy cows as measured by time-lapse photography. *J Dairy Sci* **63**:412–416 (1980).
- 15 Baumont R, Doreau M, Ingrand S and Veissier I, Feeding and mastication behaviour in ruminants, in *Feeding in Domestic Vertebrates: From Structure to Behaviour*, ed. by Bels V. CAB International, Wallingford, pp. 241–262 (2006).
- 16 Martin P and Bateson P, *Measuring Behaviour: An Introductory Guide*. Cambridge University Press, Cambridge (1993).
- 17 Galef BG Jr and Henderson PW, Mother's milk: A determinant of the feeding preferences of weaning rat pups. *J Comp Physiol Psychol* **78**:213–219 (1972).
- 18 Oostindjer M, Bolhuis JE, van den Brand H and Kemp B, Prenatal flavor exposure affects flavor recognition and stress-related behavior of piglets. *Chem Senses* **34**:775–787 (2009).
- 19 Richardson C and Hebert CN, Growth rates and patterns of organs and tissues in the ovine foetus. *Br Vet J* **134**:181–189 (1978).
- 20 Brunjes PC, Olfactory bulb maturation in *Acomys cahirinus*: Is neural growth similar in precocial and altricial murids? *Brain Res* **284**:335–341 (1983).
- 21 Seckl JR, Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. *Mol Cell Endocrinol* **185**:61–71 (2001).
- 22 Nolte DL, Provenza FD, Callan R and Panter KE, Garlic in the ovine fetal environment. *Physiol Behav* **52**:1091–1093 (1992).
- 23 Provenza FD, Postingestive feedback as elementary determinant of food preference and intake in ruminants. *J Range Manag* **48**:2–17 (1995).
- 24 Dudink S, Simonse H, Marks I, de Jonge FH and Spruijt BM, Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Appl Anim Behav Sci* **101**:86–101 (2006).