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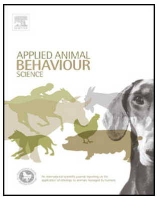

Improved acceptance of *Chromonaela odorata* by goat kids after weaning is caused by *in utero* exposure during late but not early pregnancy

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ABSTRACT

The aim of the current experiment was to study the effect of the phase of pregnancy on *in utero* learning of *Chromonaela odorata* by the goat kids by comparing mid pregnancy (day 50–99, MP) with late pregnancy (day 100–145, LP). It was hypothesized that kids born to dams fed *C. odorata* during late pregnancy (day 100–145) would show an improved post-weaning consumption of this plant. Twenty four female goats (Co breed) were synchronized, inseminated and divided randomly into 4 equal groups. All pregnant goats were fed a diet either without (control) or with 50 g of *C. odorata* leave meal (COLM) at 10:00 am during 30 min during mid and late pregnancy. The COLM diet was fed either from 50 to 99 days of pregnancy (mid pregnancy, MP), or from 100 to 145 days of pregnancy (late pregnancy, LP) or from 50 to 145 days of pregnancy (MLP, positive control). After weaning (3 months old), one kid from each goat dam was selected to measure COLM intake for 30 min over a 4-week period. Feeding activities of the individually housed goat kids were monitored with a camera system.

Post-weaning consumptions of COLM by the goat kids increased significantly ($P < 0.05$) in the LP and MLP treatments and remained essentially unchanged in the control and MP treatments. The higher consumption of COLM by kids from the LP and MLP treatment was associated with a significantly ($P < 0.05$) shorter latency to eat and a longer chewing time ($P < 0.05$). It was concluded that transmission of feeding behaviour from mother to offspring occurs between day 100 to 145 of gestation and that it remains present at least 3 months after weaning in goats.

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1. Introduction

Nowadays, governmental policy in Vietnam is oriented towards the preservation of forest and natural land. This policy implies that amongst others, meat goats have to be kept indoors. Consequently, instead of self-selecting the forages outdoors, the goats now have to be fed feedstuffs that are novel to them. However, the acceptance

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of new feedstuffs is hampered by the well-known neophobia of goats (Provenza et al., 1994; Tien, 2002). It is generally accepted that learning plays a fundamental role in the development of the animals' dietary habits. During the past few decades, the effect of prenatal exposure via maternal ingestion have been shown to influence postnatal feeding behaviour of the offspring of several animal species (Leprohon and Anderson, 1980; Smotherman, 1982; Stickrod et al., 1982; Hepper, 1988; Post et al., 1998) and humans (Schaal et al., 1998; Mennella et al., 2001; Raimbault et al., 2007).

In two previous experiments, Hai et al. (2012, 2013) demonstrated that the intake of *Chromonaela odorata* by pregnant dams stimulated the post-weaning consumption of this feedstuff by their kids. Furthermore, Hai et al. (2013) also showed that the underlying mechanism of the improved post-weaning intake of *C. odorata* can be attributed to the *in utero* period of the kids but not to the subsequent suckling period. These results can be interpreted in that repetitive *in utero* exposure to specific chemosensory cues from the maternal diet influences post-weaning feed selection. In ovine, the taste cells of foetal sheep are functionally mature and achieve adult-like form around the 17th week of gestation (Bradley and Mistretta, 1973a). Under the assumption that this condition can be extrapolated to goats, it can be suggested that *in utero* exposure to *C. Odorata* during late pregnancy versus the entire pregnancy is equally effective to improve the post-weaning consumption of *C. odorata* by kids. Therefore, the aim of the current study was to determine the importance of the phase of pregnancy on *in utero* learning of *C. odorata* by the goat kids by comparing mid pregnancy (day 50–99) with late pregnancy (day 100–145). It was hypothesized that kids born to dams fed *C. odorata* during late pregnancy (day 100–145) show an improved post-weaning consumption of this plant. *C. Odorata* was used as a test feedstuff as goats are reluctant to consume it voluntarily, thereby, resulting in high contrasts compared to control animals (Hai et al., 2012, 2013).

2. Material and methods

2.1. Animal, feeds and management

Twenty four healthy local female goats (Co breed), with an initial mean body weight of 29 ± 3.1 kg and 12–14 months of age were successfully synchronized and inseminated with fresh diluted semen (0.25 ml containing 300 to 400 million sperm diluted in homogenized-pasteurized skim milk) collected by an artificial vagina from 4 fertile Bachthao bucks. Synchronization was induced using intravaginally placed Eazi-Breed™ CIDRs impregnated with 0.3 g of progesterone (Pfizer Australia Pty Ltd) for 18 days. At the time of CIDR removal, the females received an intramuscular injection of 400 IU PMSG (Folligon, Intervet, The Netherlands). Pregnancy was confirmed by not returning to estrus and by the use of a Preg-Tone detector (Renco Corp., USA) at day 30 to 40 after insemination. Dam goats were vaccinated against "Foot and mouth disease"—Aftovax, 3 type (O, A, Asia1) and were dewormed—Ivermectin (Vinavet Co., Vietnam) one month before the experiment.

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

Parameter	Mean ± SEM
Dry matter (% as is)	87.44 ± 0.98
Crude protein (% DM)	18.08 ± 1.37
Ether extract (% DM)	2.30 ± 0.39
Ash (% DM)	6.21 ± 0.89
Crude fiber (% DM)	11.35 ± 1.52
Nitrogen free extract (% DM)	62.06 ± 2.41

Animal housing and experimental procedures were performed in accordance with European Union regulation concerning animal experimentation, including European Community directive 86/609/EEC. Forty five days after insemination, the pregnant goats were randomly divided into four groups.

All goats were individually housed in the Livestock Research Center, Hue University of Agriculture and Forestry (HUAF), Hue, Vietnam. The experimental barn was divided into 4 areas separated by a 2.5-meter wide aisle. Each area consisted of a row of 6 metal pens (1.2 × 2.0 m), which were separated by a solid dark plastic sheet, thereby, avoiding physical contact between neighboring dams. Animals were exercised once a day in four separated *C. odorata*-free yards from 15:00 to 16:00 h. The feeds used in the experiment were elephant grass and an agro-industrial by-product mixture (AIBM), which included rice bran (50%), cassava leaves and stems (20%), beer residue (20%) and cassava root residue (10%). Grass was collected from existing pastures of the Livestock Research Centre (HUAF) twice a day, at 07:00 h and 14:00 h, and subsequently chopped to a length of 10 to 20 cm, directly before feeding. All animals had free access to mineral blocks (63% NaCl, 9% Ca, 11% P, 1.26% Mg, 1.0% Fe, 0.15% Cu, 0.12% Mn, 0.05% I, and 0.01% Co) and fresh water.

2.2. 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* leave meal (COLM). Only *C. odorata* leaves at the top of the plant were used because N-oxide of pyrrolizidine alkaloids – a harmful chemical to goats – are reported to be low compared to other parts of the plant (Biller et al., 1994). Young leaves (10 cm from the top) were deliberately avoided due to the possibility of high concentrations of nitrate (Wollenweber et al., 1995). Proximate analysis of the COLM (Table 1) was performed in triplicate at the Central Laboratory of HUAF, Hue, Vietnam.

The 24 pregnant goats were randomly allocated to one of the four treatments (six animals per group). Each pregnant goat was fed 100 g of AIBM either without (control) or with 50 g of COLM at 10:00 am during 30 min. The AIBM containing COLM was fed either from 50 to 99 days of pregnancy (mid pregnancy, MP), or from 100 to 145 days of pregnancy (late pregnancy, LP) or from 50 to 145 days of pregnancy (MLP, positive control). All goats were fed fresh elephant grass (DM of 3% of body weight) twice a day at 11:00 and 16:00 h and received an additional 200 g of AIBM at 20:00 h per head per day. During the three month lactation period, AIBM was increased to 500 g or 600 g per head per day depending on the number of kids, i.e. one or two, respectively.

The kids were weaned at 3 months and one kid from each goat dam was randomly selected and subsequently housed in an individual cage. The cages were separated by dark plastic sheets in such a way that the kids could not have visual or physical contact with other kids. The ratio of male and female kids was 4:2, 4:2, 3:3 and 2:4 for the treatments control, MP, LP and MLP, respectively. Kids were fed *ad libitum* hay and 350 to 500 g/day of a mixture containing locally purchased soybean meal (50%) and rice bran (50%). At 23:00 h, 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 h the next morning. At the start of a 30 min COLM intake measurement, 50 g of COLM was provided to each kid. The total experimental period lasted 4 weeks during which time *C. odorata* intake and feeding behaviour of the kids was recorded daily (a total of 28 measurements for each kid).

2.3. Measurements

Feed intake was measured as the difference between the quantities offered and refused as measured by a digital balance (iBalance 201 (200 g/0.01 g), My weigh Co., USA). Feeding behaviour of the kids was monitored by a time-lapse video recording system (Vasilatos and Wangsness, 1980). Computer connected cameras (Logitech® Webcam Pro 9000) were placed at 1.5 m height above each individual pen (24 cameras) and controlled by i-Catcher Sentry (ver.2) software (iCode Systems Ltd.). 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.), 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 color markers to monitor the movement of different parts of the goat's head. The movement of the markers were used to calculate the duration of each movement. Eating time (min) 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 food 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 (Martin and Bateson, 1993). 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.

2.4. Statistical analyses

All the experimental data were analyzed using IBM SPSS Statistics 20.0 for Windows. Prior to statistical analysis, the daily feed intake of the kids was averaged per week. Data within each of the experimental treatments appeared to be normally distributed (Kolmogorov–Smirnov test). Data were analyzed using repeated measures ANOVA with treatment as factor. 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. Post-hoc test with Bonferroni correction was used to identify groups with different effects on the variable involved. The values in the tables represent means ± SEM and $P < 0.05$ was considered statistically significant.

3. Results

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

On average, the dams in the four groups had a mean (±SEM) litter size at birth of 1.6 (±0.15) kids/dam. Mean body weight (±SEM) of the randomly selected kids at birth was 2.65 kg (±0.26) and rose to 16.8 kg (±1.28) at weaning (3 months of age). For all groups combined, the mean body weight (±SEM) of the selected goat kids at the end of the experiment (4 months of age) was 21.7 kg (±1.05). 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.

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

The pregnant dams in the MP, LP and MLP groups consumed all the COLM/AIBM and AIBM mixture that was offered daily. In the first week of the experiment, there was no difference in *C. odorata* intake by the goat kids in the four treatment groups. However, in the subsequent weeks of the experiment, the intake of COLM by the goat kids in the LP and MLP treatments (Table 2) increased significantly. In contrast, feed intake of *C. odorata* remained low for the goat kids in the control and MP groups. Intake of *C. odorata* by dams during late pregnancy (LP and MLP groups) resulted in a 70% higher intake (week 4 *versus* 1) of COLM by their kids after weaning ($P < 0.05$, Table 2). The intake of COLM was not significantly different between the LP and MLP kids.

3.3. Feeding behaviour related to the acceptance of COLM

The higher consumption of COLM by kids from the dams fed COLM during LP and MLP was associated with a significantly ($P < 0.05$) shorter latency to eat (Table 3) and a significantly longer chewing time ($P < 0.05$). A larger meal size was also observed as a consequence of higher COLM intake but it was not statically significant. The number of visits without COLM intake, and meal frequency did not differ between the treatments. Rates of COLM intake and meal size were numerically higher on the LP and MLP treatments,

Table 2
Weekly intakes of *C. odorata* leaf meal (COLM) by goat kids born to does fed COLM during mid (MP), late (LP) and both (MLP) stages of pregnancy or not fed COLM (control). Presented values are means ± SEM in g per kid during the 30 min observation period per day.

Week	Group				*P*-value (treatment)
	Control	MP	LP	MLP	
1	14.7 ± 2.7	15.8 ± 2.9	16.1 ± 3.2^{a}	17.2 ± 4.0^{a}	Ns
2	12.8 ± 2.2^{c}	14.7 ± 2.3^{c}	24.2 ± 4.5^{bd}	19.6 ± 4.9^{ad}	<0.05
3	12.1 ± 2.2^{c}	14.4 ± 2.7^{c}	23.2 ± 4.2^{bd}	27.6 ± 5.1^{bd}	<0.05
4	13.2 ± 2.0^{c}	14.1 ± 1.8^{c}	26.4 ± 4.9^{bd}	30.1 ± 5.3^{bd}	<0.05
P-value (week)	ns	ns	<0.05	<0.05	

Means within column with different superscripts (a,b) differ significantly ($P < 0.05$); means within row with different superscripts (c,d) differ significantly ($P < 0.05$); Ns: non-significant ($P > 0.05$).

but the difference between the control and MP treatments did not reach statistical significance (Table 3).

4. Discussion

The present observations proof that the last phase of pregnancy (day 100–145) is a critical period for prenatal exposure to COLM in order to establish an improved intake of COLM by goat kids after weaning. The higher acceptance to COLM after weaning on the LP and MLP treatments is consistent with the outcome of our previous studies (Hai et al., 2012, 2013) and confirms our hypothesis.

It is generally accepted that prenatal learning on chemosensory information may play an important role in mammals to determine feed preferences after birth. Flavors from the mother's diet pass into the amniotic fluid (Mennella et al., 1995) which is swallowed by the fetus (Nolte et al., 1992; Mennella et al., 2001) or into maternal and fetal plasma (Desage et al., 1996). The fetus reacts to these chemosensory stimuli by encoding, retaining and using them as cues in their postnatal feeding behaviour. The capacity for sensing postnatal flavors commences *in utero* with the development of the gustatory and olfactory systems. Though both morphological and functional development of these systems start during the first trimester, they present functionally maturity and have achieved adult-like form around the 17 th week of gestation for the taste cells of foetal sheep (Bradley and Mistretta, 1973a) and 28⁻29th week of gestation for olfactory receptor cells of the human foetus (Chuah and Zheng, 1987; Ventura and Worobey, 2013). In the light of the outcome of the current study, it can be suggested that in goats also the taste cells of the fetus becomes functionally mature after the 14th week of pregnancy.

In the ovine fetus, swallowing responses are intact and functional at near-term (after 123 of gestation, (El-Haddad et al., 2005b). The ovine fetus can swallow amniotic fluid 79–447 ml per day during day 101 to 136 of pregnancy (Bradley and Mistretta, 1973b) and 98–577 ml per day between day 109 and 128 of gestation (Harding et al., 1984). There is also a continuous flow of amniotic fluid through the nasal cavity, which is estimated to be about twice the amount of amniotic fluids that is swallowed (Xu et al., 2001; El-Haddad et al., 2005a). This phenomenon has also been documented in rodents and rabbits (Hepper, 1988; Bilko et al., 1994). Therefore, it can be extrapolated from data in other species that the swallowing of amniotic fluid and the passage of amniotic fluid through the nasal cavity becomes substantial after day 100 of pregnancy in the goat and that the process of chemo sensing starts to function in this period. The lack of response to prenatal exposure of COLM before day 100 (treatment MP) is in line with this reasoning.

Exposure to dietary flavors in amniotic fluid may be one of the ways for the dam to teach her offspring which feeds are "safe" during gestation. That is, memories evoked by odours and taste are more emotionally charged than those evoked by other sensory stimuli (Herz and Cupchik, 1995) because of the olfactory system's intense and immediate access to the neurological substrates underlying emotion (Cahill et al., 1995). The emotional potency of odor- and taste-evoked memories, and the reward systems that encourage us to seek out pleasurable sensations together play a role in the strong emotional component of feed habits.

Table 3
Selected indices of feed acceptance and *C. odorata* leaf meal (COLM) intake by goat kids born to does fed COLM during mid (MP), late (LP) and both (MLP) stages of pregnancy or not fed COLM (control). Presented values are means over 4 weeks and based on 30 min observation periods.

Parameter	Group				*P*-value
	Control	MP	LP	MLP	
Latency to eat (min)	4.01 ± 0.67b	3.57 ± 0.48b	1.67 ± 0.45a	2.11 ± 0.55a	<0.05
Meal frequency (times)	3.71 ± 1.69	3.10 ± 1.28	4.23 ± 1.91	3.50 ± 1.55	Ns
Mean COLM intake (g DM)	13.2 ± 2.3a	14.8 ± 2.4a	22.5 ± 4.2b	23.6 ± 4.8b	<0.05
Eating bout length (min/meal)	0.97 ± 0.58	1.15 ± 0.53	1.18 ± 0.69	1.57 ± 0.63	Ns
Number of visits without intake (times)	3.60 ± 1.65	3.20 ± 1.21	3.11 ± 0.92	2.93 ± 1.19	Ns
Intake rate (g DM/min)	3.87 ± 0.52	4.09 ± 0.88	4.50 ± 0.79	4.25 ± 0.64	Ns
Meal size (g DM/meal)	3.55 ± 1.52	4.67 ± 1.95	5.58 ± 1.38	6.77 ± 1.37	Ns
Chewing time (min)	5.67 ± 1.81a	5.90 ± 2.12a	8.23 ± 2.06b	9.15 ± 2.75b	<0.05

Values are means ± SEM and means within row with different superscripts differ significantly ($P < 0.05$). Ns: non-significant ($P > 0.05$).

The improvement of COLM intake of kids on the LP and MLP treatment was associated with a significant reduction of latency to eat and an extension of the chewing time. These parameters can be considered as specific indicators for feed neophobia (Provenza, 1995). These observations are in line with the outcome of previous studies of Hai et al. (2012, 2013). However, it is interesting to note that the levels of COLM intake, mean intake rate of COLM and meal size were higher in the present study compared to the values observed in the previous study (Hai et al., 2013). The differences in intake, intake rate and meal size cannot be unequivocally explained but they are probably related to the different age of weaning and/or breed used in the current study. In the present experiment, kids were weaned at 3 months after birth, which is two weeks later than in the previous study (Hai et al., 2013). Moreover, the goats in the present experiment were cross breeds (Bachthao x Co) with higher body weights than the pure bred Co goats that were used in the previous study.

In conclusion, the current study supports the hypothesis that the transmission of feeding behaviour from mother to offspring occurs during gestation in goats and that it remains present after weaning (Hill and Przekop, 1988; Hepper, 1989; Hai et al., 2012; Hai et al., 2013). The present study shows that this process takes place during day 100 to 145 of pregnancy. Consequently, in this phase of pregnancy, the dam can be fed feedstuffs intended to be offered to their kids after weaning. Prenatal learning about feed has a long-term effect and prevents resistance to eat novel feeds by the kids. From a practical viewpoint, this finding is important when kids are transferred from outdoor to indoor systems. The current study also provides a clue about the underlying mechanism to manipulate postnatal feeding behaviour but further studies are required to proof the idea that the swallowing of amniotic fluid enriched with COLM derived cues plays a key role.

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