Caprine *in utero* learning and feed neophobia

***In utero* leren en voer neofobie bij geiten**

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 8 juni 2017 des middags te 4.15 uur

door

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geboren op 5 augustus 1973 te Ha Tinh, Vietnam

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This thesis was accomplished with financial support from:
the Asia-link project (Phase III, contract number: TH/Asia-link/014) in cooperation with the Trig Project of Hue University, Vietnam.

Content

Chapter 1

General introduction

1. Introduction

Small ruminants, including goats, contribute to an important economic and ecological niche in developing countries (Devendra, 2005) because they provide animal derived foods for human consumption (meat and milk), skins and, therefore, income for many farmers. Goat production in Vietnam, which mainly takes place on a small-holder scale, has been occurring in extensive management systems for many years and has increased markedly to keep pace with the increasing demand for goat meat. This extensive management system is characterized by browsing of the goats in forests and vacant agricultural land. However, small-holder farmers in Vietnam are nowadays encouraged to relocate their goats from an outdoor to an indoor system, mainly because of environmental reasons such as the preservation of forests. This change in goat husbandry is associated with a change in nutrition as the animals have to be fed indoors, resulting in the use of feedstuffs such as protein-rich concentrates, molasses/urea blocks, rice bran or rice straw. However, particular feedstuffs provided may be novel and it is commonly observed in sheep that the animals refuse to ingest these novel feedstuffs, leading to production losses (Chapple and Lynch, 1986). This issue was addressed by Tien (2002) who showed that in goats also, the introduction of novel feeds is hampered by the fact that they exhibit neophobia, i.e. fearful or reluctant to anything new (in this case novel feeds). Neophobia is a major factor affecting feed consumption by naïve or inexperienced herbivores (Squibb et al., 1990) and often occurs during the first period of feed adaptation (Chapple and Lynch, 1986). Upon the first exposure to novel feedstuffs, it has been estimated by Noland et al. (1975) that up to 80% of the ruminants initially reject a new feed. This initial rejection of novel feedstuffs probably has its basis as a protective mechanism to avoid toxicities (Corey, 1978).

Around the time of weaning, young animals need to make important choices regarding which feedstuffs can be consumed or should be avoided. In order to make these choices, animals have to learn the value of feedstuffs. In this process, two different types can be distinguished: an affective and a cognitive process of learning. The affective process of learning is based on post-ingestive feedback. When the animal has consumed a feedstuff, it will associate the feedback (positive or aversive) with the taste and/or metabolic feedback, thereby, learning to increase or decrease the intake of the specific feed in question (Provenza, 1995). Cognitive experiences include the use of senses, smell and sight, to learn from the mother, experienced animals and learning from "trial and error" in order to get feedback from the constituents of the feeds (Provenza et al., 1992). There is abundant evidence that, before weaning, experienced animals facilitate the process of learning about novel feeds, thereby, influencing subsequent feeding behaviour in animals (Faverdin, 1999). Thus, social learning assists young animals to establish feed preferences early in life (Lobato et al., 1980). Especially the mother is an important source of information, as young animals generally direct most of their attention to her. There is ample evidence suggesting that the mother teaches her offspring to avoid feed with a negative post-ingestive feedback and that the learning might continue for up to two to three months (Mirza and Provenza, 1994). Thus, the mother can act as a role model and enhance the willingness to try new feedstuffs, thereby, learning the offspring which feedstuffs are healthy and nutritious. The aforementioned process of learning is considered highly effective because mother and offspring are genetically related and respond similarly to smell, tastes and post-ingestive feedback of the feed (Chapple and Lynch, 1986; Provenza et al., 1995).

The transmission of feeding behaviour from mother to offspring is not restricted to the postnatal period but can start already during pregnancy (Hill and Przekop, 1988; Provenza, 1995; Hepper and Wells, 2006). Prenatal exposure to chemosensory stimuli via maternal consumption influences postnatal feeding behaviour of the neonate in a number of species including rats (Leprohon and

Anderson, 1980; Smotherman, 1982; Stickrod et al., 1982; Hepper, 1988; Post et al., 1998), dogs (Hepper and Wells, 2006), cats (Wyrwicka, 1978), rabbits (Bilko et al., 1994; Semke et al., 1995) and humans (Mennella, 1995; Schaal et al., 1998; Mennella et al., 2001; Romantshik et al., 2007). For ruminants, this effect of *in utero* learning has also been shown in sheep (Nolte and Provenza, 1992; Simitzis et al., 2008; Chadwick et al., 2009a,b,c). Maternal influence on food/feed preference of offspring can thus potentially increase the post-weaning intake of otherwise novel feeds. However, the concept of *in utero* learning on feed preference has not yet been proven in goats. The main goal of the current thesis was to verify whether the concept of *in utero* learning can also be applied to goats in order to facilitate the introduction of novel feeds.

2. *In utero* learning

The process of *in utero* learning provides the foetus with a natural and safe mechanism to begin its life-long experience to ingestion of feeds. This process can be considered natural and safe because it relies on the mother's normal feeding behaviour and knowledge of toxic and nontoxic feedstuffs. Most likely, chemosensory or olfactory cues play an important role in the process of *in utero* learning. Perhaps, these chemosensory or olfactory cues induce a process of associative learning due to the positive value given by the opioid system, thereby, influencing postnatal feed preference of the new-born (Arias and Gabriela Chotro, 2006). Alternatively, prenatal experience to chemosensory stimuli influences elements of the chemosensory system by modifying specific receptors, thereby, increasing the saliency of the stimulus when experienced after birth (Semke et al., 1995; Hepper and Wells, 2006). Transmission of chemosensory stimuli (i.e. feed cues) can occur through the transfer of these cues from maternal to foetal blood (Desage et al., 1996) but they can also be transferred via amniotic fluid (Mennella et al., 1995) and subsequent swallowing by the foetus (Nolte and Provenza, 1992; Mennella et al., 2001). It has been demonstrated (Thornburg et al., 2010; Masuyama and Hiramatsu, 2012) that dietary ingredients influence the genome of the foetus *in utero* and, thereby, causing potential epigenetic changes (temporal and spatial control of gene activity during development of complex organisms) and altering the phenotype of the adult thereafter (Fig. 1).

3. Epigenetic modification of gene expression

There are two main processes that can modify gene expression which are not related to DNA sequence, i.e. histone modification of chromatin and DNA methylation. Chromatin is located in the cell nucleus and comprised of DNA and proteins (histones). A change in the chromatin structure, for instance by the addition of amino acids, can make genes more or less accessible for transcription thereby affecting the expression of the gene. For example, in case 5-methylcytosine is attached to DNA during the DNA methylation, silence transcription by inhibiting the recruitment of repressor proteins often occurs, thereby, leading to insufficient neurobiology and behaviour (Moore et al., 2013). Gene expression can also be modified by DNA methylation, even from early development of the foetus. After fertilization, a wave of demethylation occurs which erases at least partly, the inherited parental methylation pattern. Shortly after the implantation of the embryo, *de novo* methylation occurs, thereby, establishing a new embryonic methylation pattern (Okano et al., 1999) which can lead to animal imprinting (Paulsen and Ferguson-Smith, 2001).

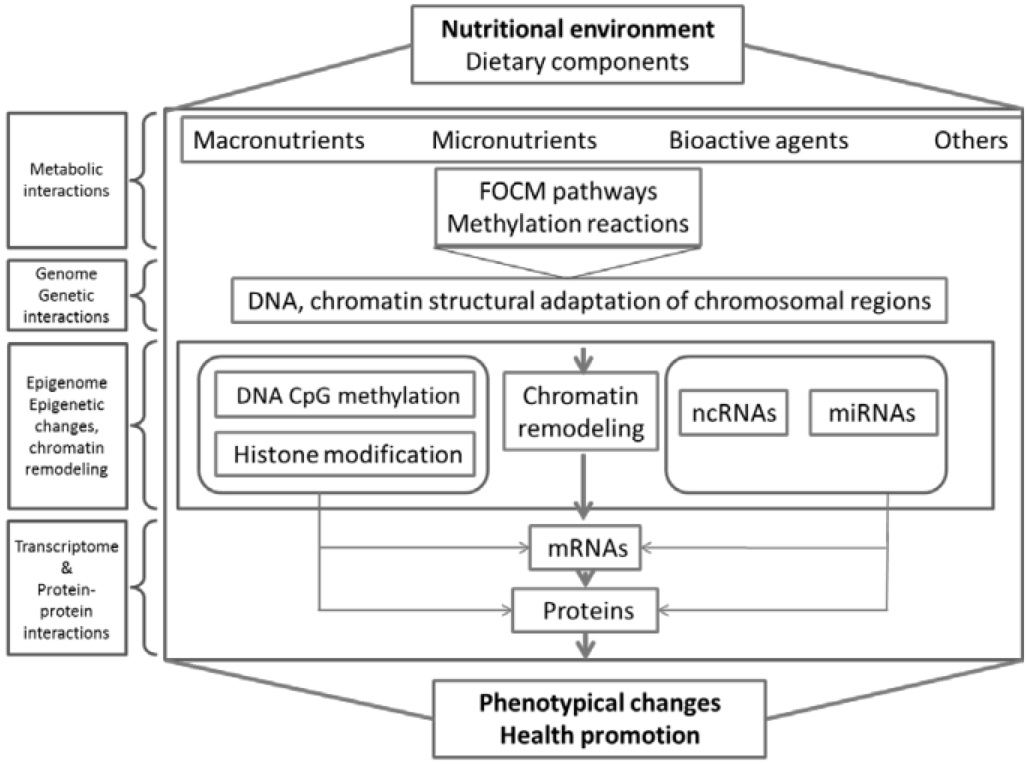

Fig. 1. Overview of epigenetic mechanisms; a link between nutritional environment and phenotypical changes. Adapted from Chango and Pogribny (2015).

The growth and development of the foetus, with long-term impacts on brain development and metabolism can be significantly affected by the mother's diet during pregnancy (Godfrey and Barker, 2001; Symonds et al., 2007; Zeisel, 2009). For example, the offspring of female rats ingesting a low protein diet during pregnancy were found to have a high expression of genes encoding for glucocorticoid and peroxisome proliferator-activated receptors in association with a reduction in DNA methylation of these genes (Lillycrop et al., 2005; Lillycrop et al., 2008).

The phenotype of the adult animal can be influenced by the maternal diet (Szyf, 2009; Hogg et al., 2012) but there is currently insufficient knowledge to understand how nutritional factors influence epigenetic mechanisms during the development of the foetus. It has been proven that dietary deficiency or excess of the methyl group donors required for the methylation reactions of the cell can change epigenetic patterns but the impact of diet on histone modification and chromatin modifying proteins is currently not yet clear. Furthermore, it is known that DNA methylation in the brain plays a role in the regulation of short-term memory storage (Sweatt, 2003) but its role in long-term memory storage is not yet clear. Nevertheless, it is generally accepted that offspring can learn *in utero* from their mother.

Maternal epigenetic effects do not only occur during pregnancy but also postnatally. Natural variations in postnatal care in rodents for instance, may cause differences in behaviour through epigenetic factors (Meaney, 2001; Szyf et al., 2005). It has been shown that a negative relationship exists between maternal licking/grooming behaviour during the early postnatal period and expression of glucocorticoid receptor (GR) in association with a decreased hippocampal GR promotor methylation and stress response in the offspring (Weaver et al., 2004). Studies on cross-fostering confirm that the aforementioned effects are mediated by the level of maternal care received postnatally (Caldji et al., 1998; Francis et al., 1999).

4. Postnatal learning by means of milk consumption

After birth, mammals start to consume their mother's milk which is facilitated by maternal odours that are experienced by the offspring during the periparturient period. Alternatively, the mother can also influence the feeding preference of her young by odours and aromas from feeds that might be transmitted through the mother’s milk (Galef and Laland, 2005). In case the mother’s milk contains flavours originating from the diet, chemosensory stimuli experienced via the mother’s milk may result in an increased preference for feed flavoured with that stimulus as shown in humans, rats, and rabbits (Galef, 1981; Bilko et al., 1994; Mennella et al., 2001). The ingestion of milk is reinforced by hedonic and post-ingestive events following the first consumption of the mother’s milk (Mennella and Beauchamp, 1998). The exposure of flavour through the milk alone, however, does not lead to increased preference in all cases. For instance, postnatal exposure to anise did not change the preference for this flavour in dogs (Hepper and Wells, 2006) and in pigs (Oostindjer et al., 2009; Oostindjer et al., 2010). The plasticity of the brain after birth and the relative behavioural maturity of the offspring in the early postnatal phase may determine whether chemosensory learning through the mother's milk occurs, either independent of, or in addition to prenatal experience (Leon et al., 1984). Thus, in fully altricial species such as humans and rats, the brain may be sufficiently plastic for postnatal exposure to result in a strong familiarization with the flavour. This notion is corroborated by observations in dogs showing a higher preference while exposed both pre- and postnatally to aniseed (Hepper and Wells, 2006).

5. Outline of the present thesis

The main objective of the studies reported in this thesis was to determine whether *in utero* learning by goat kids occurs and can be potentially used to overcome neophobia after weaning. *Chromoneala odorata* was chosen as a model feedstuff to study neophobia in goats. *C. odorata*, also known as Siam weed, is a potential feed ingredient abundantly available for goats in Vietnam and other tropical countries. The leaf fraction contains a high crude protein content (258 g kg^{-1} DM) and has a high organic matter and crude protein degradability (Apori et al., 2000). *C. odorata*, however, has a strong smell and adult goats are reluctant to consume this plant voluntarily. Therefore, *C. odorata* was selected as a test feedstuff because it was anticipated that the intake of *C. odorata* would result in a high contrasts compared to control animals.

The first experiment (Chapter 2) was designed to test the hypothesis that feeding *C. odorata* to pregnant goat dams increases the acceptance of this plant by their offspring. It was found that kids born from does that were exposed to *C. odorata* during pregnancy consumed more of this plant. This finding indicated that the kids learned *in utero* about *C. odorata*. Due to the experimental design, however, it could not be excluded that the process of adaptation to *C. odorata* was caused by the consumption of the milk instead of during pregnancy. Therefore, a second experiment was designed (Chapter 3) to investigate whether the process of adaptation to *C. odorata* was related to either the *in utero* period of the goat kids or to the subsequent suckling period. It was hypothesized that kids born to dams fed *C. odorata* during pregnancy and receiving milk from dams not exposed to *C. odorata* during pregnancy show an improved acceptance to consume this plant. This experiment corroborated the outcome of the first experiment in that kids learned *in utero* about *C. odorata*. An attempt was made to provide a clue about the underlying mechanism to manipulate postnatal feeding behaviour (Chapter 4). Therefore, the importance of the phase of pregnancy on *in utero* learning of *C. odorata* by goats was evaluated by comparing mid with late pregnancy exposure to *C. odorata* of the pregnant does. It appeared that the transmission of feeding preference from mother to offspring occurred during late gestation and that it remains present at least 3 months after weaning in goats. Finally, the idea was tested whether kids would consume more *C. odorata* after experiencing the flavour through the mother’s milk (Chapter 5). It was hypothesized that exposure of *C. odorata* to the dams during both pregnancy and lactation versus pregnancy alone, further increased post-weaning intake of *C. odorata* by their offspring. However, it appeared that the transmission of feeding behaviour from goat dams to offspring did not occur during lactation. The concept of *in utero* learning in goats, however, again was confirmed. In the last chapter of this thesis (Chapter 6), the results of these studies are summarized and discussed.

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Chapter 2

Feeding *Chromonaela odorata* during pregnancy to goat dams affects acceptance of this feedstuff by their offspring

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Applied Animal Behaviour Science (2012) 137: 30-35

Abstract

We investigated the effect of *Chromoneala odorata* ingestion by goat dams during pregnancy on intake of *C. odorata* by their kids. Alteration of prenatal feed preference may be used to increase feed intake of novel feeds and the transit from outdoor to indoor goat farming in Vietnam. Ten female goats were synchronized, inseminated and divided randomly into two equal groups. The experimental group was offered 50 g of sun dried *C. odorata* leave meal (COLM) along with a basal diet from day 45 to 135 of pregnancy while the control group was offered only the basal diet during this period. After weaning (3 mo), one kid from each goat dam in both groups was randomly selected and housed in individual cages. After a 9.5 h fast, each kid was offered 50 g of COLM daily at 8:30 am for 30 min for 4 weeks. Feeding activities were monitored with a video camera system. We determined mean COLM intake and indices of feed acceptance including latency to eat, number of visits without intake, meal frequency, eating bout length, eating time, intake rate, meal size and chewing time for each kid.

Intake of COLM by goat kids born to dams fed COLM was significantly higher over the 4 weeks compared to goat kids born to dams not fed COLM (11.32 $\pm$ 3.60 vs. 6.09 $\pm$ 1.91 g, $P < 0.028$). This difference was more pronounced during weeks 3 and 4 than weeks 1 and 2. Goat kids from mothers fed COLM had a shorter latency to eat, a larger meal size and a longer chewing time than kids from mothers not fed COLM during pregnancy. We conclude that *in utero* transmission of feeding preference occurred from does to their offspring.

1. Introduction

In Vietnam, small-scale farmers are currently moving their meat goats from an outdoor to an indoor system, mainly for preservation of forest and natural lands. This change in goat husbandry is associated with changes in nutrition as the animals must be fed indoors. In many cases, small-scale farmers have only a limited number of feedstuffs available and some such as rice straw or cassava leaves are novel to goats. Because goats are more particular than sheep in terms of feed choice (Tien, 2002) they may exhibit neophobia more profoundly and reject novel feeds more easily (Provenza et al., 1994), thereby hampering the change in husbandry practice. The acceptance of feedstuffs by animals is affected by preferences resulting from congenital flavour preferences, individual experience and imitation (Chapple and Lynch, 1986; Provenza et al., 1992). Goats learn about their feed through post-ingestive consequences, thus learning to avoid feeds containing particular secondary compounds, e.g. tannins (Bernays et al., 1989) or toxins (Burritt and Provenza, 1989). They also learn from other animals, typically from their mother (Mirza and Provenza, 1994; Saint-Dizier and Ferreira, 2007). For young kids, the mother has probably the most important influence on establishing and maintaining her offspring's dietary habits (Provenza et al., 1992).

A potential feed ingredient abundantly available for goats in Vietnam and other tropical countries is Siam weed (*Chromoneala odorata*). This weed plant grows abundantly in tropical humid areas and the leaf fraction contains high crude protein content (258 g kg^{-1} DM) and has high organic matter and crude protein degradability (Apori et al., 2000). *C. odorata* has a strong smell, however, and adult goats are reluctant to consume this plant. *C. odorata* contains a number of secondary metabolites including alkaloids, tannins, steroids, terpoids, flavanoids and cardiac glycoside with a steroidal ring or deoxy sugar (Akinmoladun et al. 2010).

In addition to post-ingestive consequences and imitation, it is increasingly accepted that maternal influence of food/feed preferences begins *in utero*. Prenatal exposure to chemosensory stimuli via maternal consumption influences postnatal feeding behaviour of the neonate in a number of animals including rats (Leprohon and Anderson, 1980; Smotherman, 1982; Stickrod et al., 1982; Hepper, 1988; Post et al., 1998), dogs (Hepper and Wells, 2006), cats (Wyrwicka, 1978), rabbits (Bilko et al., 1994; Semke et al., 1995) and humans (Mennella et al., 1995; Schaal et al., 1998; Schaal et al., 2000; Mennella et al., 2001; Raimbault et al., 2007; Romantshik et al., 2007). For ruminants, this effect has also been shown in sheep (Nolte et al., 1992; Simitzis et al., 2008; Chadwick et al., 2009a,b,c). Maternal influence on perinatal food preference can potentially increase the intake of feeds or feed ingredients consumed in low amounts by goats. The main goal of the current experiment was to verify whether the concept of in utero learning also occurs in goats, thereby contributing to the generalization of the forementioned concept. *C. odorata* was selected as a test feedstuff as goats are reluctant to consume this plant voluntarily, presumably because of its strong smell and as such would result in a high contrasts compared to control animals. We hypothesized that feeding *C. odorata* to pregnant dams increases the acceptance of this plant by their offspring.

2. Material and methods

2.1. *Animal and housing*

Ten local, 12–14 months old does with an initial mean body weight of 29 ± 3.8 kg were successfully synchronised and inseminated with semen from different bucks. Dam goats were vaccinated against “Food and mouth disease” - Aftovax, 3 type (O, A, Asia1) and were given a prophylactic treatment against gastrointestinal parasites (Bioxinil - Bio Pharmachemie, 1.5 ml/head) 1 month before the experiment. Animal housing and experimental procedures were in accordance with the European Union regulation concerning animal experimentation, including European Community directive 86/609/EEC. Pregnancy was confirmed by not returning to estrus and high blood progesterone concentrations. One month after insemination the pregnant goats were randomly assigned to either the control group or experimental group with 5 goats per group.

All goats were kept on a farm in Thua Thien Hue province, Vietnam. During the experiment, the adult goats dams were allowed to range freely in the grass land (~1 ha) each day from 12.00 h to 18:00 h. The area was enclosed by a barbed wire fence and there was no *C. odorata* available within the grass land area. From 18:00 h to 12:00 h the next day, goats were individually housed in wooden pens (1.2 x 2 m). At 20:00 h they were fed an agro-industrial by-product mixture (AIBM; 200 g/head/day) which included rice bran, cassava leaves and stems, cassava root residue, beer residue, minerals, salt and limestone. 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 was harvested at 120 days age, at 40 cm length from the top of the plant, sun dried and the leaves then were separated and manually crumbled 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). Extremely young leaves 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 Central Laboratory of Hue University of Agriculture and Forestry, Hue, Vietnam.

The five pregnant goats in the experimental group were offered 50 g of COLM for 15 min each day (at 10:00 am) from day 45 to 135 during pregnancy.

The five pregnant goats allocated to the control group did not receive COLM nor did they have any contact with the COLM. During the last 2 weeks of pregnancy (day 136-150), all goats were fed 300 g agro-industrial by-product mixture per head per day. During the 3 month lactation period goats with singles were fed 500 g of the agro-industrial by-product per head per day and goats with twins 600 g per head per day. All animals grazed outside between 12:00 to 18:00 h in the grass land area except 5 days after parturition. During this period the animals had no access to *C. odorata*.

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

Parameters	*C. odorata* leaf meal
Dry matter (% as is)	81.3
Crude protein (% DM)	20.63
Ether extract (% DM)	2.05
Ash (% DM)	6.27
Crude fibre (% DM)	11.9
Nitrogen free extract (% DM)	59.15

Goat kids were weaned at 3 months of age. One kid from each doe (control and experimental) was randomly selected and individually housed in cages. The control group consisted of 3 females and 2 male kids, while the experimental group consisted of 2 females and 3 male kids. The cages were separated by plastic sheets such that the kids did not have visual or physical contact. Kids were fed hay *ad libitum* and 350 - 500 g concentrate, which consisted of soybean meal and rice bran each day. Soybean meal and the rice bran were purchased locally and mixed every 3 days. At 23:00 h, all feed was withdrawn before measurements of voluntary COLM intake started at 8:30 am. At the start of a measurement, 50 g of COLM was offered in a different trough to each kid for 30 minutes. The total experimental period lasted 4 weeks during which time *C. odorata* intake and feeding behaviour of the kids was recorded daily for 30 min.

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/0.01g), My weigh Co., USA). Feeding behaviour of the goat 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 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. Variables were calculated from video motion analysis software: Motionpro (CyberAccess123 Inc.) which included integrated time registration to allow calculation of the various variables. Eating time (min) was defined as the total time when the goat was eating from the trough with its muzzle in the feed bowl or chewing or swallowing food with its head above the feed bowl. Latency to eat (min) defined as the time between the goat standing at the trough and taking the first bite (Martin and Bateson, 1993). If a goat kept his head in the trough for more than 15 seconds, 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 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 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 SPSS Statistics 17.0.1.1 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. The values in the tables and figures represent means ± SEM and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of offering C. odorata *to dams on the feed intake of their kids toward this feedstuff*

The dams in the control and treatment groups gave birth to 8 (4 males and 4 females) and 7 (3 males and 4 females) kids, respectively. The mean body weight (± SEM) of the 10 randomly selected kids at birth, weaning and the end of the experimental period was 2.2 (± 0.06), 13.2 (± 0.33) and 17.5 (± 0.41) kg, respectively for the control group. Values for the experimental and control group were not significantly different at any of the time (2.3 ± 0.04, 13.6 ± 0.036 and 17.6 ± 0.27 kg, respectively). Mean intake of COLM by the does in the experimental group was 8.1 g/day (SED = 3.02) from 45 to 135 days of pregnancy. The amounts of COLM consumed by the dams did not increase in the course of pregnancy.

After weaning at 3 mo age, *C. odorata* intake of the goat kids from does fed COLM during pregnancy tended ($P = 0.002$) to increase each week from 9.98 g (± 2.15) in wk 1 to 14.67 g (± 3.90) in wk 4 (Fig.1). However, only a statistically significant ($P = 0.005$) difference was observed between wk 2 and wk 4. In contrast, a gradual decline in *C. odorata* intake was observed (P = 0.031) in the kids born to dams not fed COLM (control group) from 7.50 g (± 1.89) in wk 1 to 4.50 g (± 0.93). Weeks 3 and 4 were statistically (P = 0.048) different. In general, the mean COLM intake of goat kids from does fed *C. odorata* during pregnancy over the 4 wks was significantly (P = 0.028) higher than the kids in the control group (11.32 ± 3.60 vs. 6.09 ± 1.91 g). Goat kids in the treatment group had a significant higher intake of *C. odorata* compared to the kids in the control group in wk 3 and 4.

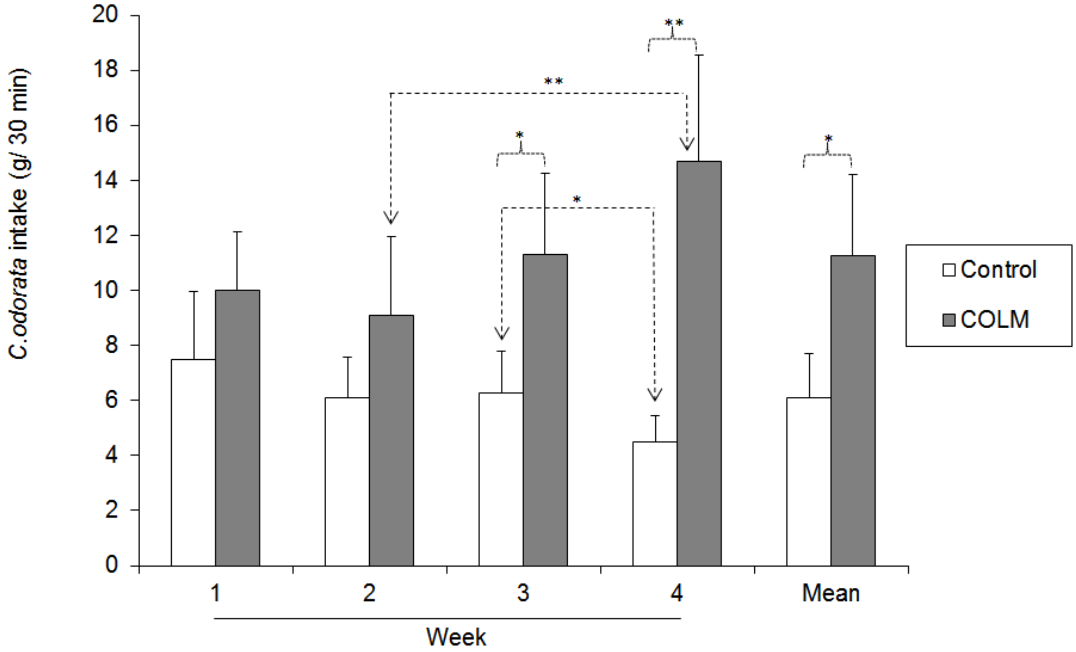

Fig. 1. Weekly mean (± SEM) intake of *C. odorata* leaf meal (COLM) during 30 min by goat kids born to does fed COLM during pregnancy or not (control). * P < 0.05; ** P < 0.01.

3.2. Feeding behaviour related to the acceptance of COLM

The higher intake of COLM by kids from dams fed COLM was associated with a significantly (P < 0.05) shorter latency to eat and a numerically lower number of visits without intake (Table 2). Meal frequency was numerically lower in the goat kids born from mothers fed COLM during pregnancy, which was associated with an increase in time spent on each meal. Consequently, total eating time was somewhat higher in the kids from mothers fed COLM during pregnancy but the difference in eating time was not statistically significant. The total amount of COLM ingested was significantly ($P < 0.05$) higher in the kids born from does exposed to COLM during gestation but the rate of intake was not different from the controls. Because of the higher consumption of COLM with a concomitant lower frequency of meals, the amount of COLM ingested per meal was significantly ($P < 0.05$) higher in the goat kids from treated dams. Finally, a significant ($P < 0.05$) increase in chewing time was observed in kids prenatally exposed to COLM.

Table 2. Selected indices of feed acceptance and short term *C. odorata* leaf meal (COLM) intake by goat kids born to does fed COLM during pregnancy or not (control).

Parameter	Control		COLM		P-value
	Mean	SEM	Mean	SEM	
Eating time (min)	3.39	1.54	4.65	1.80	Ns
Latency to eat (min)	4.1	1.34	2.8	1.21	< 0.05
Meal frequency (times)	4.14	1.87	2.48	1.27	Ns
Mean of intake (g DM)	6.09	1.91	11.32	3.60	< 0.05
Eating bout length (min/meal)	0.82	0.57	1.88	0.31	Ns
Number of visits without intake (times)	3.2	1.12	1.7	0.93	Ns
Intake rate (g DM/min)	1.80	1.02	2.43	1.14	Ns
Meal size (g DM/meal)	1.47	0.63	4.56	0.88	< 0.05
Chewing time (min)	5.6	1.21	7.8	2.07	< 0.05

4. Discussion

To the authors' knowledge, the present study provides the first experimental evidence in goats that prenatal flavour experiences influence postnatal responses to that flavour in solid feeds. This effect became more prominent during the course of the experiment (Fig.1). The increase intake of *C. odorata* over time may be due to an adaptation of the animals. This observation is in line with the data reported by Chadwick et al. (2009a,b,c) whom showed that offspring of sheep were able to adapt to a high salt load of their mothers. However, from the studies of Chadwick et al. (2009a,b,c) it is not clear whether the adaption occurred during pregnancy or lactation because the ewes were fed a high sodium ration from 60 days of pregnancy until day 21 of lactation. Contrary to the experimental group, the adaption was not seen in the control group where the intake of COLM gradually reduced. This change may be due to the post-ingestive feedback (PIF), which is regulated by food experience through specific gastric and enteric receptors (Provenza et al., 1994). With experience from PIF, lambs and heifers are able to avoid or prefer a particular feedstuff by smelling a feed (Early and Provenza, 1998; Atwood et al., 2001). This may be a likely reason for the reduction in feed intake and time for eating (Table 2) in the control group and the improvement in the experimental group.

During the first two weeks, goats initially consumed only 7.61 to 9.98 g COLM during 30 min (Fig. 1) after being fasted for 9.5 hours. Intake of COLM was remarkably low in comparison to 31.7 to 77.2 g (DM) of chopped fresh grass consumed by dwarf goats with the same body weight in 30 min (Anika, 1985). The low intake of *C. odorata* suggests animals were neophobic (Barnett, 1958). It is unlikely that low intakes were caused by poor nutritional value of *C. odorata* meal which is high in crude protein content (206 g kg^{-1} DM) and the leaf fraction has been reported to have a high organic matter and crude protein degradability

(Apori et al., 2000). Taste or smell can play an important role in determining the palatability of a feedstuff. Although palatability is improved by the sun drying (Atta-Krah and Sumberg, 1988), the smell of *C. odorata* may have caused the low intake.

The latency to eat, which was lower in the experimental group ($P < 0.05$, Table 2), indicates goat kids born to *C. odorata*-fed does were more eager to eat *C. odorata* leaf meal. Goat kids in the experimental group better adapted to the new feed. The presence of neophobia was suggested by the high number of visits without eating (goats spent a longer time sniffing and generally rejected the COLM in most cases) and resulted in a longer latency to eat in the control group (Table 2). Meal size, which is an important predictor for feed intake (Baumont et al., 2000), of the kids in the experiment group (4.56 g DM) was significant higher than the kids in the control group (1.47 g DM) indicating a higher acceptance of the feed. The decrease in intake of COLM by kids in the control group probably resulted from both a lower intake rate and a shorter meal duration.

The higher COLM acceptance of goat kids born to does fed *C. odorata* can be explained by the mother-foetus interaction (Bartoshuk and Beauchamp, 1994), in which prenatal exposure to a chemosensory stimulus via maternal consumption can influence neonate postnatal feeding behaviour. In sheep, when a pregnant ewe consumes a feed, the concentration of various feed components and metabolites including those with chemosensory properties increase in maternal and foetal plasma (Desage et al., 1996). Flavours from the mother's diet are transmitted to the amniotic fluid (Mennella et al., 1995) and swallowed by the foetus (Nolte et al., 1992; Mennella et al., 2001). These flavours stimulate olfactory and gustatory receptors via the hematogenic route or are perceived through the taste receptors located on the tongue (Hudson and Distel, 1999). Thus, unborn sheep foetuses (101–136 days of gestation) would taste these flavours, since they swallow considerable amounts of amniotic fluid (Bradley and Mistretta, 1973). This method of learning seems to be quick and more 'sustainable' because mother and offspring have a close genetic relationship and hence should respond more similarly to smell, tastes and post-ingestive feedback of feeds than unrelated individuals (Chapple and Lynch, 1986; Provenza, 1995).

The current study supports the hypothesis that the transmission of feeding behaviour from mother to offspring begins in the sensitive pregnancy period and continues long after weaning (Hill and Przekop, 1988; Hepper, 1989). Caution is warranted to generalize the outcome of the current study due to the small number of animals involved. Moreover, the experimental design of the current study does not exclude the possibility that the kids born from does fed COLM during pregnancy might have experienced effects of chemosensory COLM compounds after birth. It is possible that secondary compounds of COLM were retained by the does during pregnancy and subsequently released during the lactation period as animals experience a negative energy balance post-partum. Consequently, the milk of the does fed COLM during pregnancy might have been enriched with the characteristic chemosensory compounds of COLM due to lipolysis. It is therefore also possible that the process of adaptation to *C. odorata* was initiated by means of milk consumption instead of during pregnancy. Obviously, the current experiment does not provide information as to how chemosensory compounds of *C. odorata* can be retained during pregnancy and released during lactation. However, it can be speculated that it is associated with changes in energy stores of the does around parturition (Smith and Sherman, 2009). A study using cross fostered kids may provide further insights into this mechanism.

Acknowledgements

The study was supported by Asia-link project (Contract number: TH/Asia-link/014).

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Chapter 3

Improved acceptance of *Chromoneala odorata* by goat kids after weaning is triggered by in utero exposure but not consumption of milk

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Applied Animal Behaviour Science (2013) 146: 66-71

Abstract

The aim of the current study was to determine whether the improved post-weaning intake of *Chromoneala odorata* by goat kids is related to either the *in utero* period of the goat kids or the subsequent suckling period. It was hypothesized that kids born to dams fed *C. odorata* during pregnancy and receiving milk from dams not exposed to *C. odorata* during pregnancy show an improved acceptance to consume this plant. Twenty female goats were successfully synchronized and divided into 4 groups. Two groups (1 and 3) were offered 50 g of dried *C. odorata* leave meal (COLM) mixed with a basic diet for the last 3 months of pregnancy until 1 week before parturition. At birth the kids from the goats in group 1 and 2 were cross fostered without colostrum or milk from their own mother. While waiting for the delivery of kid from another goat, the kid was fed milk replacer or milk from any goat dam in the same treatment. Kids from groups 3 and 4 remained with their mothers. After weaning (2.5 months old), one kid from each goat dam was selected for COLM intake which was measured for 30 min over a 4 week period. Feeding activities of the individually housed goat kids was monitored with a camera system. Kids born to dams receiving COLM during pregnancy consumed higher amounts of the COLM supplemented test feed during all feeding preference tests compared to kids from the control group, particularly during week 3 and 4 ($P < 0.001$). Shorter latency, longer time spent on each meal and total eating time, chewing time and higher meal size ($P < 0.05$) were different in the kids born from does that ingested COLM during pregnancy. It is concluded that prenatal exposure to *C. odorata* via maternal ingestion significantly increases the intake of *C. odorata* by weaned goat kids. This improved intake is due to the *in utero* learning and not the transfer of (secondary) components via the milk of the mothers fed *C. odorata* during pregnancy.

1. Introduction

Nowadays, smallholder farmers in Vietnam are encouraged to move their meat goats from an outdoor to an indoor system, mainly for environmental reasons such as the preservation of forest and natural lands. The practical implication of this change is that the goats are no longer able to self-select forages outdoors and required to be fed selected forages indoors. For economic reasons, a limited number of feedstuffs is available in practice and some of these feedstuffs may be novel to goats. The introduction of these novel feeds is hampered by the fact that goats are neophobic with regards to foods leading to the rejection of novel feeds (Provenza et al., 1994; Tien, 2002).

The acceptance of feedstuffs is affected by preferences probably resulting from congenital flavour preferences, individual experience and imitation (Chapple and Lynch, 1986; Provenza et al., 1992). For young kids, the mother has a strong influence on establishing and maintaining dietary habits (Provenza et al., 1992). Apart from imitation, prenatal exposure to chemosensory stimuli via maternal consumption may influence the neonates postnatal feeding behaviour of various 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).

Recently, we demonstrated that goat kids born to dams fed *Chromoneala odorata* during pregnancy increased their voluntary consumption of *C. odorata* post-weaning (Hai et al., 2012). In that study *C. odorata* was used as a test feedstuff as goats are reluctant to consume this plant voluntarily and as such would result in the high contrasts compared to control animals. However, the experimental design of the latter study did not exclude the possibility that secondary compounds of *C. odorata* were retained by the does during pregnancy and subsequently released during the early lactation period as does experience a negative energy balance. Mobilization of maternal body fat post-partum could have released characteristic chemosensory compounds of *C. odorata* allowing for the possibility that the process of adaptation towards *C. odorata* was caused by means of milk consumption instead of *in utero* learning.

The aim of the current study was to investigate whether the improved post-weaning intake of *C. odorata* by goat kids (Hai et al., 2012) is related to either the *in utero* period of the goat kids or to the subsequent suckling period. It was hypothesized that kids born to dams fed *C. odorata* during pregnancy and receiving milk from dams not exposed to *C. odorata* during pregnancy show an improved acceptance to consume this plant.

2. Material and methods

2.1. *Animal, feeds and management*

Twenty healthy, 12–14 months old local female goats with an initial mean body weight of 28 ± 3.6 kg 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 either of 4 fertile bucks. Synchronization was induced using an intra-vaginally placed Eazi-Breed™ CIDRs device 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 goats not returning to estrus and by a Preg-Tone detector (Renco Corp., USA) used 30 to 40 days after insemination. Dam goats were vaccinated against “Foot and mouth disease” with Aftovax, 3 type (O, A, Asia1) and were dewormed using Ivermectin (Vinavet Co.) one month before the experiment. Animal housing and experimental procedures were performed in accordance with European Union regulations concerning animal experimentation, including European Community directive 86/609/EEC. Forty five days after insemination, the pregnant goats were randomly divided into 4 groups (Table 1): dams allocated to groups 1 and 3 were fed with *C. odorata* leaf meal (COLM) during pregnancy while dams in group 2 and 4 fed a COLM free ration.

Table 1. Experimental design to test whether the improved post-weaning intake of *Chromoneala odorata* by goat kids is related to the *in utero* or suckling period.

Goat dams		Cross	Goat kids	Treatment[a]		Kid group[b]
Group #	n	fostered	(n)	Utero	Milk	
1	5	Yes	5	-	+	No/Co
2	5		5	+	-	Co/No
3	5	No	5	+	+	Co/Co
4	5		5	-	-	No/No

[a]*In utero*/milk effect of *C. odorata* intake of dams on intake of this feedstuff by goat kids.

[b]Co: With the exposure of *C. odorata*; No: Without exposure of *C. odorata*.

All goats were individually housed in the same experimental barn of the Livestock Research Center, Hue University of Agriculture and Forestry (HUAF), Vietnam. The experimental barn was divided into 2 areas separated by a 4-meter wide aisle. Each area consisted of a row of 10 wooden pens (1.2 x 2.0 m), which were separated by a solid dark plastic sheet thereby avoiding physical contact between neighboring dams. COLM was fed in only one row of pens. Animals were exercised once a day in two separate *C. odorata*-free yards from 15:00 to 16:00 h. The goats were fed a basal ration consisting of elephant grass (*Pennisetum purpureum)* and an agro-industrial by-product mixture (AIBM) containing (in dry matter) 50% rice bran, 20% cassava leaves and stems, 10% cassava root residue and 20% beer residue. Fresh elephant grass was supplied at a level of 3% of body weight (DM basis) and was available between 11:00 and 15:00 h. During pregnancy, dams were provided 200 g of AIBM per head per day at 20:00 h. During the 2.5 months of lactation, AIBM was increased to 500 or 600 g per head per day for goats suckling one or two kids, respectively. The grass was collected twice daily at 07:00 and 14:00 h from existing pasture areas of the Livestock Research Centre. After harvesting, the grass was chopped to a length of 10 - 20 cm directly before supplying this meal to the animals. 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 was harvested at 120 days age at 40 cm length from the top of the plant and sun dried. Thereafter, the leaves were separated and manually crumbled to produce COLM. Only *C. odorata* leaves at the top of the plant were used because N-oxide of pyrrolizidine alkaloids – a harmful chemical to goats - has been reported to be low compared to other parts of the plant (Biller et al., 1994). Very young leaves were avoided due to the possibility of high concentrations of nitrate (Wollenweber et al., 1995). Proximate analysis of COLM (Table 2) was performed in triplicate at the central laboratory of Hue University of Agriculture and Forestry, Hue, Vietnam.

The 10 pregnant goats allocated to experimental groups 1 and 3 were offered 50 g of COLM mixed with 100 g of AIBM (a part of daily AIBM of each goat) for 15 min each day (at 10:00 am) from day 45 after insemination to a week before the expected parturition. The ten pregnant goats allocated to the control groups 2 and 4 were offered same amount of AIBM but did not receive, or had any contact with COLM throughout the experiment.

Directly after birth, all does were thoroughly washed with shampoo (Nova-Yellow Shampoo, Anova Ltd., Vietnam) and the kids born to does allocated to group 1 were transferred to does of group 2 and *vice versa* (Table 1).

Table 2. Chemical composition of the *C. odorata* leaf meal.

Parameter	Mean	SEM
Dry matter (% as is)	80.90	1.77
Crude protein (% DM)	20.46	0.46
Ether extract (% DM)	2.06	0.13
Ash (% DM)	6.29	0.27
Crude fibre (% DM)	11.21	0.64
Nitrogen free extract (% DM)	59.98	2.77

Kids born to does allocated to groups 3 – Co/Co kids and 4 – No/No kids (Table 1) remained with their biological mothers. Thus, in contrast to the latter kids, kids born to dams allocated to either group 1 – Co/No kids or group 2 – No/Co kids, did not consume any colostrum or milk from their biological mother. In case of an asynchronous delivery of a pair of does from groups 1 and group 2, the new-born kid was fed artificial commercial milk replacer (Kaeco Group, Inc.) or colostrum from any goat dam in the recipient group.

To stimulate the mother-kid bond of the animals allocated to groups 1 and 2, three initiatives were undertaken. First, manual stimulation of the vagina and cervix of the recipient mother was practiced thereby distracting the animal’s attention during the introduction of a kid (Kendrick et al., 1991). Secondly, directly after birth, kids were bandaged with a piece of fabric until the time of translocation when the fabric was used to bandage the cross fostered kid. The bandage facilitated the acceptance of the kid by the recipient because of the familiar smell. Finally, during the first 3 days after cross fostering, kids were encouraged to suckle by bringing the head of the kid close to one of the foster dam’s teats and spraying a couple squirts of milk onto the kid's lips. Meanwhile, the head of the foster doe was restrained to avoid that the kids were pushed away during suckling. Although some of the cross fostered kids were somewhat reluctant to suckle from the foster dam's teats, all animals overcame the situation within a few days.

After weaning (2.5 months of age), one kid from each goat dam was randomly selected. Consequently, the ratio of male and female kids in groups 1, 2, 3 and 4 was 2:3; 3:2; 2:3 and 4:1 respectively. The selected kids were individually housed in pens. Kids were provided hay *ad libitum* and 350 to 500 g concentrate consisting of a mixture of locally purchased soybean meal (50%) and rice bran (50%) each day. At 23:00 h, all feed provided to the kids was withdrawn before measurements of voluntary COLM intake at 08:30 h the next morning. Dark plastic sheets separated the cages such that the kids could not have visual or physical contact. At the start of a 30 min COLM intake measurement period, 50 g of COLM was provided to each kid and *C. odorata* intake as well as feeding behaviour of the kids was recorded. The total measurement period lasted 4 weeks during which time COLM intake was measured daily (a total of 28 measurements) for each kid.

2.3. Measurements

Feed intake was measured as the difference between the quantity of COLM offered and refused as measured using a digital balance (iBalance 201 (200 g/0.01g), My weigh Co., USA). Feeding behaviour of the goat 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 controlled by i-Catcher Sentry (ver.2) software (iCode Systems Ltd.) during the 30 min measurement period when COLM was offered. The calculated variables from the video motion analysis software: Motionpro (CyberAccess123 Inc.), in which specific functions such as stopwatch and movement of goat's head by dotting on the lip, jaw and forehead of goats so that the duration of each movement was calculated. Eating time was defined as the total time (min) 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 was defined as the time (min) between the goat standing at the trough and taking the first bite (Martin and Bateson, 2007). If a goat kept his head in the feed bunk for more than 15 s, this was considered a visit to the feed bunk. A visit with COLM intake was recorded as a meal; otherwise it was recorded as a visit without intake. Meal frequency was calculated as the total number of visits to the feed bunk with COLM being consumed. Eating bout length was defined 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 measurement period. Intake rate was calculated as the intake of COLM divided by the eating time and meal size was determined by 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 analyses, the daily feed intake of the kids was averaged per week. Data within each of the experimental treatments were checked for normal distribution (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 within 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 and figures 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 gave birth to 1.4 (± 0.6) kid/dam (Table 3). Mean body weight of the randomly selected kids at birth was 2.2 kg (± 0.2) and increased to 12.1 kg (± 0.6) at weaning. For all groups combined, the mean body weight of the selected goat kids at the end of the experiment was 16.1 kg (± 0.7). There was no difference in body weight gain between the treatments.

Table 3. Sex and body weights of the offspring during the experiment.

Kid group	No. of kids		Kids/dam	Body weight (kg) of the selected kids		
	Male	Female		Birth	Weaning (2.5 mo.)	End of trial (3.5 mo.)
No/Co	3	4	1.4 ± 0.6	2.2 ± 0.16	12.2 ± 0.66	16.1 ± 0.87
Co/No	3	5	1.6 ± 0.6	2.2 ± 0.24	11.7 ± 0.42	16.4 ± 0.43
Co/Co	4	3	1.4 ± 0.6	2.3 ± 0.19	12.6 ± 0.73	16.8 ± 0.77
No/No	3	3	1.2 ± 0.4	2.1 ± 0.22	11.7 ± 0.45	16.2 ± 0.72

Values are means ± SEM.

3.2. C. odorata *intake of the goat kids*

During day 45 after insemination to a week before the expected parturition, goats allocated to experimental groups 1 and 3 (Table 1) consumed all the COLM/AIBM mixture offered. Intake of *C. odorata* during pregnancy of the does resulted in a significantly higher intake of *C. odorata* by their offspring (Co/No kids and Co/Co kids) during week 3 and 4. Furthermore, intake of *C. odorata* of goat kids born to does fed *C. odorata* during pregnancy but nursed by does not exposed to *C. odorata* during pregnancy (Co/No kids) was similar to that of *C.*

odorata intake of kids born and nursed by dams that consumed *C. odorata* during gestation (Co/Co kids) (Fig. 1). Differences in *C. odorata* intake by goat kids born from dams exposed or not exposed to *C. odorata* during pregnancy were less pronounced during the first weeks of the experimental periods and became more pronounced during week 3 and 4 of the experimental period ($P_{week} < 0.001$). Nevertheless, for all 4 weeks combined, the mean intake of COLM of goat kids from dams ingesting COLM during pregnancy (Co/No kids and Co/Co kids) was significantly increased (Table 4), irrespective of the dam that nursed the kids.

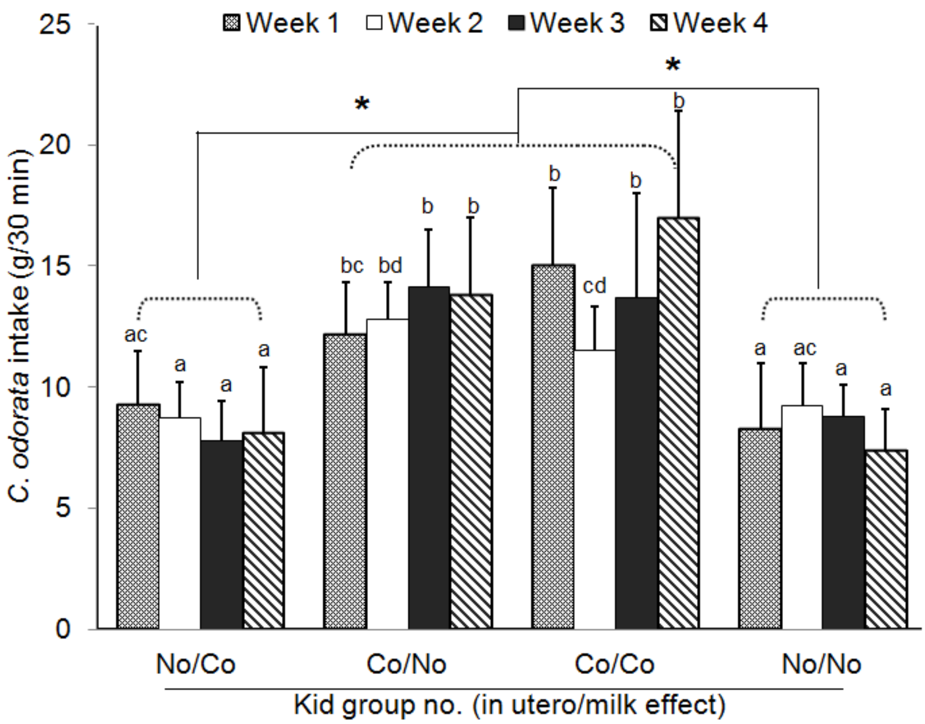

Fig. 1. Mean (± SEM) weekly intake of *C. odorata* during 30 min by goat kids. Bars with different letters (a-d) within week are significantly different ($P < 0.05$). * $P < 0.05$.

3.3. Feeding behaviour related to the acceptance of COLM

The higher intake of COLM by kids from dams fed COLM during gestation was associated with a significantly ($P < 0.05$) shorter latency to eat (Table 4).

However, the number of visits without COLM intake, and meal frequency did not differ between the groups. The total amount of COLM ingested was significantly higher (P < 0.05) in the kids born to does that ingested COLM during pregnancy but the rate of intake was only significantly increased (P < 0.05) in the kids that were born to mothers exposed to COLM during pregnancy but fostered by non-exposed does. Finally, a significant (P < 0.01) increase in chewing time was observed in the kids that were prenatally exposed to COLM.

Table 4. Selected indices of feed acceptance and *C. odorata* leaf meal (COLM) intake by cross fostered and non-cross fostered goat kids born to does fed COLM during pregnancy or not (control). Presented values are means over 4 weeks and based on 30 minutes observation periods.

Parameter	Cross fostered kids		Non-cross fostered kids		P-value
	No/Co	Co/No	Co/Co	No/No	
Latency to eat (min)	2.57 ± 0.51[a]	1.92 ± 0.54[b]	1.81 ± 0.52[b]	2.12 ± 0.47[a]	< 0.05
Meal frequency (times)	4.18 ± 1.47	3.34 ± 1.87	4.17 ± 1.20	4.22 ± 1.17	Ns
Mean COLM intake (g DM/ 30 min)	8.5 ± 2.0[a]	13.2 ± 2.3[b]	14.3 ± 3.9[b]	8.4 ± 1.9[a]	< 0.05
Eating bout length (min/meal)	1.13 ± 0.56	1.56 ± 0.74	1.26 ± 0.64	1.17 ± 0.79	Ns
Number of visits without intake (times)	2.17 ± 0.93	2.51 ± 0.92	2.23 ± 1.14	1.98 ± 1.02	Ns
Intake rate (g DM/min)	1.56 ± 0.61[b]	1.90 ± 0.59[a]	1.52 ± 0.36[b]	0.94 ± 0.38[c]	< 0.05
Meal size (g DM/meal)	2.04 ± 1.12	3.91 ± 1.19	3.41 ± 1.54	3.01 ± 1.04	Ns
Chewing time (min)	6.91 ± 3.07[a]	8.40 ± 2.39[b]	8.32 ± 2.65[b]	6.12 ± 2.21[a]	< 0.01

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

4. Discussion

The current study clearly demonstrates that the ingestion of COLM by does during gestation results in a significantly higher intake of COLM in goat kids after weaning. This outcome is corroborated by our earlier observation (Hai et al., 2012). However, kids with *in utero* experience in the present study had a slightly higher COLM intake than those in our previous experiment (13.2 ± 2.3 and 14.3 ± 3.9 g/30 min vs. 11.3 ± 3.6 g/30 min). This difference in feed intake may be due to the higher mean intake of COLM by does in the experimental groups in the present study (50 g/day) than the study of Hai et al. (2012) (8.2 g/day). The results in the present study confirm our hypothesis that COLM intake after weaning was not influenced by the dam that nursed the kids. Therefore, it appears that no chemosensory compounds of *C. odorata* were excreted with milk during the early lactation period despite the fact that the does experienced a positive energy balance during pregnancy and a negative energy balance during the suckling period. The mean loss of body weight during lactation was 4.2 ± 1.62 kg, irrespective dietary treatment. Clearly, COLM intakes of 13 to 14 g/day do not substantially contribute to the total daily nutrient intake of the goat kids but COLM was selected as a test feedstuff in the current experiment and not intended to serve as a quantitatively important source of nutrients.

Although the kids were fasted for 9.5 h, intake of COLM was low in the kids that were not prenatally exposed to COLM; i.e. 8.5 and 8.4 g for No/Co kids and No/No kids, respectively. The observed low intake of COLM in the kids that were not exposed to COLM *in utero*, suggests that they were neophobic (Barnett, 1958). The low intakes of COLM were most likely not due to a low nutritional value of *C. odorata* because it is high in crude protein (205 g/kg DM) and the leaf fraction has been reported to have a high degradability of organic matter and crude protein (Apori et al., 2000) and contains relevant levels of minerals and vitamins

(Suriyavathana et al., 2012). However, *C. odorata* also contain high levels of secondary metabolites such as tannins, alkaloids (Suriyavathana et al., 2012) and terpenes (Caballero-Gallardo et al., 2011), which may have an adverse effect on voluntary intake (Narjisse et al., 1997). Furthermore, *C. odorata* also has a strong smell (Apori et al., 2000) which probably has a negative effect on palatability. Sun drying can reduce the offensive odour and improves the palatability of *C. odorata* but it does not completely remove the typical smell.

The improved intake of COLM was associated with a significant reduced latency to eat. This indicates that kids that were prenatally exposed to *C. odorata* were more eager to consume COLM thereby suggesting that these kids were better adapted to *C. odorata.* Next to latency to eat, the number of visits without eating can be considered an indicator of neophobia. However, the number of visits without eating was not significantly different between treatments. The current observations are in line with that of Hai et al. (2012) who also could not detect a significant difference in the number of visits without eating between kids either or not prenatally exposed to COLM. Apparently, number of visits without eating is not a sensitive indicator of neophobia, at least under the experimental conditions used in the current experiment. Meal size is considered an important indicator for feed intake (Baumont et al., 2000).

The current study demonstrates that milk is not a vehicle for chemosensory compounds of *C. odorata* as no differences in mean COLM intake between the No/CO and No/No groups (Table 4). This observation is in line with other studies reporting that the flavour to which offspring had been exposed postnatal did not lead to a higher preference in kittens (Hepper and Wells, 2006) and piglets (Oostindjer et al., 2010). It can be concluded that the observed perinatal COLM preference of goat kids was induced by prenatal exposure of COLM. Likewise, prenatal exposure to chemosensory stimuli via maternal consumption also influenced neonatal feeding behaviour after birth in rats (Leprohon and Anderson, 1980; Smotherman, 1982; Stickrod et al., 1982; Hepper, 1988; Post et al., 1998), dogs (Hepper and Wells, 2006), cats (Wyrwicka, 1978) rabbits (Bilko et al., 1994; Semke et al., 1995), sheep (Nolte et al., 1992; Schaal et al., 1995; Chadwick et al., 2009a,b,c) and humans (Schaal et al., 1998; Mennella et al., 2001; Raimbault et al., 2007). The current study does not provide information about the underlying mechanism by which the higher acceptance of COLM of goat kids born to does fed COLM during pregnancy could be explained, but apparently a mother-foetus intereaction (Bartoshuk and Beauchamp, 1994) seems to be involved. In pregnant sheep, amounts of various metabolites originating from feed including those with chemosensory properties increase in maternal and foetal plasma (Desage et al., 1996). Furthermore, it has been shown by Menalla et al. (1995) that flavours from the mother's ration are transmitted to the amniotic fluid and swallowed by the foetus (Nolte et al., 1992; Mennella et al., 2001; El-Haddad et al., 2005). These flavours stimulate sensory receptors located in the upper nasal cavity and taste receptors located on the tongue, the buccal cavity and at the back of the throat (Hudson and Distel, 1999). Consequently, unborn foetuses can taste these flavours because they swallow amniotic fluid. In sheep, foetuses (101–136 days of gestation) may swallow up to 447 ml of amniotic fluid per day (Bradley and Mistretta, 1973).

The current study supports the hypothesis that the transmission of feeding behaviour from mother to offspring begins during gestation and that it remains present after weaning (Hill and Przekop, 1988; Hepper, 1989). The current results confirm that results of our previous study (Hai et al., 2012) that improved post-weaning intake of *C. odorata* by goat kids is related to the *in utero* period of the goat kids instead of the subsequent suckling period. Further studies are needed to unravel the underlying physiological mechanism of *in utero* learning.

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Chapter 4

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

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Applied Animal Behaviour Science (2014) 159: 50-54

Abstract

The aim of the current experiment was to study the effect of the phase of pregnancy on *in utero* learning of *Chromoneala 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* leaf 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 daily 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 month after weaning in goats.

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 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 intra-vaginally 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. 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 x 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 07:00h and 14:00h, 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.

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

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/0.01g), 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 was 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 sec, 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.

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 ($P > 0.05$).

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

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 ± 0.67[b]	3.57 ± 0.48[b]	1.67 ± 0.45[a]	2.11 ± 0.55[a]	< 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 ± 2.3[a]	14.8 ± 2.4[a]	22.5 ± 4.2[b]	23.6 ± 4.8[b]	< 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 ± 1.81[a]	5.90 ± 2.12[a]	8.23 ± 2.06[b]	9.15 ± 2.75[b]	<0.05

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

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. Flavours from the mother's diet pass into the amniotic fluid (Mennella et al., 1995) which is swallowed by the foetus (Nolte et al., 1992; Mennella et al., 2001) or into maternal and foetal plasma (Desage et al., 1996). The foetus reacts to these chemosensory stimuli by encoding, retaining and using them as cues in their postnatal feeding behaviour. The capacity for sensing postnatal flavours 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 17th week of gestation for the taste cells of foetal sheep (Bradley and Mistretta, 1973a) and 28th-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 foetus becomes functionally mature after the 14th week of pregnancy.

In the ovine foetus, swallowing responses are intact and functional at near-term (after 123 of gestation, (El-Haddad et al., 2005). The ovine foetus 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., 2005). 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 flavours 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 odour- 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.

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 × 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.

Acknowledgement

The study was supported by Asia-link project (Contract number: TH/Asia-link/014) and Trig Project (phase 1) of Hue University, Vietnam.

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Chapter 5

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

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Journal of the Science of Food and Agriculture (2016) 96: 2215-2219

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 postnatal exposure of *Chromonaela odorata* leaf meal (COLM) in relation to the preferences to this feedstuff by goat kids after weaning. It was hypothesized 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 behavior from goat dams to offspring does not occur during lactation. However, the concept of *in utero* learning in goats was confirmed.

1. Introduction

Nowadays, socio-economic and environmental issues such as biofuel 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 (Provenza et al., 1994; Tien, 2002).

It is well known that flavours from the diet consumed by pregnant animals can reach the foetus (Schaal et al., 1995; El-Haddad et al., 2005), thereby influencing elements of the chemosensory system of the foetus and consequently increase the saliency of the stimulus when experienced after birth (Semke et al., 1995; Hepper and Wells, 2006). 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 (Hai et al., 2012; 2013; 2014) clearly demonstrates an improved acceptance of *Chromoneala odorata* by weaned goat kids after prenatal exposure to this feedstuff. In these studies (Hai et al., 2012; 2013; 2014), *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 postnatally through the consumption of maternal milk in rabbits (Bilko et al., 1994), dogs (Hepper and Wells, 2006), and humans (Mennella et al., 2001). 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 improves the consumption of *C. odorata* by their kids after weaning. It was hypothesized 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.

2. Material and methods

2.1. Animal, 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 months old, healthy local female goats (Co breed) that had lambed once, with an initial mean (± 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 dewormed (Ivermectin (Hanvet Co., Vietnam), 1 month before the experiment. Goats were synchronized 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 estrous 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 homogenized-pasteurized skim milk) collected from four fertile Co bucks by an artificial vagina. Pregnancy was confirmed by not returning to estrus and by the use of a Preg-Tone detector (Renco Corp., Minneaplos ,MN, USA) at day 30 to 40 after insemination. At 40 days after insemination, 32 pregnant goats were randomly allocated to four groups (8 animals per group). Selected goat kids stayed 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-meter 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 neighboring 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.

2.2. 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* 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 – is 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.

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

Parameter	Mean ± 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

Each goat was fed 100 g of AIBM (as fed) either without (control) or with 50 g of COLM (as fed) at 10:00 am during 30 min. The AIBM containing COLM was fed either between the 50^{th} to 145^{th} 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 one week before farrowing, to prevent flavour exposure through the mother's skin, faeces, fur, or breath before the postnatal COLM treatment started. Kids were separated from their mothers by a dark plastic fence during the time of feeding COLM and 1 hour 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).

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., West Yorkshire, England). Feeding behaviour of the kids was monitored by a time-lapse video recording system (Vasilatos and Wangsness, 1980). 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 color 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 (Baumont et al., 2006), 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 (Martin and Bateson, 1993). If a goat kept its head in the trough for more than 15 sec, 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. Data analyses

All experimental data were analyzed 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 analyzed 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 $P < 0.05$.

3. Results

3.1. 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/dam). Mean body weight (± 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 (± 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.

3.2. 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.

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	P-value (treatment)
1	7.9	9.6^{a}	8.3	9.9^{a}	2.25	0.149
2	9.3^{A}	12.5^{bB}	9.0A	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
P-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 × week < 0.01.

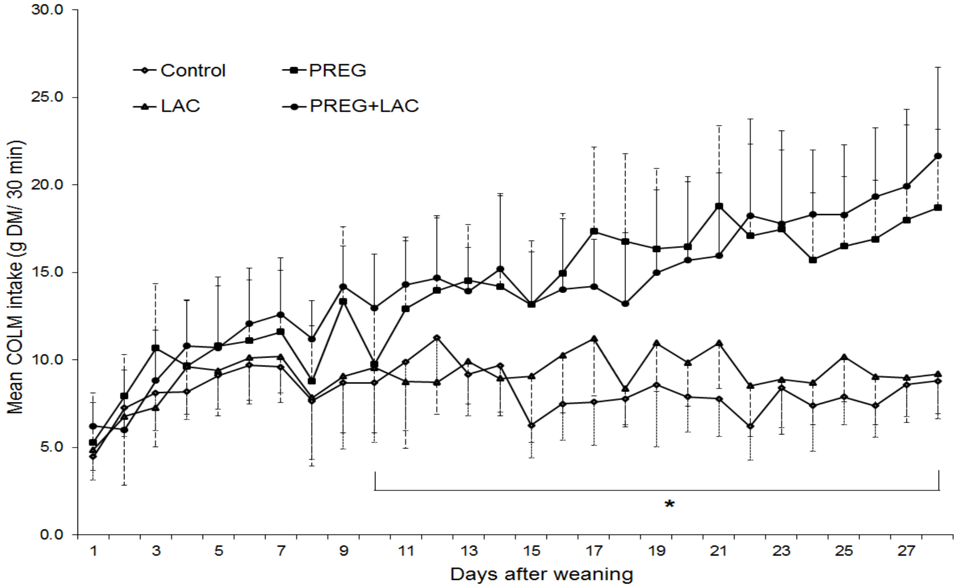

Fig. 1. Daily mean (±SEM), post-weaning intake of *Chromonaela odorata* leaf meal (COLM) during 30 min by goat kids born to does fed COLM during pregnancy (PREG), lactation (LAC), PREG+LAC or not fed COLM (Control). *indicates differences ($P < 0.05$) among groups for individual days (P treatment × days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

3.3. 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.

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.

4. 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 (Galef and Henderson, 1972; Bilko et al., 1994; Mennella et al., 2001). These observations are in line with the notion that flavours of the maternal diet 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, postnatal exposure alone may be an unimportant mechanism to modify offspring's preference. For example, postnatal exposure to anise did not change the preference for this flavour in dogs (Hepper and Wells, 2006) and pigs (Oostindjer et al., 2009). 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 (Richardson and Hebert, 1978). Such brains are probably less plastic in the period after birth compared to altricial species such as humans, rats, and rabbits (Brunjes, 1983), 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 (Brunjes, 1983). Therefore, brain related programming of feed preference after birth (Seckl, 2001), 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 (Hai et al., 2012; 2013; 2014). It is believed that flavours from the mother's diet are transmitted to the amniotic fluid and swallowed by the foetus (Nolte et al., 1992; Mennella et al., 2001). 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 (Provenza et al., 1994). Here, low palatability was overcome by ‘*in utero* learning’.

The present result is in line with the findings in a cross-fostering experiment (Hai et al., 2013), 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 (Hai et al., 2013). 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 (Provenza, 1995). Interestingly, the kids that experienced only postnatal 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 (Dudink et al., 2006).

The differences in intake, intake rate and meal size compared to the values observed in the previous study (Hai et al., 2014) 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 (Hai et al., 2014).

5. Conclusions

The outcome of the current study does not support the hypothesis that the exposure of *C. odor*ata 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.

Acknowledgements

The study was supported by Asia-link project of European Commission (Contract number: TH/Asia-link/014) and Trig Project of Hue University, Vietnam.

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Chapter 6

General discussion

The introduction of novel feeds to goats is hampered by the fact that they are susceptible to feed neophobia, i.e. fearful or reluctant to novel feeds. This issue was already addressed by Tien (2002) and it was shown that the addition of extracts from familiar grasses to a novel feedstuff improved its feed intake in goats. Likewise, mixing *C. odorata* with a familiar feed also is instrumental to overcome feed neofobia in weaned goat kids (Textbox 1). Thus, mixing novel feedstuffs with familiar feedstuffs can be considered as a practical tool to overcome feed neofobia in goats. Alternatively, observations from species other than caprine indicate that *in utero* learning also is instrumental to improve post-weaning consumption of otherwise novel feedstuffs in young animals. However, it was not known whether the concept of *in utero* learning could also be applied to goats and it was considered to be of scientific interest to verify whether the concept of *in utero* learning can also be applied to goats to facilitate the introduction of novel feeds.

1. The use of *Chromonaela odorata* as a model feedstuff

In the current studies, *Chromonaela odorata* (*C. odorata*) was selected as a model feedstuff to study neophobia in goats. *C. odorata* belongs to the family *Asteraceae* and its common names include Siam weed, triffid weed, bitter bush or jack in the bush. The plant is native to South and Central America but it has spread throughout the tropical humid areas, including Vietnam. From an agricultural perspective, *C. odorata* is considered a threat and globally many studies have been carried out to control this weed. However, *C. odorata* is difficult to control and Aro et al. (2009) opportunistically suggested to use *C. odorata* for a useful purpose such as animal feed. Fresh *C. odorata*, however, is difficult to use as an animal feed because goats are reluctant to consume this plant voluntarily probably due to its specific odour or taste. It was, therefore, anticipated that the intake of *C. odorata* would result in a high contrasts compared to control animals, thereby, facilitating the interpretation of the data. Furthermore, the use of only one test feedstuff is favourable compared to the use of different feedstuffs because it provides valuable information on repeatability of previously observed effects.

In the present studies, *C. odorata* was harvested at 120 days after planting, at 40 cm length from the top of the plant and sun dried so as to enhance the acceptability of the feed by the goats. Then, the leaves were separated and crumbled by hand to produce *C. odorata* leave meal (COLM).

Textbox 1. The effect of mixing an unknown feedstuff with a familiar feedstuff to overcome feed neophobia in weaned goat kids (Hai et al., unpublished results).

Ten, three to four month old (weaned) male goat kids were randomly divided into two groups. During a two week learning period, the experimental group was offered 50 g of *C. odorata* leave meal (COLM) mixed with 100 g of a familiar feed, an agro-industrial by-product mixture (AIBM) while the control group was offered AIBM only. After this learning period, all goats were fed only with grass and AIBM for four weeks. Then, a testing period of 14 days was initiated. After a 9.5 h fast, each kid was offered 50 g of COLM daily at 8:30 am for 30 min and COLM intake was monitored.

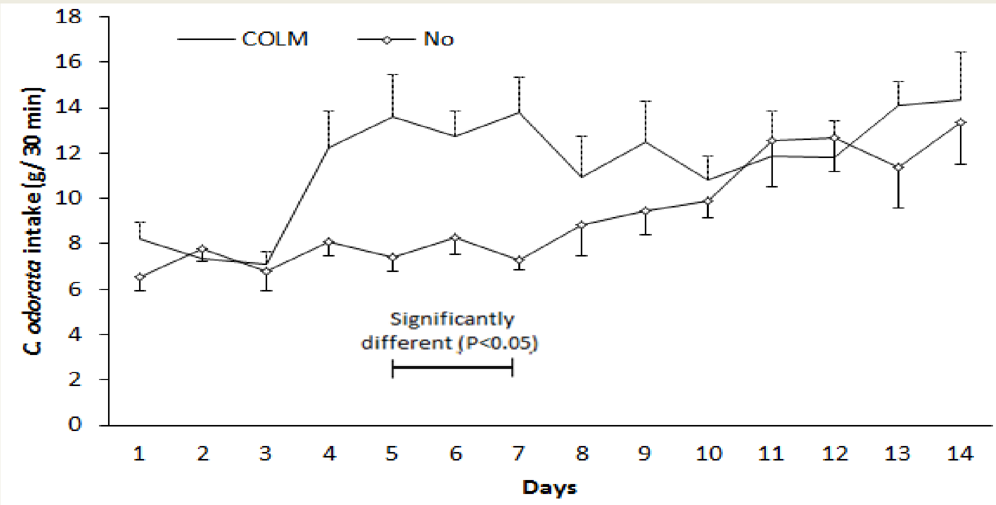

Fig. 1. Mean daily intake of COLM during 30 min by goats fed either COLM (COLM group) or not (No group) during the testing period. Error bars indicate SEM.

During the first 3 days of the testing period, the intake of COLM was similar between the two groups but COLM intake increased rapidly ($P < 0.05$) to 12-14 g/day in the kids that learned about COLM directly after weaning (Fig. 1). In contrast, COLM intake remained similar up until the 7th day of the testing period and gradually increased during the next 4 to 5 days. Overall, goats in experimental group had higher intake of COLM (11.5 g/day versus 9.4 g/day, $P < 0.05$) and showed their preference by a significantly ($P < 0.05$) shorter latency to eat (data not shown).

Only young leaves from the top of the plant were used because the N-oxides of pyrrolizidine alkaloids – a harmful chemical to goats – are reported to be low compared to other parts of the plant (Biller et al., 1994). Extremely young leaves were deliberately avoided due to the possibility of high concentrations of nitrate (Wollenweber et al., 1995). The chemical composition of COLM used in the various experiments is shown in Table 1 and was found to be rather constant between experiments (coefficient of variation ≤ 6.2%). The minor variation in chemical composition is probably due to the seasonal effects in growing conditions.

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

Item	Reference (Chapter)				Mean ± SD[1]
	2	3	4	5	
Dry matter (% as fed)	81.30	80.90	87.44	85.28	83.7 ± 3.17
	-------------- (% DM basis) ---------------				
Ash	6.27	6.29	6.21	5.64	6.1 ± 0.31
Crude protein	20.63	20.46	18.08	19.03	19.5 ± 1.21
Crude fibre	11.90	11.21	11.35	11.27	11.4 ± 0.32
Nitrogen free extract	59.15	59.98	62.06	62.12	60.8 ± 1.50
Ether extract	2.05	2.06	2.30	2.11	2.1 ± 0.12

[1]SD = Standard deviation

Across studies, the mean crude protein content of COLM was 19.5% (DM basis) and this value is somewhat lower than that reported by Apori et al. (2000), i.e. 25.8 % (DM basis). This difference is probably related to the local breed of *C. odorata*, the fertility of the soil especially N and difference in the parts of the plants analysed. Next to its relatively high contents of crude protein, *C. odorata* also contains relatively low amounts of neutral detergent fibre (i.e. 33.1 % DM basis) and acid-detergent lignin (5.3 % DM basis), thereby, indicating its potential as a feedstuff in ruminant nutrition. For obvious reasons, this potential of *C. odorata* can only be fulfilled in case the issue of acceptance is resolved. Moreover, *C. odorata* contains relevant amounts of total extractable phenolic compounds (37.1 g kg^{-1} DM) and to a lesser extent, condensed tannins (1.4 g kg^{-1} DM), with little or no phenolic-related anti-nutritive factors (Apori et al., 2000) which may prevent the use of *C. odorata* as an animal feed.

2. Intake of *C. odorata* by pregnant goats improves its post-weaning intake by goat kids

In each experiment described in this thesis, does were fed or not fed *C. odorata* during pregnancy and post-weaning intake of *C. odorata* by their goat kids was monitored. The results are summarized in Table 2 and clearly there was a higher feed intake of *C. odorata* during the 30 min test period in the treatment groups in comparison to the control groups. Across the four experiments described in this thesis, the post-weaning intake of *C. odorata* by kids that were born from does that ingested this plant during pregnancy was found to be 1.7 times higher (n = 4) compared to the control kids.

Table 2. Effect of *C. odorata* exposure during the pregnancy (experiment) on the intake of this feedstuff by their goat kids, 2.5 - 3.0 months after weaning.

Chapter	Breed	Learning period (days of pregnancy)	Post-weaning COLM[1] intake (g/30 min)		Effect (b/a)
2	Co	45-135	Control (a)	6.1 ± 1.9	1.86
			Experiment (b)	11.3 ± 3.6	
3	Ba[2]	45-135	Control (a)	8.4 ± 1.9	1.57
			Experiment (b)	13.2 ± 2.3	
4	Ba × Co	100-145	Control (a)	13.2 ± 2.1	1.70
			Experiment (b)	22.5 ± 4.2	
5	Co	50-145	Control (a)	8.2 ± 2.1	1.71
			Experiment (b)	13.9 ± 2.7	

[1]COLM: *Chromonaela odorata* leave meal.

[2]Ba: Bachthao.

It is interesting to note that the relative effect (b/a, Table 2) of *C. odorata* feeding during pregnancy on post-weaning intake was similar across the studies, but absolute post-weaning intake (g/30 min) of *C. odorata* showed some variation between studies. The feed intakes recorded in the third experiment (Chapter 4) were numerically higher than those found in the other three experiments (Chapter 2, 3 and 5). This difference cannot be unequivocally explained but it could be explained by the fact that post-weaning responses may be related to the amounts of *C. odorata* ingested by the pregnant does. However, the mean intakes of *C. odorata* by the pregnant does in the experimental groups were 8.2 g/day in the first (Chapter 2) experiment and 50 g/day in the three remaining experiments (Chapter 3, 4, and 5). It thus appears that the higher post-weaning intake of *C. odorata* observed in experiment 3 is not related to higher intakes of *C. odorata* during pregnancy. Furthermore, the difference in *C. odorata* intake cannot be explained by differences in body weight (BW) either because BW of the kids at weaning was 13.2 kg and 12.1 kg in experiments 1 and 2, respectively (Chapter 2 and 3, respectively) and in experiments 3 and 4, respective BWs were be 16.8 and 17.1 kg (Chapter 4 and 5, respectively). Perhaps, the difference in the breeds used (pure breed versus crossbreed) or the different batches of *C. odorata* and/or season were related to the observed differences in post-weaning intake of *C. odorata*. Nevertheless, the current results on post-weaning intake of *C. odorata* are in line with the notion that the kids learned *in utero* about this plant. *In utero* learning has been described previously for a number of species such as rabbits (Semke et al., 1995), lambs (Schaal et al., 1995), dogs (Wells and Hepper, 2006), rats (Hepper, 1988) and mice (Nolte and Mason, 1995) but this thesis presents the first evidence that *in utero* learning also occurs in goats.

3. Feeding behaviour related to the acceptance of *C. odorata*

To test feeding behaviour after the introduction of *C. odorata*, goat kids were fasted overnight (9.5 hours) in the current experiments. It can be expected that hunger may attenuate the aversion of consumption of a novel feed, thereby, diminishing the contrast in feed consumption compared to the control. However, this point of view is not strongly supported by scientific data. For instance, Provenza (1995) reported that the intake of a novel feed was not improved in sheep after a period of 10 days of feed restriction. This observation is in line with the results described in the current thesis. Despite the fact that the animals were fasted prior to exposure to *C. odorata*, a clear contrast in COLM intake was observed between naïve goat kids and kids that experienced *C. odorata* during pregnancy.

The improvement in post-weaning intake of *C. odorata* occurred after a certain period of adaptation with low feed intakes; i.e. 2 weeks (Chapters 2 and 3), 1 week

(Chapter 4) and 10 days (Chapter 5). These observations are in line with the idea that the kids exhibited feed neophobia. Neophobia generally is displayed as a period of low intake followed by a period in which the intake (rapidly) increases until some stable maximum intake is reached. The aforementioned results are corroborated by data from studies with sheep indicating that the period of neophobia lasts from less than 7 days (Burritt and Provenza, 1989) to over 13 days (Chapple and Lynch, 1986). Generally, the latency to consume a novel feed is considered a specific indicator to show the phenomenon of feed neophobia (Provenza, 1995). This idea is in line with the observations that in all 4 experiments it was shown that kids from dams fed *C. odorata* had a significantly ($P < 0.05$) shorter latency to eat in comparison with the controls. However, in case the results on the latency to eat COLM are combined for the 4 studies, a weak, non-significant, relationship was found between the latency to eat and COLM intake (Fig. 2, panel B). The different time of weaning (3 versus 2.5 month) and breed (Co and Bach Thao) among studies could have had an effect on the COLM intake but not on the latency to eat, leading to a strong effect of outliers. Moreover, the number of visits without intake (Fig. 2, panel A), meal frequency (Fig. 2, panel C) and eating boat length of goat kids (Fig. 2, panel D) were found to be poorly related with COLM intake of weaned goat kids during the testing periods. In contrast, COLM intake significantly ($P < 0.01$) correlated with meal size (Fig. 3, panel A), chewing time (Fig. 3, panel B), and intake rate (Fig. 3, panel C). The strongest relationship was found between COLM intake and meal size but this is inherent to the calculation of meal size, i.e. intake of COLM divided by the number of meals.

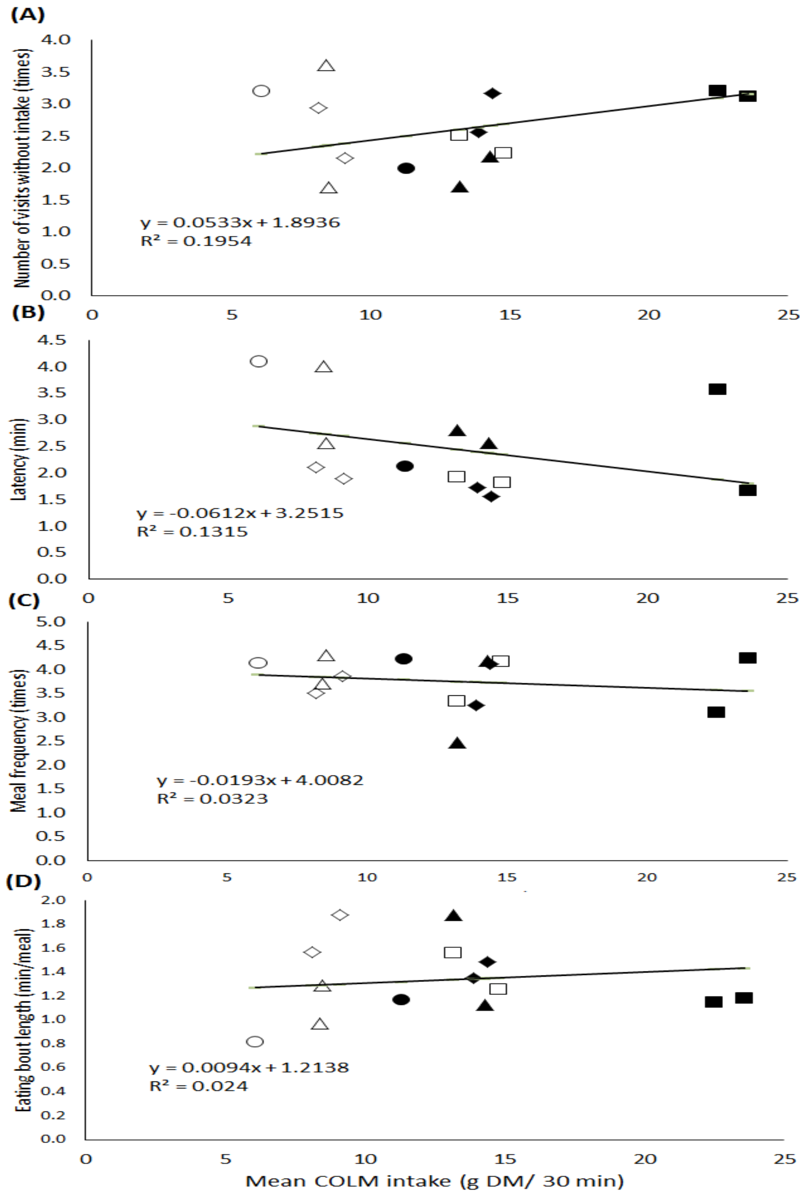

Fig. 2. Relationship between COLM intake and number of visits without intake (A), latency to eat (B), meal frequency (C) and eating boat length of goat kids (D). Symbols:

	Chapter			
	2	3	4	5
Control (naïve kids)	○	Δ	□	◊
Test (prenatally exposed to COLM)	●	▲	■	♦

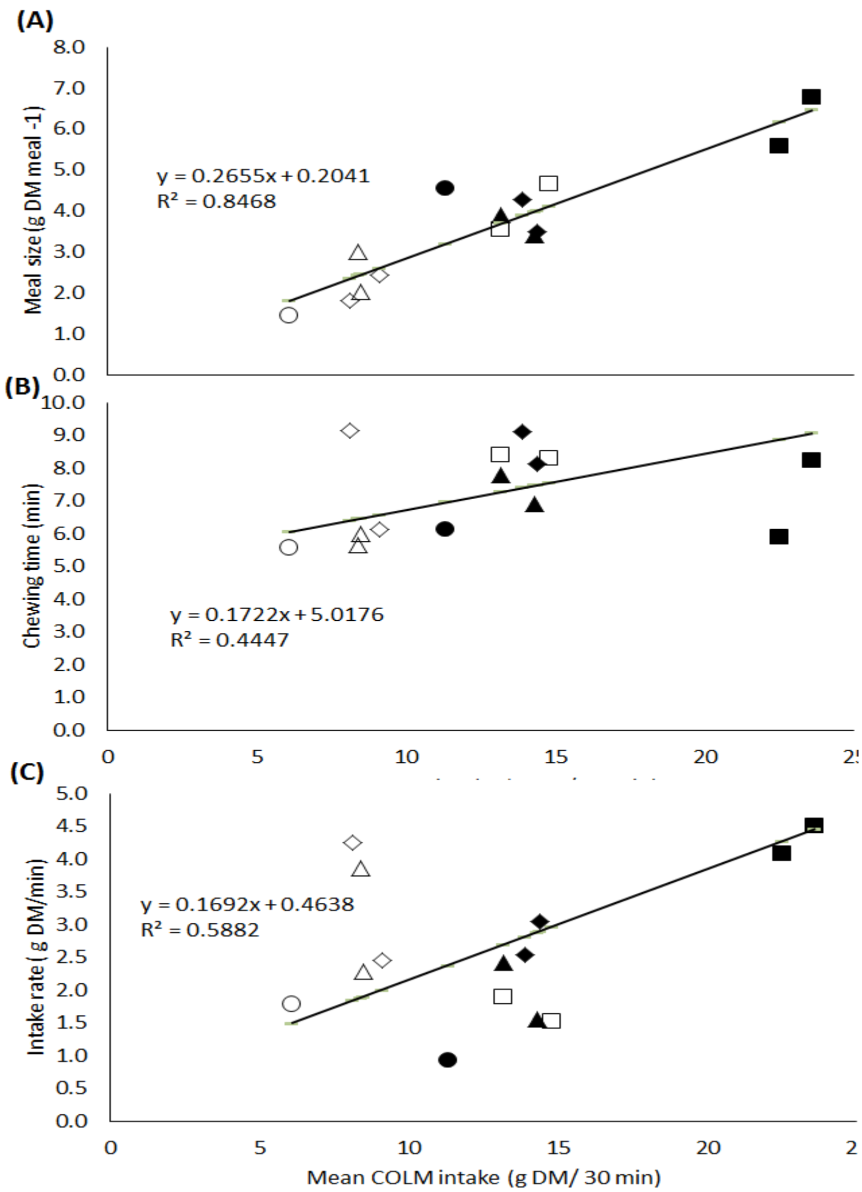

Fig. 3. Relationship between COLM intake and meal size (A), chewing time (B) and intake rate (C). Symbols:

	Chapter			
	2	3	4	5
Control (naïve kids)	○	Δ	□	◊
Test (prenatally exposed to COLM)	●	▲	■	♦

4. Components in *C. odorata* that may affect *in utero* learning

The results of the current studies (Hai et al., 2012; 2013; 2014, 2016) clearly show that the intake of *C. odorata* by pregnant dams increased preference and stimulated the intake of this feedstuff by their kids. The underlying mechanism cannot be derived from the current studies but it is known that flavours/chemosensory cues from the maternal diet can enter the foetal blood stream after crossing the placental barrier (Nolte et al., 1992) and may be perceived through the foetal nasal capillaries (Schaal et al., 1998). Alternatively, flavours/chemosensory cues can be transmitted to amniotic fluid and subsequently perceived by the foetus during mouth movements and ingestion of the fluid (Nolte et al., 1992; Mennella et al., 1995, 2001; El-Haddad et al., 2005). The present observations (Chapter 4) indicate that the last phase of pregnancy (day 100-145) is a critical period for prenatal exposure to *C. odorata* in order to establish an improved intake of *C. odorata* by goat kids after weaning. This finding is corroborated by research in humans showing that there is a continuous turnover of amniotic fluid through the nasal passages such that by the last trimester of pregnancy, the foetus swallows significant amounts of amniotic fluid (Hepper, 1989). Furthermore, human foetuses begin to respond to odours, which are an important component of flavour, at around 28 weeks (de Vries et al., 1985). These observations are in line with data from rodents and rabbits (Hepper, 1988; Bilko et al., 1994). In sheep, foetuses (101–136 days of gestation) swallow 79–447 ml of amniotic fluid per day (Bradley and Mistretta, 1973) and this coincides with the development of taste and smell. Thus, *in utero* experience, especially during the last trimester of pregnancy, can potentially influence elements of the chemosensory system, thereby, increasing the saliency of the stimulus when experienced after birth (Semke et al., 1995; Hepper and Wells, 2006).

In case a mother consumes a feed, the concentration of various metabolites, including those with chemosensory properties, increase in maternal and foetal plasma (Mennella et al., 1995; Desage et al., 1996). Amniotic fluid, the first material consumed by infants, contains a wide range of nutrients such as glucose, fructose, lactic acid, fatty acids, and amino acids, that have particular tastes (Liley, 1972), as well as flavours (for which the odours are perceived retro-nasally) of the foods consumed by the mother (Mennella et al., 1995; Mennella et al., 2001). Currently, the flavours/odours of *C. odorata* related to the transmission of the acceptance of *C. odorata* by goat kids are unknown.

The understanding of amniotic fluid formation, reabsorption, and regulation is important in order to understand concepts affecting maternal-foetal amniotic feed component transfer. It was found that direct transfer of solute and water across the chorio-amnion between maternal and amniotic compartments is very small because of the lack of vascularization and perfusion of this exchange surface (Seeds, 1980). Small lipid soluble substances can rapidly diffuse across the placental barrier, resulting in similar concentrations in both amniotic fluid and foetal plasma. Larger, water soluble compounds that are transferred more slowly, are incorporated into the amniotic fluid (Seeds, 1981). Basic substances may accumulate in the amnion due to ion trapping, resulting in, for example drug concentrations in excess of those found in foetal or maternal plasma (Tucker and Mather, 1979). Therefore, water or lipid soluble components could be responsible for the learning effect.

In terms of nutrient composition of *C. odorata*, the recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. Solvent extraction is a frequently used technique for isolation of plant antioxidant compounds. The most suitable of these solvents are water, ethanol, methanol, acetone, and ethyl acetate (Peschel et al., 2006). Then, extracts can be tested for the presence of certain phytochemicals (Harborne, 1998).

Phytochemical screening was used in the present study to obtain qualitative information on the chemical composition of aqueous and ethanol extracts of COLM using different solvents and coloration to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, amino acids, quinones, coumarins and steroids. This analysis revealed the presence or absence of these compounds in the COLM extracts (Table 3). For future studies, it would be interesting to investigate if there is post-weaning learning by goat kids toward COLM with added lipophilic, ethanol or aqueous COLM extracts to a familiar feed. In this manner, the chemical components from *C. odorata* which are important in the process of adaption of goats to this plant can be narrowed down.

Table 3. Phytochemical screening for chemicals of *C. odorata* extracts.

Chemical groups	COLM extract		
	Ethanol*	Aqueous*	Methanol**
Oils and fats	++	-	
Alkaloids	++	-	p
Lactones and coumarins	-	-	
Triterpenoids and steroids	+	-	p
Resins	+++	-	
Reducing agents	++	++	
Phenols and tannins	+	++	
Amino acids	-	++	
Quinones	+++	-	a
Flavonoids	++	+	p
Astringents	+	+	
Saponins	+	+++	a
Tannins	+++	+++	p
Phlorotannins			p
Terpenoids	++	-	p
Cardiac glycosides:			
With steroidal ring			p
With deoxy – sugar			p

* Chemical analyses performed by the Central Laboratory Section of Hue University of Agriculture and Forestry, Hue, Vietnam; ** Adapted from Akinmoladun et al (2007).

+ = present; ++ = present in relatively high concentrations; +++ = present in relatively extremely high concentrations; - = absent; p = present; a = absent.

5. Transfer by milk (direct and indirect via body reserves)

The studies described in this thesis were designed to test the concept of *in utero* learning by goats. It was demonstrated (Chapter 2) that goat kids born to dams fed *C. odorata* during pregnancy increased their voluntary consumption of *C. odorata* post-weaning. However, the experimental design of the latter study did not exclude the possibility that feed cues of *C. odorata* were retained by the does during pregnancy and subsequently released during early lactation when does experience a negative energy balance. In that case, post-partum mobilization of maternal body fat could have released characteristic chemosensory compounds of *C. odorata* that were excreted with milk which allows for the possibility that the process of adaptation towards *C. odorata* was caused by means of milk consumption instead of *in utero* learning. The results of Chapter 3 clearly show that the post-weaning adaptation towards *C. odorata* was not related to milk consumption because the post-weaning intake of *C. odorata* was only improved for kids born to does that consumed *C. odorata* during pregnancy. It thus appears that effective amounts of *C. odorata* related feed cues were not consumed by the kids during the suckling period. Obviously, the study described in Chapter 3 does not provide clues to explain the absence of effective amounts of *C. odorata* in milk but several explanations may be put forward. First, *C. odorata* related feed cues are not retained during pregnancy. Secondly, the characteristic chemosensory compounds of *C. odorata* are not excreted with milk. A third explanation would be that components from *C. odorata* retained in body fat differ chemically from *C. odorata* flavour that was experienced by the foetus *in utero*. Fourth, the period of learning in goats is confined to only the *in utero* period when neural pathways are established and post-partum neurological development is no longer sensitive to components associated with milk.

Moreover, one of my strategies was to determine whether goat kids would consume more *C. odorata* after experiencing the flavour through the mother's milk (Chapter 5). From this study, it was concluded that incorporation of *C. odorata* to the maternal diet during lactation alone, that is, without exposure during gestation did not lead to a clear increased intake of *C. odorata*. It thus appears that no vertical transmission of flavour occurs during the suckling period in goats. This result is in contrast to studies in humans, rats, and rabbits, in which exposure through mother's milk alone led to an increased preference (Galef and Henderson, 1972; Bilko et al., 1994; Mennella et al., 2001). It is possible that the *C. odorata* ingested by the dams did not appear in their milk or in too low concentrations to have any effect on the offspring. This result, however, is in line with studies in dogs and pigs where postnatal exposure to anise did not change the preference for this flavour in both species (Hepper and Wells, 2006; Oostindjer et al., 2009; Oostindjer et al., 2010). This indicates that, in some species, postnatal exposure alone may not be an important mechanism to modify the offspring's preference, whereas in others it is.

The goat, a precocial animal that has a brain similar to sheep which has a prenatal growth spurt (Richardson and Hebert, 1978), is probably less plastic in the period directly after birth compared to altricial species such as humans, rats, and rabbits (Brunjes, 1983). As such, they may be less sensitive to postnatal modification of flavour preference through milk. Precocial animals, on the other hand, generally start exploring and feeding relatively early in life and, therefore, postnatal programming of preference, that is, changing the structure and function of tissues such as the brain through early experiences (Seckl, 2001), may be less beneficial for those species. Under (semi)natural conditions, goat kids leave the pens a few days after birth to start exploring feed and other items together with the dams. It may be adaptive to have a preference for healthy and available feed types already before this period to avoid the intake of toxins, and this may be established mainly by prenatal experience.

The result of Chapter 5 confirms the findings in a cross-fostering experiment (Chapter 3), showing that the consumption of milk without exposure during pregnancy does not improve the intake of COLM by the kids after weaning. The observation clarified that there is no indirect transfer of the flavour preferences via body reserves of the dam (Chapter 3) and milk is not a vehicle for chemosensory compounds of *C. odorata*.

6. Conclusion and implications

The results of this study clearly show that the ingestion of *C. odorata* by goats during the second half of gestation is advantageous to increase the post-weaning intake of *C. odorata* by the offspring. Thus, the goats' flavour preferences can be modulated by providing *in utero* exposure to *C. odorata*. It is, therefore, possible to utilise the mechanism(s) of *in utero* learning to reduce feed neophobia in goat kids, thereby, increasing post-weaning production performance and welfare of the goat kids. The principle of *in utero* learning in goats is of biological interest and importance, but its practical relevance to overcome neophobia may be somewhat limited because already during pregnancy a decision has to be made on the feedstuffs to be fed in future. Thus, the mixing of known and novel feedstuffs remains of interest to overcome feed neophobia.

In relation to *in utero* learning of feedstuffs by goats, however, the following further insights are needed:

(i) generalization of the current results obtained using *C. odorata* as test feedstuff;

(ii) how the learning process exactly works and which components (feed cues) are responsible for the *in utero* learning process;

(iii) the persistence/memory of *in utero* leaning.

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Summary

Environmental driven changes in meat goat production systems in Vietnam required the use of new (by-) products in the diets for goats. The introduction of novel feedstuffs to young goats is often associated with a reduced feed intake, daily gain and welfare. The work presented here investigated different learning strategies to increase the preference for flavour cues and ingredients after weaning. It was hypothesized that goat kids may learn flavour preferences from their mother during pregnancy or during lactation and that this learning improves the intake of novel ingredients after weaning.

Chapter 2 evaluated the effect of *Chromoneala odorata* ingestion by goat dams during pregnancy on intake of *C. odorata* by their kids after weaning. Ten pregnant female goats were used: five goats (treatment group) were fed 50 g of sun dried *C. odorata* leave meal (COLM) along with a basal diet from day 45 to 135 of pregnancy while the other five goats (control group) were fed only the basal diet during this period. After weaning at 3 months, one kid from each goat dam (control and treatment) was selected and was offered each day 50 g of COLM for 4 weeks. The intake of COLM by goat kids born to dams fed COLM was significantly higher over the 4 weeks compared to goat kids born to the control dams (11.32 ± 3.60 vs. 6.09 ± 1.91 g per day, P < 0.028). Goat kids from mothers fed *C. odorata* during the pregnancy showed a shorter latency to eat, a larger meal size and a longer chewing time than kids from mothers who did not ingest this plant during pregnancy. The results from this trial indicated that the foetus *in utero* could be primed by their mother's diet.

In Chapter 3 it was studied whether the improved post-weaning intake of *C. odorata* by goat kids is related to either the *in utero* period of the goat kids or the subsequent suckling period. It was hypothesized that kids born to dams fed *C. odorata* during pregnancy and receiving milk from dams not exposed to *C. odorata*

during pregnancy showed an improved acceptance to consume this plant. Twenty female goats were successfully synchronized and divided into 4 groups. Two groups (1 and 3) were offered 50 g of dried COLM mixed with a basic diet for the last 3 months of pregnancy until 2 weeks before parturition. At birth, the kids from the goats in groups 1 and 2 were cross-fostered without receiving colostrum or milk from their own mother. Kids from groups 3 and 4 remained with their mothers. After weaning at 2.5 months, one kid from each goat dam was selected for the test. Daily COLM intake of kids was measured for 30 min during 4 weeks. Feeding activities of the individually housed goat kids was monitored via a camera system and subsequently analyzed. Kids born to dams receiving COLM during pregnancy consumed higher amounts of COLM during all feeding preference tests compared to kids from the control group, particularly during week 3 and 4 ($P < 0.001$). Shorter latency, longer time spent on each meal and total eating time, chewing time and higher meal size ($P < 0.05$) was observed in the kids born from does that ingested COLM during pregnancy. It was concluded that prenatal exposure to *C. odorata* via maternal ingestion significantly increases the intake of *C. odorata* by weaned goat kids. This improved intake is due to the *in utero* learning and not due to the transfer of (secondary) components via the milk of the mothers fed *C. odorata* during pregnancy.

Chapter 4 investigated the effect of exposure phase of the goat's pregnancy on *in utero* leaning of *C. odorata* by goat kids by comparing mid (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 pregnant goats (Co breed) were divided randomly into 4 equal groups. Goat dams were fed a basal diet either without (control) or with 50 g of COLM during mid/late/mid-late period of pregnancy (MP, LP and MLP, respectively). After weaning (3 months), one kid from each goat dam was selected for the daily measurement of voluntary COLM intake over a period of 4 weeks. Feeding activities of the individually housed goat kids were monitored with a camera system. The treatments where the consumption of *C. odorata* took place during late pregnancy of the dams (LP and MLP), resulted in a 70% higher intake (week 4) of COLM by their kids after weaning ($P < 0.05$). 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 significantly longer chewing time ($P < 0.05$). It was concluded that transmission of feeding behaviour from mother to offspring in goats occurs during late gestation.

Chapter 5 aimed to assess the role of postnatal COLM exposure in relation to the preferences to this feedstuff by goat kids after weaning. It was hypothesized 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. At 40 days after insemination, 32 pregnant goats were randomly allocated to four groups. Each pregnant goat was fed 100 g of an agro-industrial by-product mixture (AIBM) either without (control) or with 50 g of COLM. The AIBM containing COLM was fed either between the 50^{th} to 145^{th} 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 during pregnancy ended on day 141 of gestation, approximately one week before farrowing. At the time of weaning (3 months), one kid from each goat dam was selected to measure voluntary intake of COLM. 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. As the goat is a precocial animal with a prenatal growth spurt and probably a less plastic brain in the period after birth than that of altricial species, it is

suggested that the goat is, therefore, less sensitive to postnatal modification of flavour preference (e.g. milk). It was concluded that the transmission of feeding behaviour from goat dams to offspring does not occur during lactation. However, the concept of *in utero* learning in goats was reconfirmed.

The general discussion in Chapter 6 provides an explanation and interpretation of the results as well as the implication for using *C. odorata* as a model to answer the question: how to improve feed intake of unknown feedstuffs in goats who are well known to have neophobia.The results clearly show that the ingestion of *C. odorata* by pregnant goat during the last stage of pregnancy increases the amount of this feedstuff consumed by goat kids after weaning whereas feeding dams during the lactation does not lead to improvement in intake. The goats' flavour preferences can be modulated by providing an early exposure, starting *in utero*, to a wide variety of flavours. In practice, this learning can reduce stress related to the introduction of new feedstuffs around weaning and increase post-weaning performance and welfare of goat kids. A limitation of this *in utero* learning is that it must be decided during pregnancy which feedstuffs are fed to the kids later on. Therefore, the option of mixing known with unknown feedstuffs seems a more practical solution for the problem of neophobia in goats.

Samenvatting

De extensieve productie van geitenvlees in Vietnam vindt traditioneel plaats door de dieren onder andere te laten foerageren in het regenwoud. Vanuit ecologische overwegingen staat deze traditionele wijze van geitenhouderij onder druk en daarom stimuleert de Vietnamese overheid geitenhouders de dieren op te stallen en te voeren met andere, voor de geit vaak onbekende voedermiddelen. De acceptatie van onbekende voedermiddelen verloopt juist bij geiten over het algemeen moeizaam en gaat meestal gepaard met een (sterk) verlaagde voeropname (neofobie) en daardoor een verminderde groei van de dieren. In dit proefschrift is het fenomeen van *in utero* leren, het beïnvloeden van voedselvoorkeur van dieren na de partus tijdens hun verblijf in de baarmoeder, in geiten onderzocht. Daartoe is de voeropname, en het daaraan gerelateerde gedrag, van een onbekend voedermiddel bij gespeende lammeren geevalueerd die afkomstig waren van geitenmoeders die al dan niet gevoerd werden met hetzelfde voedermiddel tijdens de dracht.

Bij andere diersoorten is het bestaan van het *in utero* leervermogen met betrekking tot de voedselvoorkeur reeds aangetoond, maar bij de geit is dit nog niet bewezen. In hoofdstuk 2 is onderzocht in hoeverre de opname van *Chromoneala odorata*[1] door drachtige geiten resulteert in een verhoogde voeropname van deze plant door gespeende geitenlammeren. De resultaten wezen uit dat de lammeren die afkomstig waren van moedergeiten die niet gevoerd waren met *C. odorata* tijdens de dracht, zeer terughoudend waren om deze plant te eten en een significant lagere hoeveelheid ervan consumeerden vergeleken met de lammeren die afkomstig waren van moedergeiten die tijdens de dracht wel gevoerd waren met *C. odorata*.

[1] Deze eiwitrijke plant (ook wel bekend als Siamkruid) komt veel voor in de tropen, maar wordt vanwege zijn onsmakelijkheid zeer slecht gegeten door geiten. Deze plant is in de proeven als model gebruikt.

Deze resultaten zijn in overeenstemming met het idee dat geitenlammeren een *in utero* leervermogen met betrekking tot de voedselvoorkeur hebben. Door de gekozen proefopzet was echter niet uit te sluiten dat *C. odorata* gerelateerde signaalstoffen via de moedermelk door de lammeren werden opgenomen en dat de gespeende lammeren daardoor meer *C. odorata* opnamen. In een vervolgexperiment (hoofdstuk 3) is daarom “cross-fostering” van lammeren toegepast om na te gaan in hoeverre de opname van de genoemde signaalstoffen via moedermelk bijdragen aan de geobserveerde verhoogde opname van *C. Odorata* na het spenen. In dit experiment werden lammeren afkomstig van geitenmoeders die tijdens de dracht *C. odorata* opnamen, gezoogd door geitenmoeders die geen *C. odorata* tijdens de dracht verstrekt kregen, en andersom (cross-fostering). De uitkomsten van dit experiment bevestigden de uitkomsten van het vorige experiment (hoofdstuk 2). De lammeren die afkomstig waren van geitenmoeders die tijdens de dracht met *C. Odorata* gevoerd werden, maar gezoogd door geitenmoeders gevoerd met een *C. odorata* vrij rantsoen tijdens de dracht, realiseerden een significant hogere *C. odorata* opname dan de controlelammeren waarvan de moeders geen *C. odorata* kregen tijdens de dracht. Ook lammeren afkomstig van moeders die tijdens de dracht geen *C. odorat*a kregen, maar gezoogd werden door moeders die tijdens de dracht wel *C. odorata* hadden gegeten, namen na het spenen niet meer *C. odorata* op dan de controlelammeren. In deze studie werd geconcludeerd dat *in utero* leren verantwoordelijk was voor de verhoogde opname van *C. odorata* door gespeende geitenlammeren.

Bij schapen is bekend dat de smaakcellen van de foetus functioneel zijn vanaf de 17de week van de dracht. Het is niet bekend in hoeverre deze waarneming bij schapen geëxtrapoleerd kan worden naar geiten. Indien echter wordt aangenomen dat ook bij foeten van geiten de smaakcellen functioneel zijn in het laatste deel van de dracht, zou verstrekking van *C. odorata* aan drachtige geiten alleen tijdens het laatste deel van de dracht de opname van deze plant kunnen stimuleren. Dit idee is getoetst in hoofdstuk 4 en het bleek dat de opname van *C. odorata* bij gespeende lammeren niet verschillend was tussen lammeren geboren uit geitenmoeders die deze plant opnamen tijdens de gehele dracht of alleen tijdens het laatste gedeelte van de dracht (100 tot 145 dagen dracht). Deze bevinding wijst erop dat de aan *C. odorata* gerelateerde signaalstoffen via foetale smaakcellen de voedselvoorkeur van de lammeren beïnvloedt.

Tijdens de experimenten beschreven in hoofdstukken 2, 3 en 4 werd *C. odorata* alleen tijdens de dracht verstrekt. Het kan daarom niet worden uitgesloten dat verstrekking van *C. odorata* aan geitenmoeders tijdens zowel de zoogperiode als de dracht, de opname van *C. odorata* bij gespeende lammeren nog verder kan stimuleren. De resultaten van het experiment beschreven in hoofdstuk 5 laten echter zien dat de opname van *C. odorata* door gespeende geitenlammeren niet verder gestimuleerd wordt als ook tijdens de zoogperiode *C. odorata* wordt gevoerd aan geitenmoeders. De uitkomsten van dit experiment bevestigden het concept van *in utero* leren bij geiten en suggereren dat er geen transmissie van voer gerelateerd gedrag plaats vindt tijdens de zoogperiode. Dit laatste kan verklaard worden door het feit dat de geit, als nestvlieder, geboren wordt met hersenen die minder ontvankelijk zijn voor postnatale signalen die leiden tot een bepaalde voedselvoorkeur. Dit in tegenstelling tot de meeste nestblijvers.

In de algemene discussie (hoofdstuk 6) zijn de resultaten van de experimenten samengevat en hieruit komt duidelijk naar voren dat de opname van *C. odorata* bij gespeende geitenlammeren consistent verhoogd is wanneer hun moeder deze plant tijdens de dracht heeft opgenomen. Het concept van *in utero* leren is dus ook bij de geit aanwezig en kan derhalve gebruikt worden in de geitenhouderij in Vietnam. Deze kennis kan door geitenhouders worden toegepast om de introductie van onbekende voedermiddelen te vergemakkelijken. Een nadeel van het concept van *in utero* leren is wel dat tijdens de drachtperiode al bekend moet zijn welke voedermiddelen in de toekomst gevoerd gaan worden aan de gespeende geitenlammeren. Het mengen van het onbekende voedermiddel met een smakelijk en reeds bekend voedermiddel is een alternatieve strategie om voer neofobie bij geiten tegen te gaan.

Acknowledgements

I wish to express my sincere appreciation to the following persons who made the completion of this thesis possible:

Dr. Henk Everts, my copromotor, for all of his valuable advices and supports as well as for his prompt editing of the manuscripts. The thesis could not have been completed without his constructive corrections and advises.

Dr. Jan Thomas Schonewille, my copromotor, who gave me enthusiastic encouragement, guidance, support and his friendship which enabled me to develop an understanding of the research work.

Prof. Dr. Wouter H. Hendriks, my promotor, for accepting me as his PhD student, and for advice and support during my study and preparation of the manuscripts and thesis.

Associate Prof. Dr. Dam Van Tien, former Dean of FAS - HUAF, who gave me the opportunity to enter the Asia-Link project as the way to do a PhD study at Utrecht University. I also would like to express my appreciation for his advice and support during my study.

Dr. Robert Paling, former director of the Bureau of International Cooperation and his team for helping me during my stay in the Netherlands.

Associate Prof. Nguyen Van Linh - Rector of Hue University, Associate Prof. Le Van An - Rector of HUAF, Associate Prof. Nguyen Xuan Ba - Dean of FAS - HUAF and Dr. Lai Xuan Thuy - former head of Trig Project - Hue University, who gave me the opportunities and favourable official procedures to follow the PhD program in Utrecht University.

My colleagues at HUAF: Associate Prof. Le Dinh Phung, Drs. Nguyen Hai Quan and Drs. P.H. Son Hung, Dr. Le Xuan Hoa, Associate Prof. Du Thanh Hang and Drs. Le Xuan Anh; PhD students of the Asia-link project: Jaturong Wonsanit, Rittichai Pilachai, Hasliza Abu Hassim and Paiwan Srenanul for their warm kindness and friendship.

My students: Mr. Quang, Mr. Son, Mr. Tho, Ms. Han, Ms. Tinh, Ms. Van… who helped me with the conductance of my experiments.

My family is the most important reason why I have been able to fulfill my studies.

Lastly, I offer my regards and blessings to all of those who supported me in any way during the completion of the thesis.

Curriculum Vitae

Mr. Phan Vu Hai was born on the 5th August 1973 in Ha Tinh province, Vietnam. He attended the Hue University of Agriculture and Forestry, Hue, Vietnam and obtained his BSc degree in Animal Science in 1996. In 2002, he completed his MSc in Tropical Animal Sciences at James Cook University, Townsville, Queensland, Australia. From 1997 to 2017 he was a lecturer and researcher at the Department of Veterinary Medicine in the Faculty of Animal Husbandry and Veterinary Medicine at the University of Agriculture and Forestry, Hue, Vietnam. From 2010 to 2017 he was head of this Department. In 2001 he obtained funding for a two months International Course on Animal Production and Veterinary Public Health in Utrecht University.

From 2010 to 2016, he received several funds from the Asia-link project (Contract number: TH/Asia-link/014) and Trig Project of Hue University, Vietnam for his studies in the field of *in utero* learning of goat kids to overcome feed neophobia.

Besides his studies and career as a lecturer, Phan Vu Hai is married and has three children.

Publications

Peer-reviewed journals

Phan Vu Hai, Ho Trung Thong and D. V. Tien. 2011. Matching ingestive behaviour of goats to local feed resources. Journal of Science - Hue University (English version) 67:57-67.

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