

# **Food for Ruminantion**

Developing novel feeding strategies  
to improve the welfare of veal calves

Laura Webb

## **Thesis committee**

### **Promotor**

Prof. Dr I.J.M. de Boer  
Professor of Animal Production Systems  
Wageningen University

### **Co-promotors**

Dr E.A.M. Bokkers  
Assistant professor, Animal Production Systems Group  
Wageningen University

Dr C.G. van Reenen  
Researcher, Animal Sciences Group  
Wageningen UR Livestock Research

### **Other members**

Prof. Dr M. Naguib, Wageningen University  
Dr G. van Duinkerken, Wageningen UR Livestock Research  
Dr B.J. Lensink, ISA Group, Lille, France  
Prof. Dr D.M. Weary, University of British Columbia, Canada

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# Food for Ruminantion

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Laura Webb

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## ABSTRACT

Veal calves are typically fed high quantities of milk replacer supplemented with solid feed, which tends to contain a relatively small portion of roughage. Feeding strategies used in veal production have been associated with welfare issues, including the development of abnormal oral behaviours (AOB) and poor gastrointestinal health. AOB mainly include tongue playing and excessive oral manipulation of the environment, and are thought to develop in calves when chewing activity (i.e. eating and rumination) is not adequately stimulated. The aim of this thesis was to develop novel feeding strategies to improve the welfare of veal calves, i.e. to minimise the development of AOB and gastrointestinal health disorders. Increasing solid feed provision stimulated chewing activity and reduced AOB frequency, although this was less true for solid feed mixtures comprising a large proportion of concentrate (i.e. 80%). The relationship between the amount of solid feed provided and AOB, however, was not straightforward. If calves experience a decrease in chewing activity as they grow older, their welfare may be compromised. Solid feed provision should be increased throughout the fattening period to meet the growing need of calves for structure in their feed. Moreover, ad libitum provision of hay, a roughage source with both high levels of structure and fermentable fibre, seemed to meet all three objectives of encouraging rumination and rumen development without exacerbating abomasal damage. If hay is omitted in veal production due to its high iron content, then multiple roughage sources should be provided to calves that together provide sufficient structure and fermentable fibre. The simple addition of ad libitum long straw to a typical veal diet (with a high concentrate proportion) seemed to improve behaviour, and therefore, welfare, significantly. Calves preferred milk replacer, concentrate and hay over straw and maize silage, although preferences varied across age and depended on the variable considered to assess preference (i.e. intake, time spent eating or frequency of visits). Calves were willing to work for hay and straw, despite being fed a high-energy diet of milk replacer and concentrate. In addition, they showed a preference for long over chopped hay, but not long over chopped straw. Calves voluntarily selected an average of 1000 g DM roughage and 2000 g DM concentrate on top of milk replacer (provided ad libitum), and seemed to select a diet that enabled them to meet their needs in terms of chewing activity. Novel feeding strategies aimed at improving the welfare of veal calves should comprise sufficient roughage to meet every individual's needs in terms of chewing activity, and this throughout their lifetime, whilst stimulating good gastrointestinal health.



# Contents

1	General introduction . . . . .	9
2	Effects of roughage source, amount and particle size on behaviour and gastrointestinal health of veal calves . . . . .	27
3	Behaviour and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth . . . . .	49
4	Dietary preferences of Holstein-Friesian calves: What do calves choose to eat and how do preferences affect behaviour? . . . . .	67
5	Methods for cross point analysis of double-demand functions in assessing animal preferences . . . . .	91
6	Chopped or long roughage: what do calves prefer? Using cross point analysis of double demand functions . . . . .	111
7	Does temperament affect learning in calves? . . . . .	129
8	Can tongue playing in veal calves be linked to cortisol or temperament? . . . . .	141
9	The role of solid feed amount and composition, and milk replacer supply in veal calf welfare . . . . .	157
10	General discussion . . . . .	183
	Summary . . . . .	213
	Samenvatting (Dutch summary) . . . . .	217

<b>References . . . . .</b>	<b>222</b>
<b>Acknowledgements . . . . .</b>	<b>241</b>
<b>About the author . . . . .</b>	<b>245</b>
<b>List of publications . . . . .</b>	<b>247</b>
<b>Education certificate . . . . .</b>	<b>251</b>
<b>Colophon . . . . .</b>	<b>256</b>



Chapter **1**

General introduction

*“... the motivation of animal behaviour is extremely diverse.”* (Dawkins, 1983)



# 1 Introduction

Veal calves are raised for the production of (white) veal, with a fattening period generally ranging from 2 to 27 weeks of age in the Netherlands. Feeding strategies used in veal production systems are thought to be associated with poor animal welfare.

Veal calves were originally fed a feeding strategy consisting of milk replacer (**MR**) only. Following societal concern about veal calf welfare, the 1997 EU Directive was put into place, stipulating a minimum fibrous feed amount for calves ranging from 50 g/d at 8 weeks to 250 g/d fibrous feed at 20 weeks of age (EU Council, 1997). In 2008, a new Directive was created which stipulated a minimum of 50 g fibrous feed from 2 weeks of age instead of 8 weeks (EU Council, 2008). It is unclear whether these amounts refer to fresh or dry matter (**DM**) amounts, and these Directives allow calves older than 20 weeks of age to receive no fibrous feed whatsoever. Moreover, it is unclear why the amount of 250 g of fibrous feed was chosen, which sources should be used, and in what form or particle size they should be fed to calves. In the remainder of this thesis, ‘fibrous feed’ will be referred to as solid feed. Solid feed comprises of concentrate, generally in the form of pelleted feed, and roughage, such as straw, hay, and maize silage.

In a feeding context, poor welfare in veal calves is signalled by the occurrence of abnormal behaviours (Leruste, 2014) and poor gastrointestinal health (Brscic et al., 2011). Abnormal behaviours displayed by calves, thought to be associated with poor feeding conditions, are often referred to as abnormal oral behaviours (**AOB**). AOB include excessive, repetitive oral manipulation of the pen structure and trough or bucket, sham chewing (i.e. chewing without any substrate inside the mouth), as well as rolling and unrolling of the tongue inside or outside of the mouth, i.e. tongue playing (Wiepkema et al., 1987; Kooijman et al., 1991; Veissier et al., 1998; Mattiello et al., 2002). Low levels of eating and rumination (hereafter referred to as chewing activity) are often considered the main causal factor for the development of AOB in calves (Veissier et al., 1998; Mattiello et al., 2002).

The main aim of this research was to develop novel feeding strategies to improve the welfare of veal calves, i.e. to minimise the development of AOB and gastrointestinal health disorders (involving the rumen and abomasum), as well as maximise chewing activity. To this end, behavioural indicators of welfare, in particular the monitoring of AOB, and preference tests were used. Therefore, it is important to first understand the causal mechanisms mediating AOB and the best method available to investigate feed preferences in calves. These two aspects are described in the next two

sections. Following this, past research on feeding strategies developed for veal calves and their implications for calf welfare will be addressed.

## 2 Stereotypies as indicators of poor welfare

When well established in calves, AOB become a form of stereotypic behaviour. Stereotypies are “behavioural elements that have a very constant form, that are repeated over and again, (may) differ from individual to individual and that seem to have no function” (Wiepkema, 1987). Stereotypies are used in applied animal behaviour research as indicators of poor welfare because they tend to be displayed by animals kept in sub-optimal environments (Mason, 1991a). They develop in animals that are believed to be ‘frustrated’ (Duncan and Wood-Gush, 1972), ‘bored’ (Wemelsfelder, 1993) or ‘aroused’, or in situations leading to unavoidable stress and fear (Wiepkema et al., 1987; Mason, 1991a).

However, the link between stereotypies and poor welfare, supposedly including suffering through unpleasant mental states, is not a straightforward one (Mason, 1991b). In fact, stereotypies can develop in non-aversive situations, be associated with anticipation rather than negative mental states, and aversive situations do not necessarily lead to the development of stereotypies (Mason, 1991a). Despite exceptions to the rule, stereotypies generally develop in environments that are restricted and associated with causing stress to animals. Inter-individual differences in the development of stereotypies may reflect the coping/adaptive value of these behaviours, which have been suggested to reduce stress or have a calming effect on animals that perform stereotypies in sub-optimal environments (Wiepkema, 1987; Wiepkema et al., 1987). This further implies that animals that do not develop stereotypies in sub-optimal environments might actually be worse off than stereotyping animals (Mason and Latham, 2004).

The pathway towards the understanding of factors leading up to the development of stereotypies has seen two approaches: 1) studying underlying mechanisms for the expression of behaviour, and 2) focusing on the association between stereotypies and stress (Lawrence and Terlouw, 1993). This section will first investigate current theoretical frameworks for the underlying mechanisms of feeding behaviour, with the aim of understanding how feed-related stereotypies develop in animals. After this, I will look more closely at AOB in veal calves, attempting to understand the underlying mechanisms for AOB by using the previously described theoretical framework for the motivation system of feeding behaviour.

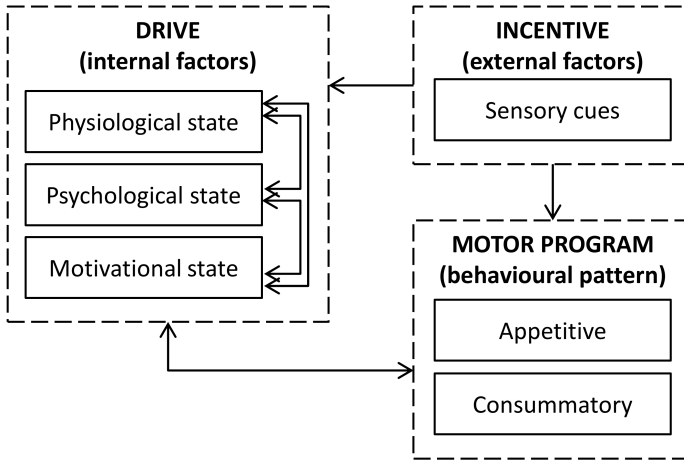


Figure 1.1: Basic motivation system (based on Toates, 1986; Jensen and Toates, 1993; Lawrence and Terlow, 1993). We apply this model to feeding behaviour in the text, but this model is compatible with any behavioural pattern aimed at attaining a particular goal.

## 2.1 Basics of behaviour control: normal and thwarted feeding behaviour

Because stereotypes generally develop in restricted environments, it is thought that stereotypes come about through frustration of particular motivation systems (Hughes and Duncan, 1988a; Rushen et al., 1993). Motivational systems are an attempt to explain which factors (internal and external) mediate activities (i.e. behavioural patterns) performed by individuals (e.g. feeding). Motivation is described by Toates (1986) as “the strength of the tendency to engage in behaviour when taking into account not only internal factors but also appropriate external factors”. In the context of motivation, external factors are also referred to as ‘incentive’, while internal factors are referred to as ‘drive’ (Toates, 1986). Behavioural patterns, such as feeding, include two motor programs: 1) appetitive behaviours, which are all behaviours linked to the search for and manipulation of food (i.e. foraging), and 2) consummatory behaviours, which are the behaviours linked to ingesting food (Hinde, 1953). A simple theoretical framework illustrating the basics of behaviour control is shown in Fig.1.1 and described in the following section.

## Motivational system for feeding behaviour

Simple homeostatic models of motivation propose that motivation is directed by some physiological measure (e.g. nutrient) falling below the optimal level and when this level reaches the optimum again, following activation of some motor program (e.g. feeding), the motivation ceases (Toates, 1986). Accordingly, if an animal's stomach is injected with nutrients, the animal will accommodate for this by reducing food intake (Pekas 1983 in Lawrence et al., 1993). Physiological measures that may affect feeding behaviour include nutrient levels, energy state, or feedback from the gastrointestinal tract (e.g. stomach wall stretch). This model is, however, too simplistic and fails to explain, for example, why animals choose to work for food when the same food is freely available (i.e. contrafreeloading: Inglis et al. 1997). In addition, some animals may simply start eating after being disturbed, and, thus, being aroused (Lawrence and Terlouw, 1993). The latter demonstrates the importance of external factors in the form of sensory cues for the activation of behaviour (Lawrence et al., 1993).

In addition to physiological state and external stimuli, the psychological state of an individual may influence feeding behaviour. Psychological state includes cognition (Toates, 2004) (e.g. memory of previous meal or learning of post-ingestive consequences), emotions (Toates, 2004) (e.g. neophobia), preferences (e.g. positional preference), and temperament (e.g. generalist versus specialist, or social animals influenced by social facilitation). The physiological and psychological states both impact on the motivational state (tendency to start feeding) (Fig.1.1).

## Why stereotypies develop

Stereotypies are thought to emerge from naturally occurring behaviours (Toates, 2004). Cronin (1985 in Wiepkema, 1987) described four stages for the development of stereotypies in tethered sows: 1) aggression and escape attempts (around 1 hour), 2) immobility potentially due to exhaustion (around 1 day), 3) new, less vigorous escape attempts that are repeated (around 16 days), and 4) ritualisation of the escape attempts, with a reduction in flexibility, complexity and vigour, and even sometimes a loosening from objects (e.g. sham chewing). In other words, stereotypies occur through the following mechanisms: a frequently performed behaviour pattern in a restricted environment will limit variation ("minimal cognitive input", Toates, 2004) and will limit termination of behaviour. As a consequence, the said behaviour will subsequently become more and more independent from external stimuli (in terms of control): a process called emancipation (Mason, 1991b). Stereotypies are thought to occur through a shift of control from higher to lower-levels of control, i.e. less complex cognitive processes to initiate behaviour, associated with positive reinforcement

(Toates, 2004).

Stereotypies may be mediated by long-term or repeated impairments at the physiological state level (Fig.1.1) (Lawrence and Terlouw, 1993). For example, crib-biting in horses (repetitive biting of some aspect of the environment) might develop in response to gastric acidity, resulting from a feeding strategy low in roughage (Wickens and Heleski, 2010). Moreover, stereotypic air-pecking in hens may relate to a calcium deficiency (Hughes and Wood-Gush, 1973). Psychological state might also mediate the emergence of stereotypies. For example, anticipation of an upcoming meal in undernourished sows may cause high levels of arousal and subsequently high levels of activity in a restricted environment, which might cause sows to start biting the pen structure (Lawrence and Terlouw, 1993).

A number of authors have suggested that stereotypies develop in sub-optimal environments as an attempt by individuals to ‘adapt’ to, or ‘cope’ with the environment (i.e. coping hypothesis) (Wiepkema, 1987). Although some controversy existed relating to the coping hypothesis of stereotypies (Mason, 1991b; Rushen, 1993), there does seem to be some improvement in welfare associated to the performance of these behaviours (e.g. lower prevalence of gastric ulceration or lower heart rate: reviewed in Mason and Latham, 2004). Individual differences in the performance of these behaviours may be associated with differences in so-called ‘temperament traits’, for example, differences in ‘coping style’ (Mason, 1991b). Koolhaas et al. (2007) defines coping styles as “alternative response patterns in reaction to a stressor”. These styles may depend on sex, species or individual variation (Mason, 1991b), and comprise of a proactive and reactive style, which can be considered as the two extremities of a continuum (Benus et al., 1991). The proactive style has been associated with less behavioural flexibility and habit or routine formation (Mason, 1991b; Bolhuis et al., 2004). The idea is that proactive animals may be more likely to develop high levels of stereotypic behaviour, compared to reactive animals, because they are more likely to respond to stress with activity and may be more prone to ritualise repetitive behaviours (Mason, 1991b). Mason and Latham (2004) state that “stereotypies should always be taken seriously as a warning sign of potential suffering” but “non-stereotyping or low-stereotyping individuals should not be overlooked or assumed to be faring well”.

## 2.2 Why abnormal oral behaviours develop in veal calves

I will now discuss potential reasons why AOB develop in veal calves, including: frustration leading to chronic stress, lack of stimulation, arousal and iron deficiency. Other causal factors may exist, particularly at the neurological level (e.g. Wickens

and Heleski, 2010), but discussing all of these is not within the scope of this thesis.

Some may argue that not all AOB in calves are stereotypies, but instead abnormal behaviours that are not ritualised. Calves may excessively lick the pen structure in a manner that does not appear repetitive and invariant. Moreover, one may wonder what ‘excessive’ truly means, and at which level of licking we can say with absolute certainty that we are talking about abnormal behaviours and not normal exploration. I will assume here that most AOB in veal calves are in fact stereotypies, or behavioural patterns soon to become stereotypies. The work presented in the next chapters of this thesis does not attempt to understand what level of, for example, oral manipulation of the trough is ‘abnormal’, but instead compares AOB levels observed in calves fed different feeding strategies.

### **Abnormal oral behaviours and chronic stress**

If AOB develop in calves in response to frustration from limited chewing opportunity, one would expect to find a relationship between AOB level and (non-behavioural) measures of chronic stress (Wiepkema, 1987). In most chronic stress studies, researchers investigate some aspect of the hypothalamic-pituitary-adrenal (**HPA**) axis (Mormède et al., 2007). Other possible measures of chronic stress include: changes in body weight, immune system impairments, mechanisms of the autonomic nervous system, or health problems (Dantzer and Mormède, 1983). A detailed description of the role and mechanisms of the HPA axis during acute and chronic stress can be found in Tsigos and Chrousos (2002). In brief, acute stress stimulates an increase in amplitude and synchronisation of corticotropin-releasing hormone (**CRH**) and arginine-vasopressin from the paraventricular nuclei located in the hypothalamus, which stimulates the release of adrenocorticotrophic hormone (**ACTH**) from the anterior pituitary gland. In turn, ACTH stimulates the release of glucocorticoid hormones (in most mammals, including calves, these are mainly cortisol) from zona fasciculata cells in the adrenal cortex. The function of cortisol during acute stress is, among other things, to increase blood sugar, suppress the immune system, as well as provide feedback to the HPA axis (Tsigos and Chrousos, 2002; Mormède et al., 2011). In the absence of acute stress, the HPA axis has a regulatory function in maintaining homeostasis (Dallman et al., 1993; Tsigos and Chrousos, 2002).

Despite a comprehensive understanding of the HPA axis’ response to acute stress, measuring changes driven by chronic stress is much more difficult, and when it comes to identifying impairments to animal welfare, measuring chronic stress is a central focus (Mormède et al., 2007). When a stressor is initially applied, cortisol levels increase rapidly (in plasma and saliva this is counted in minutes), but after continued



exposure to the same stressor, cortisol levels tend to decline back to baseline levels despite maintenance of behavioural indicators of stress (Dellmeier et al., 1985; Friend et al., 1985; Jensen et al., 1996). Adrenal function has been suggested to be modified during repeated exposure to stressors, as seen by a higher cortisol peak after ACTH injection, suggesting a heightened sensitivity of the adrenal cortex or increased activity of the hypothalamus (Friend et al., 1985; Bhatnagar and Dallman, 1998). This initial heightened sensitivity may subsequently desensitise after a period of time (Mormède et al., 2007). Failure to observe differences in baseline cortisol may result from increased clearance of glucocorticoid hormones following increased secretion (Friend et al., 1985). Alternatively, this could be a result of the timing of the measurement in relation to the first application of the stressor. If the HPA axis is moving from sensitisation to desensitisation, then at a certain point in time, no difference may be observed between chronically and non-chronically stressed animals (Mormède et al., 2007). The episodic secretion of cortisol and ACTH add further challenge to the evaluation of HPA axis changes following chronic stress (Ladewig and Smidt, 1989).

Attempts were made in the past to link AOB in veal calves with chronic stress. When groups of calves were considered, i.e. for the comparison of different feeding treatments on welfare, no effect of treatment was found on cortisol response to ACTH (Seo et al., 1998b; Veissier et al., 1998; Mattiello et al., 2002). However, one study reported higher baseline plasma cortisol and ACTH in 150-day-old calves fed chopped versus long hay (Seo et al., 1998b). In these studies, treatment effects, i.e. differences in levels or types of solid feed allowances, were compared, and individual differences between calves in terms of their propensity to perform AOB were mostly ignored. As mentioned previously, stereotypies have been suggested as having a ‘calming effect’ on animals that perform them, and as such might actually improve welfare in sub-optimal environments (Mason, 1991a; Mason and Latham, 2004). This is supported by the finding of a decrease in heart rate during the performance of AOB or tongue playing in calves, suggesting a so-called ‘de-arousal’ effect for these behaviours (Seo et al., 1998a; Van Reenen et al., 2001). When individual differences are considered, higher AOB levels were linked to lower baseline cortisol and ACTH, and to lower cortisol response to ACTH and CRH in calves and heifers (Redbo, 1998; Van Reenen et al., 2001), suggesting they play a role in lowering chronic stress.

In conclusion, chronic stress does not seem to consistently cause AOB to develop in all calves, but calves that do develop AOB in sub-optimal environments may cope better with these conditions.

### **Lack of stimulation**

Barren environments, as are frequently used to hold farm and laboratory animals, may be associated with negative affective feelings including frustration (as previously mentioned), boredom, helplessness, weakness or depression (Green and Mellor, 2011). These negative affects are thought to come about through the limitation in the variation and frequency of behavioural expression (Wemelsfelder, 1993). Boredom and depression have recently received more and more attention in the search for valid methods to assess animal welfare (Meagher and Mason, 2012; Harfeld, 2013). Although defining depression in humans is complex, anhedonia and apathy are two potential symptoms of depression that are defined: anhedonia is a “reduced capacity to experience pleasure, typically measured in terms of the decreased consumption of rewards” (Willner et al., 1996; Meagher and Mason, 2012), whilst apathy is a “lack of interest or concern” and “a state of generally reduced motivation or participation in activities” (Meagher and Mason, 2012). In practice, these two negative affects will result in reduced interest in rewards (anhedonia) or in stimuli in general (apathy) (Meagher and Mason, 2012). Boredom is difficult to define but could lead to lethargic inactivity on the one hand, or restlessness and stereotypies on the other hand (Meagher and Mason, 2012) and it may be associated with ‘sensation seeking’, hence an increased interest in stimuli in general (whether rewarding or aversive) (Meagher and Mason, 2012). For example, mink from non-enriched cages show an increased interest in aversive, ambiguous or rewarding stimuli compared to mink from enriched cages (Meagher and Mason, 2012). Similarly, cattle from more barren environments show a higher motivation to interact with a novel object than cattle in an enriched environment (Schulze Westerath et al., 2009).

Ruminants would naturally spend large proportions of their time budget in chewing activities, whether grazing or rumination (Kilgour et al., 2012). This suggests that limited access to roughage might result in much ‘unused time’, which could be experienced as a state of boredom (Wemelsfelder, 1993). Boredom, due to feed restriction, may then be responsible for the development of abnormal behaviours (Wemelsfelder, 1993).

### **Arousal/anticipation and positive feedback**

Arousal may explain the development of certain stereotypies in feed restricted animals (Lawrence et al. 1993). Arousal is described by Lawrence and Terlouw (1993) as “these nonspecific internal effects that modulate the expression of specific motivational states by affecting the general activity of the animals”. If nonspecific stimuli (i.e. not feed related) are able to elicit feed-related stereotypies, this may support the role

of arousal in the development of stereotypies (Lawrence and Terlouw, 1993). It is possible that the short meal time of veal calves actually leads to behavioural arousal, increasing the propensity of calves to engage in active behaviour immediately following meals. AOB do seem to be most common around feeding time (Veissier et al., 1998), which is also the time when animals are most active/aroused (Veissier et al., 1998). Animals fed below their ad libitum intake, such as broiler breeders and sows, display stereotypic behaviour mostly after a meal, pointing to a higher, instead of lower feeding motivation following a meal (Lawrence and Terlouw, 1993). This suggests an initial positive feedback reinforcing feeding motivation (Lawrence et al., 1993). If the meal ends before adequate negative feedback was exerted on the motivation to feed, then the high feed motivation will continue after the meal is ended. This could also be the case in veal calves. Moreover, certain stereotypies are known to develop in response to anticipation of a meal, instead of distress and suffering per se (Mason, 1991b; Lawrence and Terlouw, 1993).

### **Iron deficiency**

Another possible cause for AOB is the existence in veal calves of some sort of nutritional deficiency. The most obvious deficiency in veal calves is iron (Lindt and Blum, 1994). Iron deficiency (or anaemia) in veal calves could come about via a combination of the following pathways: low iron availability in the solid feed and MR, iron depletion due to infection, and/or iron depletion due to rapid growth. These processes have been related to anaemia in humans (Centres for Disease Control (CDC), 1998). Rose veal calves are fed only on solid feed, as they are weaned off MR around 8 weeks of age. Meat colour is related to iron uptake, with a higher uptake leading to darker meat. In (white) veal production, the iron provided in the feeding strategy is kept low because pale-coloured meat is wanted. Therefore, the level of iron in the MR and concentrate is kept low and these animals are not fed roughages high in iron content, such as hay. In rose veal production meat color is less of an issue, and these calves receive roughage sources regardless of iron content. Rose veal calves were found to develop less AOB compared with (white) veal calves (Brscic et al., unpublished data). However, it is unclear whether this difference stems from differences in iron uptake or differences in solid feed provision, and subsequent rumination levels.

## **2.3 Conclusions on stereotypies**

Motivational drive to perform AOB in calves could stem from disturbances in the psychological or physiological state of calves, and in external (environmental) factors. Possible disturbances are most likely related to the limited opportunity to perform

adequate levels of chewing activity (resulting from limited solid feed provision), and include chronic stress, lack of stimulation, arousal and iron deficiency. This thesis aimed to investigate these possible disturbances in more detail.

### 3 Methodological aspects of investigating feed preferences

In order to develop animal-friendly feeding strategies for veal calves, calves' feed preferences were investigated. The most common methods used to assess preferences in animals are choice tests and operant conditioning. Both these methods have advantages and limitations, and these are discussed below in an attempt to find the best method to evaluate feed preferences in calves.

#### 3.1 Choice tests

According to Forbes and Kyriazakis (1995), we can reasonably assume that animals can orient their feeding preferences in an attempt to maximise their comfort. Feeding behaviour is most sensitive to differences in the sensory characteristics of feed when feed types are presented simultaneously in a choice situation (Baumont, 1996). The most straightforward method to assess animal preferences is the so-called choice test, where animals are presented with a choice between two or more resources for a certain period of time, and frequency of visits, or amount of time spent with each available resource is recorded (Hutson, 1984). For feed preferences in ruminants, short choice tests are mainly used with two types of solid feed presented simultaneously and intake being recorded (Morandfehr et al., 1987; Cooper et al., 1995, 1996; Commun et al., 2009; Favreau et al., 2010). Many limitations are commonly associated with this type of study (Kirkden and Pajor, 2006).

First, intake, as a measure of preference, can be rather limiting, especially when feed types that vary in speed of intake rate (e.g. concentrate versus long straw) are compared. Whilst intake may be high for easily and rapidly ingested concentrate, time spent with this feed may be short, when compared to roughages. Second, if test durations are short, in that they last only a few hours or days, preferences recorded are specific to the time of day or age at which they were monitored. In addition, ruminants must learn the long-term post-ingestive consequences of their diet choices (Provenza, 1995), which also requires longer test periods (or adaptation periods). Third, it is common for animals to be tested for their dietary preferences in isolation. When social animals, such as cattle or sheep, are removed from a large group to be

tested in isolation, preferences monitored could be affected by stress resulting from an unfamiliar environment and social isolation. Motivation for food in pigs has been demonstrated to be related to presence of peers (Pedersen et al., 2002). Competition for trough space may result in less-dominant animals making less optimal dietary choices (Forbes and Kyriazakis, 1995), although this may be less relevant in calves. However, it is more realistic to investigate preferences of social animals in a group, as this is closer to a natural context and enables social learning and facilitation (Provenza and Balph, 1987). Fourth, offering a choice between similar feeds may not allow ruminants to avoid toxin accumulation or nutrient imbalances, and may result in an overall depressed intake (Forbes and Kyriazakis, 1995).

Another limitation of choice tests in general is that no cost is imposed on choices, therefore, the strength of the preference is not assessed (Hutson, 1984). Imposing a cost on choices makes such testing more similar to natural settings, where foraging patches may be associated to different search times, travel distances, and may deplete over time (Charnov, 1976). Rats with ad libitum access to feed, for example, will feed frequently and in small quantities, whereas rats who have to work for access to food will reduce meal number and increase meal size (Collier 1980, in Toates, 1986).

### 3.2 Operant conditioning

Derived from microeconomics, behavioural demand function provides a way to estimate the importance of a given behavioural pattern in a given species (Jensen and Pedersen, 2008). Animals are asked to work (price) for access to a resource or to the ability to perform a particular behavioural pattern (reward). If the number of rewards accessed by the animal remains relatively constant with increasing price, because the animal increases its responding at a rate that allows it to maintain a constant reward level (inelastic demand), the reward is considered essential to the animal (e.g. water). If the number of rewards accessed by the animal decreases with increasing price, because the animal does not increase its responding or increases it at a rate that means it gets less rewards at higher prices (elastic demand), the reward is considered to be less important to the animal (Hursh, 1984). When comparing two resources, these can be either substitutes (e.g. two types of water), complements (e.g. salty food and water) or independent (e.g. food and social contact) (Hursh, 1993). Whether the animals can access the rewards outside the operant conditioning (open versus closed economy) might affect the elasticity of the demand. Closed economy appears a better tool in identifying differences in elasticity between different resources, because this makes differences more obvious (Hursh, 1984). “A prerequisite for animal’s motivation to be uniquely expressed in operant responding is that the operant responding

is the only, and sufficiently good strategy to get access to perform the behaviour” (Jensen and Pedersen, 2008).

When animals have only one choice of resource to work for, they work on a single demand, and when animals are given a choice between two or more resources, they work on a double demand (Hursh, 1984, 1993; Jensen and Pedersen, 2008). Single demand involves a number of limitations: 1) animals press the lever because there are no other alternatives provided, even though the reward is of little or no interest, 2) when two resources of different attractiveness are compared on single demand the response of the animal may appear similar for both resources and no preference may be found (Holm and Ladewig, 2007; Holm et al., 2007). Double demand is thus more appropriate when preference for two resources is to be evaluated (Sørensen et al., 2004; Holm et al., 2007). In double demand, the cross point between the response rates for both choices, according to the increasing fixed ratio of one of the choices is considered (as opposed to elasticity of the curve in single demand). Two options are available in double demand operant conditioning: 1) single alternating (lever) procedure and 2) double alternating procedure (Holm et al., 2007). Single alternating procedure refers to a double demand situation where the workload of one lever does not vary across sessions and the workload of the other lever varies (Sørensen et al., 2001, 2004). In a double alternating procedure the workloads on both levers vary in opposite directions (Pedersen et al., 2005; Jensen and Pedersen, 2007). There is a higher risk in the single alternating procedure that animals will show a preference for one lever (regardless of rewards) (Holm et al., 2007). In the double alternating procedure, the two resources to be compared must be substitutes (e.g. two types of water), whereas a single alternating procedure can help show that two resources are not substitutes for each other (Holm et al., 2007).

Taking these limitations into consideration for the design of an experiment using double demand operant conditioning, one needs to address possible methods for statistical analysis of the data. However, a good review of possible methods for cross point analysis of double demand function is currently lacking.

### 3.3 Conclusions on methods to assess feed preferences

Both choice test and operant conditioning methods to assess animal preferences have advantages and limitations. This thesis aimed to address limitations often associated with choice tests to assess feed preferences in calves. Furthermore, this thesis investigated how best to train calves on a double demand operant conditioning paradigm and how best to carry out cross point analysis of double demand functions.

## 4 Developing novel feeding strategies for veal calves

Once AOB and their relationship to welfare are better understood, and adequate methods for assessing feeding preferences in animals are developed, this thesis aimed to provide practical advice regarding the feeding of veal calves to improve animal welfare. Very little is currently known about the relationship between different feeding strategies and veal calf welfare. Previous findings relating to effects of feeding strategies on veal calf behaviour and gastrointestinal health are briefly described below. But first, I will investigate the meaning and validity of behavioural and health measures used in this context in past studies.

### 4.1 Measures of behaviour and health used to assess veal calf welfare

The main behavioural elements or categories considered when assessing welfare in veal calves in the context of feeding include standing, grooming, social contact, sniffing, cross-sucking, and playing (Veissier et al., 1998; Morisse et al., 1999; Mattiello et al., 2002). Standing is a measure of activity often used in welfare studies. However, studies investigating feeding in veal calves have found no connection between standing time and feeding treatments (Morisse et al., 1999; Mattiello et al., 2002). Self-grooming was excessively performed by single-housed calves (Bokkers and Koene, 2001), possibly as self-stimulation in a sub-optimal environment with restricted feeding and social contact opportunities. Grooming was also found to be positively correlated with tongue playing in dairy calves, implying that similar motivations mediate these two behaviours (Seo et al., 1998b). Grooming is especially interesting when investigating ruminal hairball prevalence in veal calves (see below), as excessive grooming was denied as an explanation for high hairball prevalence (Osborne, 1976; Morisse et al., 1999). Social contact (Mattiello et al., 2002), but also sniffing, may both reflect feed searching. Sniffing could be considered a measure of exploratory behaviour, and exploration was linked to welfare in cattle in the past (Schulze Westerath et al., 2009). Cross-sucking is used mostly in studies looking at effects of weaning in dairy calves. This behaviour is thought to mainly occur in calves younger than 8 weeks (Wiepkema, 1987). Moreover, cross-sucking is thought to be linked to the provision of a teat for milk drinking and onset by milk provision (De Passillé and Rushen, 1997) rather than differences in solid feed provision. Finally, play behaviours are used as indicators of good welfare, as they are considered as ‘luxury activities’, which would not be performed, or be performed less, in sub-optimal conditions (Lawrence, 1987).

Main health issues linked to the veal production system concern the respiratory

and gastrointestinal tract (Brscic et al., 2011, 2012). Although it may seem logical that feeding strategies only affect gastrointestinal health, certain studies have also suggested relationships between different feeding strategies and respiratory disorders (Brscic et al., 2012). Main gastrointestinal issues in veal calves include poor rumen development, abomasal damage (i.e. lesions including ulcers, erosions and scars), ruminal hairballs, and plaque (Wiepkema et al., 1987; Breukink et al., 1991; Veissier et al., 1998; Morisse et al., 1999, 2000; Cozzi et al., 2002; Mattiello et al., 2002; Suárez et al., 2007; Brscic et al., 2011). Plaque is defined as “rumen mucosa containing focal or multifocal patches with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris” (Suárez et al., 2007). The digestive tract of young calves acts as a monogastric tract in that the rumen is bypassed (via the effect of the oesophageal groove) by the ingested milk and only the abomasum (or true stomach) is fully functional (Heinrichs, 2005). As calves slowly switch from monogastrics to ruminants, through an increase in solid feed intake, their rumen increases in size, the ruminal papillae grow and darken, and there is an increase in muscularisation of the rumen wall (Heinrichs 2005). In veal calves, however, due to restricted solid feed provision, poor rumen development may be common (Cozzi et al., 2002, 2010; Brscic et al., 2011).

Before it was compulsory to feed solid feed to calves, hairballs were consistently found in the rumen of veal calves (Toofanian, 1976). They were suggested as acting “as a physical replacement for normal roughage” (Osborne, 1976), probably in that they help improve rumen capacity and muscularisation (Harrison et al., 1960). These hairballs likely develop because in the absence of roughage, hairs are not continually cleared out of the rumen, and instead may accumulate in the papillae (Morisse et al., 1999). Plaque formation may limit nutrient uptake in the rumen, and it seems to occur when large amounts of concentrate with little roughage are fed to veal calves (Suárez et al., 2007). Most likely, coarse, abrasive roughage particles are needed to remove the small concentrate particles from between the rumen papillae (Suárez et al., 2007). Abomasal damage is thought to initially occur in veal calves due to the over-stretching of the wall caused by overfilling with MR (due to large, infrequent meals). This stretching results in local ischemia and subsequent lesioning of the wall (Breukink et al., 1991). This damage is generally, but not always (Veissier et al. 1998), exacerbated by solid feed provision (Welchman and De Baust, 1987; Breukink et al., 1991; Mattiello et al., 2002).



## 4.2 Amount, source and particle length of solid feed

Most studies compared the effect of no provision of solid feed to a small amount of solid feed on veal calf welfare (Kooijman et al., 1991; Veissier et al., 1998; Mattiello et al., 2002). Although Prevedello et al. (2010) looked at ‘large amounts’ of solid feed (i.e. 170 kg for the entire fattening period), the absence of a control treatment with less solid feed makes it difficult to truly understand the adequacy of feeding this amount to veal calves. Different amounts of solid feed (0, 10 or 25 kg of concentrate for the entire fattening period) were also investigated by Morisse et al. (1999), who found no effect of amount on behaviour, most likely because concentrate offers little chewing opportunity. Morisse et al. (1999) did, however, find that the larger amount of feed resulted in better rumen development, as seen in increased papilla length. No studies providing a choice of solid feed and MR to calves, aimed to evaluate voluntary intake, could be found.

Different sources of roughage seem to have different effects on both behaviour and gastrointestinal health. Straw, as compared with beet pulp, offers a higher chewing opportunity and subsequently results in lower levels of AOB, most likely because it is a coarser roughage (Mattiello et al., 2002). However, coarser roughages also seem to result in worse abomasal damage (Mattiello et al., 2002). Feeding high levels of concentrate and little roughage results in plaque (Suárez et al., 2007; Prevedello et al., 2010; Brscic et al., 2011), although there is some suggestion that feeding only concentrate at a young age may ultimately minimise the exacerbation of abomasal damage caused by roughage provision later on (Veissier et al., 1998).

No previous research investigating the effect of different particle sizes of roughage on veal calf welfare could be found.

## 5 Summary of thesis objectives

The first and foremost objective of this thesis was to develop novel feeding strategies to improve the welfare of veal calves. To this end, feeding strategies varying in amount, source and particle size of roughage were fed to calves, and the outcome in terms of both behaviour and gastrointestinal health was assessed (Chapters 2 and 3). Following this, calves’ dietary preferences were evaluated in a free choice (Chapter 4) and operant condition set-up (Chapter 6). The latter was done using cross point analysis of double demand functions, as this was thought to be the most adequate method to assess animal preferences for substitutable resources. However, this method first needed an investigation as to the best statistical method to be used (Chapter 5). Following this, the importance and relevance of individual differences in calves

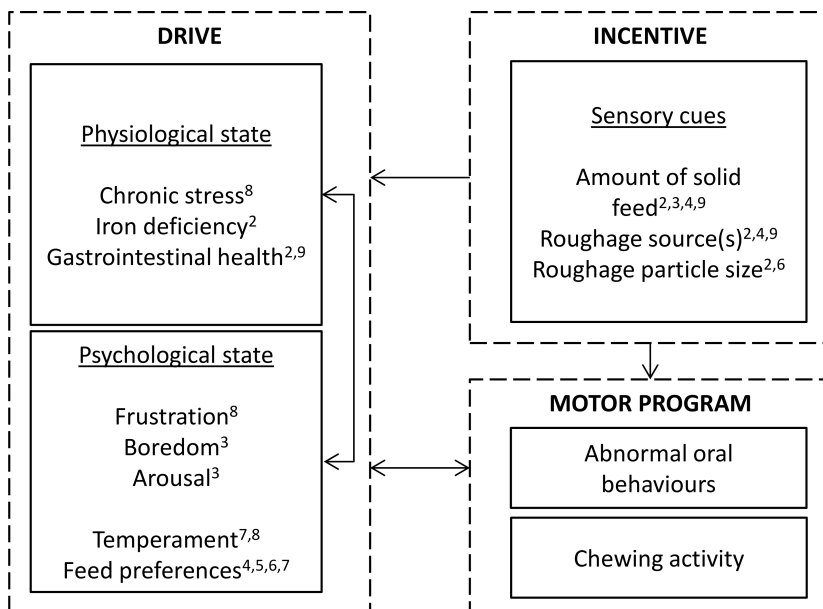


Figure 1.2: Summary of the research objectives of this thesis, presented as a motivational system (compare with Fig.1.1). Superscripts indicate in which chapters of the thesis these aspects are investigated in.

regarding learning of double demand operant tasks (Chapter 7) or tendency to develop abnormal oral behaviours was considered (Chapter 8). All this work was then finally brought together in the final chapter of this thesis, where different feeding strategies, varying in solid feed quantity and composition, were imposed on some calves, whilst others were given the opportunity to choose their own feeding strategy (Chapter 9). These objectives are summarised in Fig.1.2, and presented as a motivation system.

# Chapter 2

## Effects of roughage source, amount and particle size on behaviour and gastrointestinal health of veal calves

Laura Webb, Eddie Bokkers, Leonie Heutinck, Bas Engel, Willem Buist, Bas Rodenburg, Norbert Stockhofe-Zurwieden, Kees van Reenen

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*“If recommendations concerning the welfare of calves are to be based on an appreciation of behaviour in relation to environment, they must in no circumstances be incompatible with good health.” (Webster et al. 1985)*

### Abstract

The EU 1997 Directive, stipulating that veal calves should be fed a minimum of 50 to 250 g of fibrous feed from 8 to 20 wk of age, is vague. A fibrous feed ration maximum of 250 g has been implicated in welfare issues, namely the occurrence of abnormal oral behaviours and poor gastrointestinal health. Past research suggests that this amount is insufficient to prevent the development of abnormal oral behaviours and enabling good rumen development. Different sources and particle sizes of roughage could lead to very different welfare outcomes. In a  $3 \times 2 \times 2$  factorial design, 240 group-housed calves ( $10 \pm 1$  d;  $46.1 \pm 0.1$  kg) were fed different roughage sources (straw, maize silage or maize cob silage; the latter two were dried and provided no extra moisture compared with straw), in two amounts (250 or 500 g dry matter [DM] per day), and two particle sizes (chopped or ground). Roughage was supplemented to milk replacer (MR) from 2 wk after arrival. In addition, 60 calves were fed one of three additional control treatments: MR only ( $n = 20$ ), MR plus an iron supplement ( $n = 20$ ) or MR plus ad libitum hay ( $n = 20$ ). Oral behaviours were recorded using instantaneous scan sampling at 2 min interval for 2 h in three periods per day, at 12 and 22 wk of age. Calves were slaughtered at 24 wk of age and rumen and abomasal health parameters were recorded. Limited provision of straw resulted in comparable behaviour as unlimited provision of hay, with reduced tongue playing and oral manipulation of the environment, as well as increased chewing compared to diets with no roughage supplement. Straw prevented ruminal hairballs, but impaired rumen development and increased abomasal damage. A higher ration of roughage increased chewing (12 wk), decreased oral manipulation of the trough (12 and 22 wk), and the pen (22 wk), and increased rumen weight. However, more roughage led to increased abomasal damage for certain parameters. Longer feed particles had no obvious benefits for behaviour, but decreased hairball prevalence. Overall, unlimited hay had the highest benefit for both behaviour and gastrointestinal health. Adding iron to the milk replacer did not alter behaviour or gastrointestinal health compared to milk replacer without iron supplement. This study demonstrated that different roughage sources, amounts, and particle sizes have different effects on veal calf behaviour and gastrointestinal health, and hence on veal calf welfare.

## 1 Introduction

Surplus dairy calves are generally transported to fattening farms and reared under intensive conditions for the production of veal. In order to produce the pale coloured meat preferred by consumers, veal calves are fed a diet low in iron, which typically translates to low levels of solid feed relative to milk replacer and in particular low levels

of roughage. The European Council 1997 Directive (EU Council, 1997) stipulates that veal calves should be fed a minimum of 50 to 250 g per day of 'fibrous feed' from 8 to 20 wk of age. However, no clarification is made as to which source or particle size of fibres should be fed to veal calves. Moreover, it is unclear whether solid feed amounts stipulated in the EU Directive refer to dry matter or fresh product. Previous research has demonstrated that these amounts are insufficient in preventing the development of abnormal oral behaviours in veal calves (Mattiello et al., 2002; Morisse et al., 1999; Webb et al., 2012). These behaviours are thought to mainly result from a frustrated drive to chew and ruminate on solid feed (Veissier et al., 1998). Abnormal oral behaviours in veal calves include tongue playing and rolling, excessive oral manipulation of trough, bucket and pen structure, sham chewing, and grazing of the coat of other calves (Veissier et al., 1998; Morisse et al., 1999; Webb et al., 2012). Abnormal behaviours are generally considered to be an indication of chronic stress and poor welfare (Broom and Fraser, 2007). Abrasive and coarse feed sources, and longer feed particles may increase chewing and rumination, and consequently reduce abnormal oral behaviours in calves. For example, straw seems more effective than beet pulp in reducing abnormal oral behaviours (Mattiello et al., 2002). Moreover, larger amounts of solid feed, kept constant relative to metabolic weight, were shown to improve chewing and rumination (Webb et al., 2012).

The production of veal, and especially the feeding strategies used, have been implicated in a number of gastrointestinal health problems, e.g. abomasal damage, poor rumen development, and in some cases the development of hairballs in the rumen (Morisse et al., 1999; Brscic et al., 2011). In a cross-sectional European study, Brscic et al. (2011) showed that veal farms that fed more solid feed were associated with a higher prevalence of abomasal lesions. A link between the provision of solid feed and abomasal damage in veal calves was also confirmed experimentally, although milk replacer provision itself can lead to abomasal damage in veal calves (Breukink et al., 1991; Mattiello et al., 2002). It remains unclear whether certain sources of solid feed may be a greater risk for abomasal damage. Straw, grains, straw pellets and maize silage pellets have been associated with abomasal damage (Breukink et al., 1991; Mattiello et al., 2002; Brscic et al., 2011). Rumen development is affected by the fermentation value of the solid feed, with microbial digestion end-products, namely volatile fatty acids (VFA), enabling papillae growth (Flatt et al., 1958). In addition, the physical action of the coarse and abrasive solid feed on the rumen wall, increased rumen capacity and muscularisation (Harrison et al., 1960; Tamate et al., 1962) as well as reduced the incidence of a condition labelled plaque. Plaque involves a layer of particles and debris being stuck to ruminal papillae, which reduces VFA uptake (Haskins et al., 1969; Suárez et al., 2007). Therefore, different sources of solid feed,

differing in fermentation value and physical structure, may have different effects on rumen development. Finally, low levels of solid feed in veal calf diets have been associated with ruminal hairball development, which may impair digestion (Morisse et al., 1999).

This study assessed how different sources, amounts and particle sizes of roughage might affect the behaviour and gastrointestinal health of Holstein-Friesian calves, in order to provide a basis for an animal-friendly feeding strategy. In order to quantify the effects of roughage supplementation on behaviour and health, a control group fed only milk replacer was included. Roughage supplementation usually involves higher iron intake. Therefore, an additional control group was fed milk replacer only, with an iron supplement. This provided a control for potentially confounding effects of iron intake. Finally, a positive control was included in the design to provide a basis for high welfare in the current study. This group of calves was fed hay in unlimited quantities. Hay provides both structure and fermentable fibre and should minimise abnormal oral behaviours and enable optimal rumen development.

## 2 Materials and methods

The study was conducted at the experimental cattle farm of Wageningen University and Research Centre, Lelystad, The Netherlands. All procedures met the terms of the Dutch law for animal experiments, which complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and was approved by the Wageningen University and Research Centre, Lelystad, Committee on Animal Care and Use.

### 2.1 Animals and management

Two batches of 150 Holstein-Friesian bull calves ( $10 \pm 1$  d;  $46.1 \pm 0.1$  kg) were studied in two successive experiments, each lasting for 6 months. Each batch comprised of two groups of 75 calves housed in separate barns. Each group within each batch comprised of one pen per treatment. The calves were housed throughout the experiment in the same 3 m  $\times$  3 m pens with wooden slatted floors (5 calves per pen). During the first 6 wk after arrival, partitions (allowing visual and tactile contact between calves) were placed in each pen separating individual animals in order to minimise cross-sucking and disease transmission. Partitions were removed at 6 wk and calves were group-housed until slaughter at 24 wk. The temperature, ranging from 15 to 25°C, was controlled using mechanical ventilation and heating. Calves were given an antimicrobial treatment when they arrived at the experimental facilities (colistin for 10 d and oxytetracycline for 5 d). Blood samples were taken every 4 wk to monitor

Table 2.1: Average daily gain (ADG, g/d) from 2 to 24 wk of age and haemoglobin levels (mmol/L) at 24 wk of age in veal calves fed different feeding strategies (n = 300).

	ADG		Haemoglobin	
	Mean	SEM <sup>1</sup>	Mean	SEM
Straw	1149 <sup>a</sup>	7.7	4.8 <sup>a</sup>	0.18
Maize silage	1212 <sup>b</sup>		7.1 <sup>c</sup>	
Maize cob silage	1212 <sup>b</sup>		7.1 <sup>c</sup>	
Milk only	1121 <sup>a</sup>	15.4	4.6 <sup>a</sup>	0.09
Milk + iron supplement	1134 <sup>a</sup>		5.9 <sup>b</sup>	
Hay	1260 <sup>c</sup>		5.9 <sup>b</sup>	
Chopped	1183	6.3	5.4	0.07
Ground	1199		5.6	
250 g DM/d	1169 <sup>a</sup>	6.3	5.2 <sup>a</sup>	0.07
500 g DM/d	1213 <sup>b</sup>		5.8 <sup>b</sup>	

<sup>1</sup> SEM = standard error to the mean.

<sup>a-c</sup> Means with different superscripts within a column and between the horizontal lines differ significantly ( $P < 0.05$ ).

haemoglobin levels. Calves were injected with extra iron when required to ensure that average haemoglobin levels were above 4.5 mmol/L when slaughtered at 24 wk of age (Table 2.1). This is the minimum level stated in the EU legislation for veal calves.

All calves were bucket-fed with milk replacer twice a day at 07:00 and 16:00 h following a commercial scheme to produce veal (i.e. starting with 3 L/d, the milk allowance was increased linearly throughout the study to end at 17 L/d). During the first 6 wk calves were fed starter milk replacer and thereafter, fattening milk replacer (Table 2.2). The powder to water ratio was on average 1:8.0 at the beginning and 1:5.8 at the end of the study. The treatments were started from the second week after arrival at the experimental farm. Before that all calves were fed only milk replacer. Roughage was fed in the morning after milk replacer was consumed.

## 2.2 Treatments

The study was a  $3 \times 2 \times 2$  complete factorial design with roughage supplement source: wheat straw (straw) vs. maize silage (**MS**) vs. maize cob silage (**MC**), amount: 250 vs. 500 g DM/d, and particle size: chopped (4 to 5 cm) vs. ground (1 cm) as factors. Composition of roughage used is shown in Table 2.3. Dried MS and MC were used to improve the quality of the grinding process. MS and MC were dried in a dryer at

Table 2.2: Composition<sup>1</sup> of milk replacers used in the study. Starter milk replacer was fed for the first 6 wk, and fattening milk replacer was fed to the calves for the rest of the study. Values are percentages unless otherwise specified.

	Starter	Fattening
<i>Composition</i>		
Skimmed milk powder	51	27
Whey powder	26	21
Delactosed whey powder <sup>2</sup>	-	6
Whey powder concentrate-35 <sup>3</sup>	-	20
Starch	4	4.5
Fat	18	21.5
Vitamins/minerals	1	-
<i>Chemical composition</i>		
Dry matter	96.7	97.0
Fe (mg/kg)	53.3	9.9
Crude protein	22.5	20.5
Crude fiber	< 0.5	< 0.5
Crude ash	7.0	7.3
Fat	17.9	20.7
ME (MJ/kg) <sup>4</sup>	18.8	18.9

<sup>1</sup> Values were provided by feed manufacturer.

<sup>2</sup> Whey powder with lactose removed using crystallization.

<sup>3</sup> Whey powder concentrate with 35% protein, created using infiltration.

<sup>4</sup> ME = metabolisable energy.

350°C for 6 min. Three control treatments were included: milk replacer only (**MR**), milk replacer plus an iron supplement diluted in the milk (**MR+**), and a diet of MR plus ad libitum hay (hay). The factorial design involved a total of 300 animals (240 treated and 60 control) in 15 groups of 4 pens each. The MR+ calves received 58 mg/kg DM Fe in the starter milk, and 116 mg/kg DM Fe in the fattening milk (Table 2.2).

### 2.3 Measurements

Calves were weighed at arrival and before slaughter, and average daily gain (ADG) is shown in Table 2.1. Milk refusals were weighed daily, but were below 0.5% on average. Therefore, provision and intake were considered identical. The chemical composition of the milk was reported by the feed manufacturer (Table 2.2). Roughage refusals



Table 2.3: Composition<sup>1</sup> of roughages supplemented to milk replacer during the study. Roughage treatments started at 2 wk. The values are in g/kg DM unless stated otherwise.

	Straw	Straw-G <sup>2</sup>	MS <sup>3</sup>	MS-G	MC <sup>4</sup>	MC-G	Hay
Dry matter, %	91 ± 0.9	91 ± 0.6	86 ± 1.1	85 ± 1	91 ± 0.4	90 ± 0.4	85 ± 1.1
Fe, mg/kg DM	137 ± 12	188 ± 35	278 ± 20	261 ± 16	318 ± 23	282 ± 27	292 ± 28
Crude protein	41 ± 4	45 ± 4	79 ± 1	78 ± 1	86 ± 1	86 ± 1	146 ± 2
Crude fiber	415 ± 7	395 ± 6	168 ± 3	163 ± 3	100 ± 3	104 ± 3	287 ± 6
Crude ash	106 ± 2	105 ± 3	51 ± 1	50 ± 1	34 ± 1	33 ± 1	93 ± 5
VC-os <sup>5</sup>	44 ± 1	45 ± 1	77 ± 0	77 ± 0	82 ± 0	82 ± 0	65 ± 2
VEVI <sup>6</sup>	348 ± 5	342 ± 3	1021 ± 8	1028 ± 7	1163 ± 8	1156 ± 7	706 ± 29
DVE <sup>7</sup>	4 ± 1	6 ± 2	60 ± 1	59 ± 1	62 ± 1	62 ± 0	71 ± 3
OEB <sup>8</sup>	-31 ± 2	-30 ± 2	-44 ± 0	-44 ± 0	-28 ± 1	-29 ± 1	-4 ± 3
ME, MJ/kg DM <sup>9</sup>	5.53	5.50	11.04	11.10	11.95	12.45	9.68

<sup>1</sup> Mean analysis of six samples taken every 4 wk throughout the experiment.

<sup>2</sup> -G = ground roughage, columns without -G stand for chopped.

<sup>3</sup> MS = maize silage.

<sup>4</sup> MC = maize cob silage.

<sup>5</sup> VC-os = fermentability organic matter.

<sup>6</sup> VEVI = net energy for growing cattle according to Dutch standards (Van Es, 1978).

<sup>7</sup> DVE = true protein digested in the small intestine according to Dutch standards (Tammenga et al., 1994).

<sup>8</sup> OEB = rumen degraded protein balance according to Dutch standards (Tammenga et al., 1994).

<sup>9</sup> ME = metabolisable energy, calculated from averages.

Table 2.4: Ethogram of oral behaviours, based on De Wilt (1985).

Behaviour	Description
Chewing and rumination	Repetitive movements of upper and lower jaw moved in a regular fashion in the lateral plane, with or without substrate in mouth.
Tongue play	Tongue playing/rolling. Repeatedly turning, rolling and unrolling tongue extended outside or inside of mouth.
Oral manipulation of trough	Licking, nibbling or sucking bucket or trough.
Oral manipulation of pen	Licking, nibbling or sucking partitions, wall or floor of pen.
Oral manipulation of pen mate	Licking, nibbling or sucking the coat or part of another calf in the pen.
Groom	Tongue is extended and shifted across own body repeatedly.

were collected daily but only weighed weekly. Roughage intake was calculated per pen per week. Roughage samples were collected on a weekly basis. The samples were pooled and analysed by a private laboratory every 4 wk (Table 2.3).

### Behavioural observations

Calf behaviours (Table 2.4) were recorded by four observers. Direct observations were done across 1 wk at 12 and 22 wk of study. The observer sat on a high chair and observed two pens simultaneously. Observations were done using instantaneous scan sampling at a 2 min interval for 2 h: “a whole group of subjects is rapidly scanned [...] at regular intervals and the behaviour of each individual at that instant is recorded” (Martin and Bateson, 1993). Observations were carried out during three periods: 06:30 to 08:30 h, 11:00 to 13:00 h, and 15:30 to 17:30 h.

### Gastrointestinal health measurements

Calves were slaughtered at 24 wk in an experimental slaughterhouse 3 km away from the experimental cattle farm. The rumens and abomasa were collected, rumen fluid was extracted and the rumens and abomasa were rinsed under water for inspection by a veterinarian pathologist, and weighed. The presence of ruminal hairballs and abomasal lesions in the pyloric area was recorded. Lesions were classified as ulcers, erosions, or scars. Ulcers were characterised by focal loss or necrosis of the epithelial layer down to the submucosal or muscular layer of the stomach wall. Erosions were

characterised as inflammations with partial superficial or profound loss of epithelium without clear disruption of the epithelial layer. Scars were characterised as focal, longitudinal or round fibrous contractions of the mucosa. The number of hairballs and abomasal lesions was recorded. For hairballs, the diameter of the biggest hairball was measured, and for lesions, the size was calculated as horizontal diameter  $\times$  vertical diameter. When both diameters of a given lesion were less than 0.5 cm, an arbitrary size of 0.1 cm<sup>2</sup> was allocated to the lesion. Finally, rumen fluid pH was recorded.

## 2.4 Statistical analysis

Roughage intake up to and including week 12, as well as week 12 alone, was analysed, using a linear model of variance (ANOVA) with fixed factors source, amount and particle size, as well as two-way interactions between them. Post-hoc comparisons were carried out using Fisher's LSD. All other data were analysed in two steps, involving separate analyses 1) Effects of the three treatments of the factorial design, i.e. source, amount and particle size, were analysed; 2) Levels of the factor source were compared to the three added controls. The first step does not involve the three added controls. The second does involve the three added controls and depends upon the results of the first step as explained below. All statistical analyses were run in GenStat (VSN-International, 2012).

### Factorial Design

The set-up was a  $3 \times 2 \times 2$  factorial design with source, amount and particle size of roughage as the factors. Behavioural and gastrointestinal health data were first analysed for effects of these factors. Behavioural observations were expressed as proportions of total scans, per age (wk 12 and 22), and averaged per pen. Data for the presence of abomasal lesions and ruminal hairballs were expressed as binary data. Data for number of lesions or hairballs were expressed as counts. Data related to size of lesions were treated as continuous data. Proportions were analysed at pen level with a logistic regression model, an instance of a generalised linear model (**GLM**) comprising an additional multiplicative (over) dispersion parameter in the binomial variance function. Binary and count data were analysed at animal level with a generalised linear mixed model (**GLMM**), comprising random pen effects. For the binary data, a Bernoulli distribution and logit link function were specified. For the count data, a Poisson distribution and logarithmic link function were specified. In addition, for count data, the model included a multiplicative (over) dispersion parameter in the Poisson variance function. Continuous data were analysed with a linear mixed model (**LMM**), comprising random pen effects. All models included fixed (i.e. sys-

tematic non-random) main effects for batch and main effects and interactions for the experimental factors source, amount and particle size. Parameters were estimated by maximum quasi-likelihood for GLM (McCullagh and Nelder, 1989), penalised quasi-likelihood for GLMM (Breslow and Clayton, 1993), and restricted maximum likelihood (**REML**) for LMM (McCulloch, 2006). For observations with a high frequency of zeros, to the extent that the average value for an entire treatment group was zero, and when the algorithm that was used failed to converge, non-parametric significance tests were used to compare treatments. Thus, prevalence of abomasal scars and ruminal hairballs were analysed with Fisher's exact test. Number and size of scars as well as hairball number and maximum hairball diameter were analysed using the Mann-Whitney U test (Wilcoxon's two-sample test).

### Pairwise Comparisons with Controls

Appropriate tables of means were identified for further analysis through pairwise comparisons with Fisher's LSD method. For instance, when no significant ( $P > 0.05$ ) interactions involving factor source were found in the first step, in the second step pairwise comparisons were performed between the means of the added controls and the three means for the levels of factor source. However, if an interaction between source and amount was found, means for the controls were compared with the means for the combinations of source and amount. The basic model used for pairwise comparisons comprised a single factor at 15 levels: the three added controls plus the 12 combination of factors source, amount and particle size from the  $3 \times 2 \times 2$  factorial design.

## 3 Results

### 3.1 Roughage intake

Average intakes of straw, MS and MC are shown in Fig.2.1. There was an interaction between source and amount for roughage intake up to and including study wk 12 ( $P = 0.006$ ). Calves fed 250 g DM/d straw had lower roughage intakes compared to calves fed 250 g DM/d MS ( $P = 0.005$ ) or MC ( $P = 0.029$ ). Calves fed either 500 g DM/d straw or MC per day had lower roughage intakes compared to those fed 500 g DM/d MS ( $P < 0.001$ ). Roughage intake during the first 12 wk of study was similar for calves fed 250 or 500 g DM/d straw or MC ( $P > 0.1$ ), but was higher in calves fed 500 g DM/d MS ( $P < 0.001$ ). However, during wk 12, calves fed straw, MS or MC consumed more in the 500 g DM/d treatment compared with the 250 g DM/d treatment ( $P < 0.001$ ). Therefore, treatments 250 and 500 g DM/d were considered

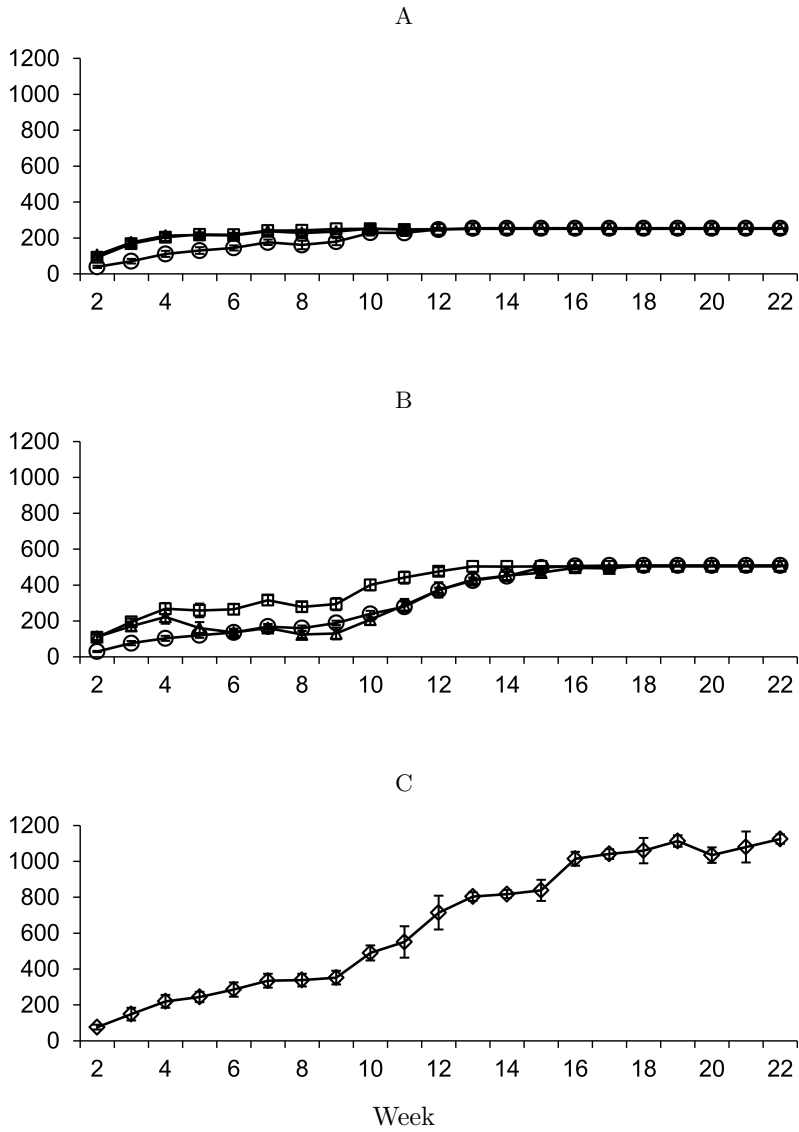


Figure 2.1: Mean ( $\pm$  SEM) intake (g DM/d) of straw (circles), maize silage (squares) and maize cob silage (triangles), or hay (diamonds) across wk, in calves fed 250 (A) or 500 (B) g DM/d, or hay ad libitum (C). Roughage provision started at 2 wk. Milk replacer was switched from starter to fattening after the initial 6 wk.

different for behavioural measurements carried out at 12 wk. Calves fed ad libitum hay reached 250 g DM/d after 5 wk and 500 g DM/d after 10 wk (Fig.2.1). At 22 wk, these calves consumed 1125 g DM /d on average.

## 3.2 Behavioural observations

Results of pairwise comparisons between the three levels of the factor source (i.e. straw, MS and MC) and the three control treatments (i.e. MR, MR+ and hay) are shown in Fig.2.2 (12 wk) and Fig.2.3 (22 wk).

### 12 weeks

An interaction was found between roughage source and amount ( $P = 0.008$ ) for chewing and rumination. Within the MS and MC treatments, calves fed 500 g DM/d chewed more than calves fed 250 g DM/d ( $P < 0.001$  and  $P = 0.001$  respectively), whereas chewing was similar with the two amounts of straw ( $P = 0.181$ ). Moreover, regardless of the amount, calves fed straw chewed more than calves fed MS or MC and calves fed MS chewed more than calves fed MC ( $P < 0.05$ ). Particle size tended to affect chewing and rumination ( $P = 0.088$ ). Calves fed chopped roughage ( $10.1 \pm 0.76$  % of total scans) tended to chew more than calves fed ground roughage ( $8.5 \pm 0.68$  % of total scans). An effect of source of roughage was found on tongue playing ( $P = 0.004$ ). Calves fed straw tongue played less than calves fed MS or MC. Both source ( $P = 0.018$ ) and amount ( $P = 0.009$ ) of roughage affected oral manipulation of the trough. Calves fed straw orally manipulated the trough less than calves fed MC. Calves fed 250 g DM/d roughage ( $11.1 \pm 0.7$  % of total scans) orally manipulated the trough more often than calves fed 500 g DM/d ( $8.2 \pm 0.6$  % of total scans). Neither source, amount nor particle size ( $P > 0.1$ ) of roughage affected oral manipulation of the pen. Similarly, no effect of source, amount or particle size ( $P > 0.1$ ) was found on oral manipulation of pen mates. No effect of source or particle size ( $P > 0.1$ ) was found on grooming. There was, however, a tendency for calves fed 250 g DM/d ( $3.9 \pm 0.22$  % of total scans) to groom less compared with calves fed 500 g DM/d ( $4.7 \pm 0.24$  % of total scans) ( $P = 0.083$ ).

### 22 weeks

At 22 wk, the source of roughage affected chewing and rumination ( $P < 0.001$ ) as well as tongue playing ( $P = 0.036$ ). Calves fed straw chewed more than those fed MS or MC and tongue played less than calves fed MS. An effect of amount of roughage was found on oral manipulation of the trough ( $P = 0.036$ ). Calves fed 250 g DM/d

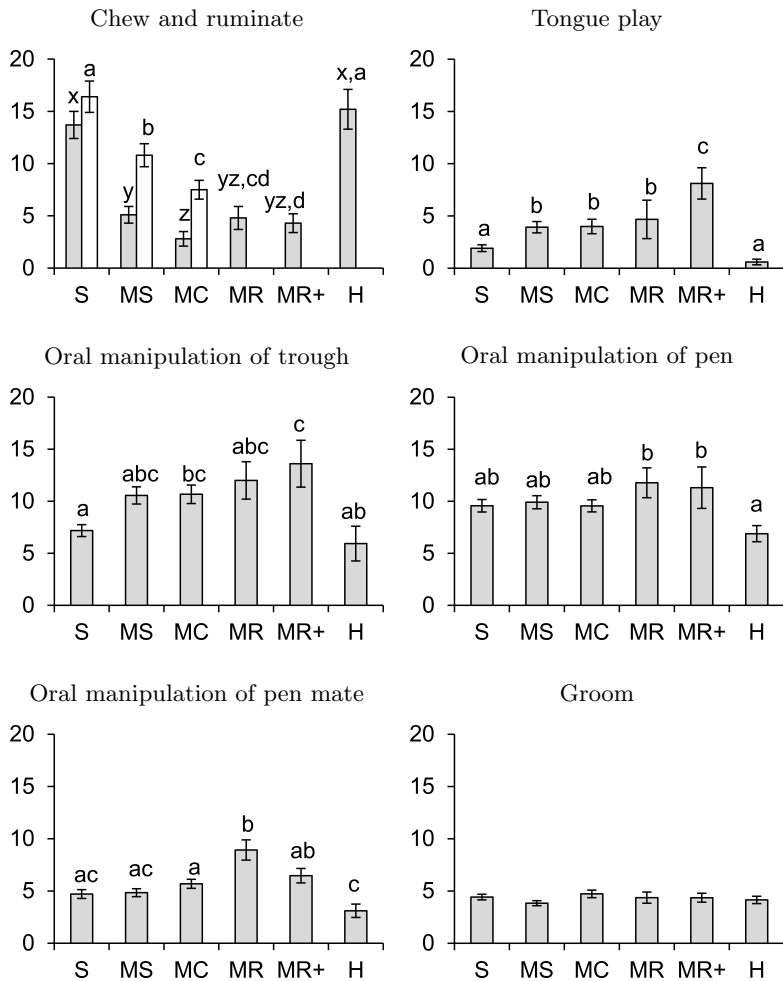


Figure 2.2: Mean ( $\pm$  SEM) % total scans for behaviours recorded at 12 wk in calves fed straw (S), maize silage (MS), maize cob silage (MC), milk replacer only (MR), milk replacer plus iron supplement (MR+), or hay ad libitum (H) ( $n = 300$ ). Bars with different superscript differ ( $P < 0.05$ ). For chewing, there was an interaction between source and amount for straw, MS and MC. Differences between levels of the factor source and controls are therefore divided between 250 (gray, superscripts x-z) and 500 g DM/d (white, superscripts a-d) for straw, MS and MC. For MR, MR+ and hay there was no effect of amount so one gray bar is shown.

( $13.4 \pm 0.8$  % of total scans) orally manipulated the trough more often than calves fed 500 g DM ( $10.8 \pm 0.6$  % of total scans). Calves fed 250 g DM/d ( $8.2 \pm 0.4$  % of total scans) tended ( $P = 0.053$ ) to orally manipulate the pen more than calves fed 500 g DM/d ( $6.9 \pm 0.4$  % of total scans). The source of roughage was found to affect oral manipulation of pen mates ( $P = 0.011$ ), with calves fed straw or MS orally manipulating pen mates less than calves fed MC. Finally, the source, amount and particle size ( $P > 0.1$ ) of roughage had no effect on grooming.

### 3.3 Gastrointestinal health

Results of pairwise comparisons carried out between the three levels of the factor source (straw, MS and MC) and the three control treatments (MR, MR+, and hay) on gastrointestinal health measurements are described in Table 2.5.

#### Abomasum

The source of roughage affected the weight of the abomasum ( $P = 0.001$ ), which was lighter in calves fed straw than in those fed MS or MC. None of the factors had an effect on ulcer or scar prevalence ( $P > 0.1$ ). The source of roughage tended to have an effect on the prevalence of erosions ( $P = 0.057$ ), with the straw treatment resulting in a higher erosion prevalence than MS or MC treatments. An interaction between amount and source of roughage on the number of abomasal ulcers was found ( $P = 0.005$ ). Calves fed 250 g DM/d MS had less ulcers than calves fed straw ( $P = 0.001$ ) or MC ( $P = 0.044$ ). Moreover, calves fed MS had more ulcers when fed 500 instead of 250 g DM/d of roughage ( $P = 0.009$ ). An interaction between source and amount of roughage was found on the number of erosions ( $P = 0.011$ ). With treatments including 250 g DM/d, calves fed MC had less erosions than calves fed straw ( $P = 0.004$ ) or MS ( $P = 0.028$ ) (Table 2.5). With treatments including 500 g DM/d calves fed MS had less erosions than calves fed straw ( $P = 0.007$ ) or MC ( $P = 0.042$ ) (Table 2.5). Finally, calves fed a smaller amount of MC had less erosions ( $P = 0.012$ ) (Table 2.5). No effect of source, amount or particle size was found on the number of scars ( $P > 0.1$ ). The amount of roughage affected ulcer size ( $P = 0.015$ ), which was smaller in calves fed 250 g DM/d ( $1.3 \pm 0.2$  cm<sup>2</sup>) than in calves fed 500 g DM/d ( $2.0 \pm 0.2$  cm<sup>2</sup>). Moreover, roughage source affected erosion ( $P = 0.001$ ) and scar size ( $P = 0.030$ ). Calves fed straw had larger erosions than calves fed MS or MC, and larger scars than calves fed MC.



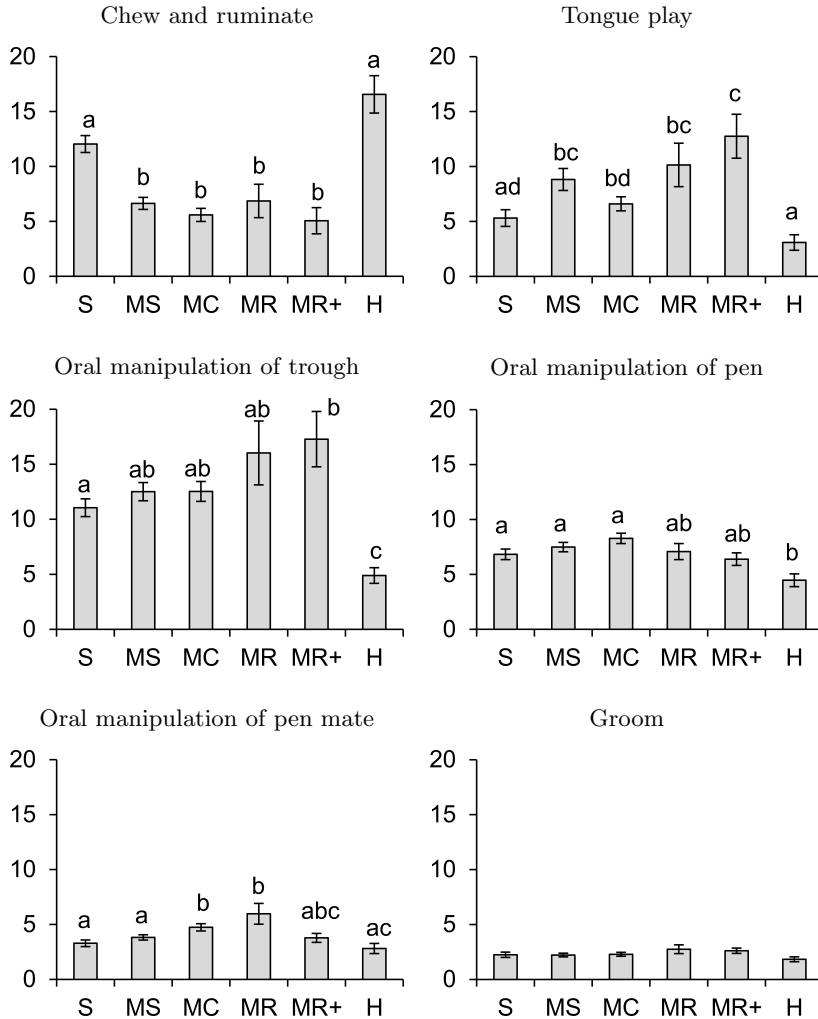


Figure 2.3: Mean ( $\pm$  SEM) % total scans for behaviours recorded at 22 wk in calves fed straw (S), maize silage (MS), maize cob silage (MC), milk replacer only (MR), milk replacer plus iron supplement (MR+), or hay ad libitum (H) ( $n = 300$ ). Bars with different superscript differ ( $P < 0.05$ ).

Table 2.5: Results of pair wise comparisons between the levels of the factor source (straw, MS and MC) and the control treatments (MR, MR+ and hay) on gastrointestinal health in 300 veal calves (24 wk of age).

Parameter	Treatment <sup>1</sup>					
	straw	MS	MC	MR	MR+	Hay
<i>Abomasum</i>						
Weight (g)	847 ± 17 <sup>a</sup>	900 ± 12 <sup>b</sup>	934 ± 12 <sup>b</sup>	835 ± 21 <sup>ab</sup>	824 ± 16 <sup>ab</sup>	931 ± 23 <sup>b</sup>
Ulcer prevalence (%)	73 <sup>a</sup>	65 <sup>a</sup>	78 <sup>a</sup>	25 <sup>b</sup>	35 <sup>b</sup>	40 <sup>b</sup>
Erosion prevalence (%)	39 <sup>a</sup>	25 <sup>b</sup>	20 <sup>b</sup>	15 <sup>b</sup>	30 <sup>ab</sup>	30 <sup>ab</sup>
Scar prevalence (%)	23 <sup>a</sup>	18 <sup>ab</sup>	16 <sup>ab</sup>	0 <sup>b</sup>	20 <sup>ab</sup>	30 <sup>a</sup>
Ulcer number <sup>2</sup>						
250g	3.5 ± 0.5 <sup>a</sup>	1.1 ± 0.3 <sup>b</sup>	2.2 ± 0.4 <sup>ac</sup>	0.9 ± 0.5 <sup>bc</sup>	0.7 ± 0.4 <sup>b</sup>	1.7 ± 0.6 <sup>bc</sup>
500g	2.2 ± 0.4 <sup>ab</sup>	2.7 ± 0.5 <sup>a</sup>	2.8 ± 0.5 <sup>a</sup>	0.9 ± 0.5 <sup>bc</sup>	0.7 ± 0.4 <sup>c</sup>	1.7 ± 0.6 <sup>ac</sup>
Erosion number <sup>2</sup>						
250g	1.7 ± 0.4 <sup>a</sup>	1.1 ± 0.3 <sup>ac</sup>	0.2 ± 0.1 <sup>b</sup>	0.3 ± 0.2 <sup>bc</sup>	0.8 ± 0.4 <sup>ab</sup>	0.4 ± 0.1 <sup>bc</sup>
500g	1.9 ± 0.4 <sup>a</sup>	0.4 ± 0.2 <sup>b</sup>	1.3 ± 0.4 <sup>ac</sup>	0.3 ± 0.2 <sup>bc</sup>	0.8 ± 0.4 <sup>ab</sup>	0.4 ± 0.1 <sup>bc</sup>
Scar number	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.1 ± 0.1	0.5 ± 0.2
Ulcer size (cm <sup>2</sup> )	1.6 ± 0.2	1.5 ± 0.2	1.9 ± 0.3	0.3 ± 0.2	1.3 ± 0.6	1.3 ± 0.6
Erosion size (cm <sup>2</sup> )	0.7 ± 0.2 <sup>a</sup>	0.4 ± 0.1 <sup>b</sup>	0.2 ± 0.1 <sup>b</sup>	0.2 ± 0.2 <sup>b</sup>	0.2 ± 0.1 <sup>b</sup>	0.5 ± 0.3 <sup>ab</sup>
Scars size (cm <sup>2</sup> )	0.6 ± 0.3 <sup>a</sup>	0.2 ± 0.1 <sup>ac</sup>	0.1 ± 0.0 <sup>bc</sup>	0.0 ± 0.0 <sup>b</sup>	0.1 ± 0.1 <sup>a,b,c</sup>	0.3 ± 0.2 <sup>a</sup>
<i>Rumen</i>						
Weight (g)	1825 ± 35 <sup>a</sup>	2038 ± 47 <sup>b</sup>	2094 ± 45 <sup>b</sup>	1454 ± 43 <sup>c</sup>	1481 ± 26 <sup>c</sup>	2454 ± 76 <sup>d</sup>
pH	6.7 ± 0.1 <sup>ab</sup>	6.6 ± 0.1 <sup>a</sup>	6.8 ± 0.0 <sup>ab</sup>	6.8 ± 0.1 <sup>a,b</sup>	7.0 ± 0.1 <sup>b</sup>	6.5 ± 0.1 <sup>a</sup>
Hairball prevalence (%)	0 <sup>a</sup>	14 <sup>b</sup>	30 <sup>c</sup>	85 <sup>d</sup>	85 <sup>d</sup>	0 <sup>a</sup>
Hairball number	0 ± 0 <sup>a</sup>	0 ± 0 <sup>ab</sup>	0.5 ± 0.2 <sup>b</sup>	6.9 ± 1.7 <sup>c</sup>	6.2 ± 1.4 <sup>c</sup>	0 ± 0 <sup>ab</sup>
Max. hairball diameter (cm)	0 ± 0 <sup>a</sup>	0.2 ± 0.2 <sup>ab</sup>	0.6 ± 0.2 <sup>b</sup>	6.3 ± 1.2 <sup>c</sup>	8.2 ± 1.0 <sup>c</sup>	0 ± 0 <sup>ab</sup>

<sup>1</sup> MS = maize silage; MC = maize cob silage; MR = milk replacer only; MR+ = milk replacer with an iron supplement; Hay = ad libitum hay.

<sup>2</sup> Interaction found between source and amount ( $P < 0.05$ ).

<sup>a-d</sup> Means or percentages within a row with a different superscript differ ( $P < 0.05$ ).

## Rumen

Both source ( $P < 0.001$ ) and amount ( $P < 0.001$ ) affected rumen weight. Calves fed straw had lighter rumens compared to calves fed MS or MC. Calves fed 500 g DM/d ( $2,120.0 \pm 34.6$  g) had heavier rumens than calves fed 250 g DM/d roughage ( $1,852.0 \pm 33.5$  g). None of the factors had an effect on ruminal pH ( $P > 0.1$ ). The prevalence of hairballs was affected by both source ( $P < 0.001$ ) and particle size ( $P = 0.011$ ). Fewer calves fed straw had hairballs compared to those fed MS and MC, and fewer calves fed MS had hairballs compared to MC. Calves fed chopped roughage were less likely to have hairballs in their rumen (9% calves) compared to calves fed ground roughage (20% calves) ( $P = 0.008$ ). The source of roughage affected hairball number ( $P = 0.018$ ), with calves fed straw having fewer hairballs than calves fed MC.

## 4 Discussion

This study to some extent confirmed the previously described conflict between behaviour and abomasal health when feeding roughage to veal calves (Mattiello et al., 2002). Roughage sources and amounts that improved behaviour, e.g. increased chewing and rumination and decreased abnormal oral behaviours, exacerbated abomasal damage. The present results indicate that straw provision, probably due to its coarser nature, was more efficient in increasing chewing and rumination in the calves than MS and MC, and subsequently better at reducing tongue playing, consistent with previous findings (Mattiello et al., 2002). Moreover, increasing roughage ration from 250 to 500 g DM/d also increased chewing and rumination and decreased abnormal oral behaviours. Roughage provision in itself, and larger amounts of roughage were associated to worse abomasal damage in this study, consistent with previous research (Breukink et al., 1991; Mattiello et al., 2002). Calves fed only milk replacer also showed some level of abomasal damage, consistent with previous research (Breukink et al., 1991; Mattiello et al., 2002). The proposed explanation for this damage is overloading of the abomasum, due to large quantities of milk fed to calves in few meals, causes stretching of the wall and local ischemia (Breukink et al., 1991). The provision of straw, in particular, led to increased erosion prevalence, number, and size compared to most other treatments. Exacerbation of ulcers, however, was associated with all roughage supplements. This difference in factors affecting erosions and ulcers may suggest that they have separate aetiologies. This was previously suggested due to a difference in numbers and distribution of erosions and ulcers in veal calf abomasa (Wiepkema et al., 1987). The present study suggests that straw and hay lead to increased scar prevalence, number and size, compared to MR. Wiepkema et al. (1987)

suggested that scars were healed ulcers based on distribution and numbers. Proposed factors involved in the aetiologies of abomasal ulceration in beef and veal calves include the abrasive action of coarse feed stuffs and hair or hairballs in the abomasum (Tulleners and Hamilton, 1980; Katchuik, 1992; Cozzi et al., 2002; Mattiello et al., 2002), as well as the transitional phase between preruminant and ruminant digestion, hence forestomach development (Jelinski et al., 1996). Roughage that could be characterised as coarser, such as straw, did seem to lead to worse abomasal damage, at least for parameters relating to erosions, and this would somewhat be consistent with the “abrasion theory” (Jelinski et al., 1996). If the abrasive nature of roughage was one of the main factors leading to abomasal damage, then one would expect particle size to play a role, which was not seen in the present study. Straw provision also led to the smallest rumen weight. Therefore, poor rumen development could also be a factor relating to abomasal damage in veal calves (Berends et al., 2012b). This is consistent with ad libitum hay ration resulting in lowest damage for ulcer parameters, because hay provision resulted in the heaviest rumens. Rumen development could protect to some extent against exacerbation of abomasal damage by minimising entry of under-digested coarse feed particles into an already sensitised abomasum. If this were the case, greater amounts of roughage leading to heavier rumens should have been associated to reduced abomasal damage. In the present study, the opposite was found: ulcers were larger when more roughage was provided (500 compared to 250 g DM/d), a greater number of ulcers was present in calves fed more MS, and a greater number of erosions was present in calves fed more MC. These findings are consistent with Brscic et al. (2011). We, therefore, suggest that abomasal lesions, whether ulcers or erosions, may come about from a combination of factors including: 1) overloading of the abomasum resulting in local ischemia and subsequent lesions, 2) exacerbation of existing damage due to the passage of under-digested feed particles from a poorly developed rumen to a sensitised abomasum, and 3) exacerbation of existing damage with coarse feed stuffs due to their greater abrasive quality. The exact mechanisms involved in the aetiologies of erosions and ulcers in veal calves, whether similar or not, should be investigated further in the future.

Particle size of roughage in the present study only had an effect on hairball prevalence, with chopped roughage resulting in a lower prevalence compared with ground roughage. Morisse et al. (1999) suggested that ruminal hairballs in calves were minimised by continuous removal of ingested hair as a result of good rumen motility. Larger particles were previously found to have a greater abrasive value than smaller particles of feed (Greenwood et al., 1997). In addition, larger particles were found to result in higher ruminal fluid pH, light gray epithelial layers, smaller papillae, a larger reticulorumen, and a smaller and lighter omasum, but no effect of particle size was

found on rumen or abomasum weight (Greenwood et al., 1997; Beharka et al., 1998), the latter being consistent with the present findings. Moreover, although rumen papillae grow in response to microbial fermentation end-products, mainly butyrate and propionate, rumen capacity and muscularisation can be significantly increased by inert materials through physical stimulation alone (Harrison et al., 1960; Tamate et al., 1962). Larger particles, also because of a higher abrasive value, may additionally lead to the physical removal of particles wedged between papillae, including feed particles, dead epithelial cells and ingested hair (Greenwood et al., 1997). Therefore, by increasing rumen muscularisation and physical removal of particles, larger feed particles would reduce the incidence of hairballs significantly, which is what the present findings point towards, and which is consistent with the hypothesis of Morisse et al. (1999). The different roughage sources also had a marked effect on hairball development, with straw and hay provision resulting in the absence of ruminal hairballs. These two types of roughage most likely had a higher abrasive value compared to MS and MC.

A larger particle size was expected to increase rumination (Heinrichs, 2005), and this was found in the present study. In contrast with expectation based on previous research, the current study did not show that larger amounts of straw increased chewing and rumination levels (Mattiello et al., 2002). Increased levels of chewing and rumination in response to greater amounts were, however, found in calves fed MS or MC. The provision of MS or MC resulted in similar behaviour and gastrointestinal health, but for a few exceptions. At 12 wk, MS provision was associated with higher chewing and rumination levels compared to MC, which could suggest that MS better met the chewing and rumination needs of calves. However, calves fed MC had a lower intake at the beginning of the study, which would explain why chewing was lower, and why chewing differences disappeared at 22 wk when intake was similar. MC also resulted in higher oral manipulation of pen mates at 22 wk. Oral manipulation of pen mates has been suggested to represent redirected feed searching (Mattiello et al., 2002), which might indicate that calves fed MC in the current study were more frustrated from limited feed ration than calves fed MS, at 22 wk. Chewing tended to be lower in the MC-fed calves at 22 wk. Straw and hay treatments also resulted in reduced oral manipulation of pen mates, hence lower redirected feed searching. Both MS and MC resulted in heavier abomasum and rumen weights than those of straw-fed calves. Since the rumen wall was probably physically stimulated more when a more abrasive roughage was provided (Greenwood et al., 1997), it is believed that straw was more likely to lead to a higher muscularisation than MS or MC. MS and MC might have led to a higher volatile fatty acid production because of a lower crude fiber and higher crude protein content compared to straw, and would have most likely

led to greater papillae growth. Furthermore, differences in rumen weight may reflect the lower straw intake at the beginning of the study compared to MS and MC.

Not many differences were observed between calves fed MR or MR+, for both behaviour and gastrointestinal health parameters. Calves fed MR+ showed higher levels of tongue playing at 12 wk compared to calves fed MR. Iron deficiency anaemia in rats and humans can result in lower activity levels (Lozoff, 1987; Felt and Lozoff, 1996), which might explain the increase in tongue playing in calves fed MR+, compared to calves fed MR. However, it does not explain why both liquid-fed treatments did not differ on any other behavioural measurement.

Calves fed unlimited hay, reached an intake of over 4.5 times the EU minimum requirement for fibrous feed, i.e. 1,125 g DM/d, on top of milk replacer. Hay contains relatively high levels of iron and is, therefore, incompatible with the production of (white) veal. Unlimited supply of hay, however, resulted in most chewing and rumination, the least tongue playing and the least oral manipulation of the environment, including pen structure, trough, and pen mates. It also resulted in the heaviest rumens, the absence of ruminal hairballs, and abomasal lesion prevalence, lesion number and lesion size comparable to that of milk-fed calves (except for scars). Calves were, thus, able to select an amount of hay that resulted in improvements in behaviour and gastrointestinal health, when compared to all other treatments. The amount of hay that ad libitum fed calves chose in this study may give an indication of how much roughage veal calves should actually be fed in practice, when welfare is to be maximised, although different sources of roughage, or a combination of different roughages might lead to differences in voluntary intake. It may be useful to give calves free choice of a variety of roughages, and potentially concentrate, in order to evaluate what, and how much, solid feed calves would choose to consume on top of milk replacer.

## 5 Conclusions

The present study suggests that the EU 1997 Directive on veal calf minimum solid feed requirements need further specification as to source(s) and particle size of roughage to be fed, and an increase in the minimum amount stated, if the welfare of veal calves is to be improved. Different sources of roughage and even different roughage particle sizes, affected the welfare of veal calves, in terms of both behaviour and gastrointestinal health, differently. In particular, straw improved behaviour and reduced ruminal hairball prevalence, but increased abomasal damage. More roughage, i.e. 500 g instead of 250 g DM/d, increased chewing and rumination and reduced abnormal oral behaviours. Although this study did not investigate feeding a combination of different

roughage sources, none of the single sources used in the present study improved both behaviour and gastrointestinal health. A combination of different roughage sources may be necessary to improve both behaviour and health, including those that facilitate good rumen development (e.g. maize silage), encourage chewing and rumination, and effectively eliminate ingested hair (e.g. straw).

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# Chapter 3

## Behaviour and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth

Laura Webb, Eddie Bokkers, Bas Engel, Walter Gerrits, Harma Berends, Kees van Reenen

Webb et al. 2012, Applied Animal Behaviour Science, 136, 108-116

*“The indicators of poor welfare are of two general types. The one demonstrating that an individual has failed to cope with an environment, the other indicating the effort involved and the extent of an individual’s attempts at coping.” (Broom, 1986)*

### Abstract

Veal calves in Europe are typically fed large quantities of milk replacer and small amounts of solid feed, a diet known to lead to the development of abnormal oral behaviours in these animals. These abnormal oral behaviours are thought to be an indication of frustration, chronic stress, and hence poor welfare. The present study investigated how different feeding strategies, differing in solid feed and milk replacer provision, affected the behaviour and welfare of veal calves across time.

Four treatment groups (A, B, C, D) comprising of 12 Holstein-Friesian bull calves each ( $7.6 \pm 0.1$  wk old and  $54.7 \pm 0.3$  kg at arrival), penned in groups of three, were fed one of four amounts of a solid feed mixture, i.e. 50% concentrate, 25% fresh maize silage, and 25% wheat straw (on dry matter (DM) basis): A = 0, B = 9, C = 18, and D = 27 g DM/kg<sup>0.75</sup>/d. Provision of milk replacer was adjusted to achieve similar average daily gain across treatments. Behaviour was recorded around feeding (10 min continuous focal observations of individual calves) and throughout the day (7 sessions of 30 min scan sampling at 5 min interval every 2 h from 06:30 h) every week for 4 months. In an attempt to find an easy practical method to measure behavioural response to feeding strategy, two 3-min behavioural tests were carried out: 1) in months 1 and 3, calves were presented with a ball and latency to make oral contact with it was recorded; 2) in month 1, calves were presented with an overall and time spent orally manipulating (i.e. chewing and licking) it was recorded using scan sampling every 10 s.

Calves in treatment D displayed less abnormal oral behaviours around feeding, less tongue playing throughout the day, and more chewing in the first two months, compared to treatment A. Treatment B only led to lower tongue playing levels compared to A and treatment C had no benefit in terms of reducing abnormal oral behaviours. Although a solid feed dose response was expected on the display of abnormal behaviours in veal calves, treatment C did not fit within this expectation.

These findings point to a more complex relationship between solid feed and abnormal oral behaviour frequency in veal calves. The two behavioural tests distinguished the different treatments as expected, and thus showed a solid feed dose-response. Because of an increase in chewing and ruminating efficiency over time, amounts of solid feed should be increased with age to maintain high levels of chewing and ruminating. Moreover, high levels of chewing and ruminating may have to be maintained long enough at the beginning of the fattening period to lead to a reduction in abnormal oral behaviours.

# 1 Introduction

In Europe, calves fattened for the production of veal are generally fed relatively small amounts of solid feed supplemented to large volumes of milk replacer. This feeding strategy is a result of consumer demand for pale meat, hence low iron levels in the diet. The European legislation set down the minimum fibrous feed provision for calves at 50 to 250 g from 8 to 20 wk of age (EU Council, 1997). This amount of feed, regardless of the structure, fibre content, or dry matter content, has been repeatedly shown to be insufficient in terms of meeting the behavioural needs of calves throughout the entire fattening period (Kooijman et al., 1991; Morisse et al., 1999; Mattiello et al., 2002). Calves fed this amount of solid feed display abnormal oral behaviours, including excessive oral manipulation of the pen structure and trough, tongue rolling and tongue playing, and sham chewing and ruminating (Kooijman et al., 1991; Morisse et al., 1999; Mattiello et al., 2002). Abnormal behaviours are indicative of poor welfare resulting from frustration due to an inadequate environment or lack of control over their environment (Broom and Fraser, 2007), and demonstrate that one of the Farm Animal Welfare Council's Five Freedoms, i.e. freedom to express normal behaviour, is not fulfilled (Farm Animal Welfare Council (FAWC), 1992).

The frustration leading to the display of abnormal oral behaviours in veal calves is accepted as deriving mostly from a lack of chewing and ruminating opportunity (Veissier et al., 1998), although housing conditions could also play a role (Bokkers and Koene, 2001). Increasing the time calves spend on chewing or ruminating appears to decrease time spent performing abnormal oral behaviours (Kooijman et al., 1991; Mattiello et al., 2002), but small increases in chewing time at the beginning of the fattening period do not lead to lower abnormal oral behaviour levels compared to groups of calves fed only milk replacer (Mattiello et al., 2002).

Furthermore, veal calves show a number of health problems related to the feeding strategy including: 1) the leaking of milk into the rumen that can lead to excessive gas formation (Van Weeren-Keverling Buisman et al., 1991), 2) plaque formation in the rumen impairing nutrient uptake (Suárez et al., 2006, 2007), 3) poor rumen development (Suárez et al., 2006, 2007), and 4) abomasal damage in the form of erosions and ulcers (Mattiello et al., 2002). It has been suggested that the consumption of large volumes of milk by veal calves may result in strong contractions and local ischaemia in the abomasal wall, which make it sensitive to ulceration (Welchman and De Baust, 1987; Breukink et al., 1991). The present study aimed to investigate the effects of increasing amounts of solid feed on the general behaviour and welfare of veal calves, including variations across time. This was done in combination with restricting the potentially harmful effects of large volumes of milk replacer by decreasing the milk

allowance with increased solid feed provision, for similar average daily gain (**ADG**) between groups.

One of the expected outcomes of this study was an inverse relationship between the amount of solid feed provided and the frequency of abnormal oral behaviours displayed by veal calves. In addition, we attempted to find an easy practical method to measure the behavioural response of calves to their feeding strategy. We hypothesised that the feeding strategy could affect calves' motivation to orally manipulate novel objects. We tested this by presenting calves with two objects (i.e. a ball and an overall), differing in the chewing opportunity they provided, and by recording the interaction of the calves with the objects.

## 2 Materials and methods

This study was conducted at Wageningen University's experimental facilities in The Netherlands. All procedures met the terms of the Dutch law for animal experiments, which complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and was approved by the Wageningen University Committee on Animal Care and Use. The present study was part of a larger (main) metabolism study aimed at quantifying responses of body protein and fat deposition rates of veal calves provided incremental amounts of a mixed solid feed, which ran from March to August 2010 (Berends et al., 2012b). The behavioural part of the study was carried out in 4 months, from April to July 2010. The aim of the main study required all calves to be tethered in metabolic cages (only visual contact of pen mates maintained) at 16 and 24 wk of age for a period of 10 d. Behavioural data collection took place before and between these two periods. Because tethering has been shown to exacerbate abnormal oral behaviours in dairy cows (Redbo, 1992, 1993), absolute levels of abnormal oral behaviours in the calves were not taken into account. Only relative differences between treatments were of interest in this study.

### 2.1 Animals and husbandry

Holstein-Friesian bull calves ( $N = 48$ ), aged 6-10 wk ( $7.6 \pm 0.1$  wk;  $54.7 \pm 0.3$  kg) were purchased from one commercial Dutch veal farm. Calves were housed in groups of three in  $2.35 \times 2.45$  m pens with wooden slatted floors. Partitions between adjacent pens and front partitions were open and enabled visual and tactile contact between calves from adjacent pens. The barn was mechanically ventilated and lit by TL-lamps switched on between 06:00 and 23:00 h. Average temperature ( $^{\circ}\text{C}$ ) and relative humidity (%) in the stable were for month 1:  $17.9 \pm 0.3$  and  $61.2 \pm 2.3$ ; month 2:  $17.8$

$\pm 0.2$  and  $67.1 \pm 1.1$ ; month 3:  $21.9 \pm 0.3$  and  $65.3 \pm 1.3$ ; month 4:  $22.7 \pm 0.4$  and  $68.7 \pm 1.5$ . Calves were habituated to the environment and to the observer for the first 2 wk after arrival. They were given antibiotic treatment as and when required, according to a veterinarian protocol. Haemoglobin levels were controlled three times during the study. Calves were provided additional iron in the milk replacer to ensure that their haemoglobin levels did not go below 5.5 mmol/L throughout the study and that they reached 5.5 mmol/L at the end of the study.

## 2.2 Feeding treatments

Calves were blocked by age and within blocks randomly allocated to one of four treatments (**A**, **B**, **C**, or **D**). The treatments included different amounts of a solid feed mixture (50% concentrate, 25% fresh maize silage, and 25% chopped wheat straw on the basis of dry matter [DM]) and milk replacer. The solid feed was provided to each treatment as follows: A = 0, B = 9, C = 18, and D = 27 g DM/kg<sup>0.75</sup>/d. The milk replacer allowance for each pen within one block was adjusted to maintain similar ADG between treatments and stimulate solid feed intake. Milk schemes were adapted so that each block reached the same weight at 16 and 24 wk of age, in accordance with the aims of the main study. Calves were weighed once a week. Mean solid feed and milk replacer provision per treatment per month are described in Table 3.1. During the first week after arrival, calves were fed according to the feeding regime of the veal farm they were purchased from. From the second week onwards, calves were fed according to their given treatment. Calves were fed their allowance of milk replacer and solid feed twice daily (07:00 and 16:00 h). Water was provided ad libitum from drinking nipples.

## 2.3 Measurement

### Behavioural observations

Direct observations were done by a single observer using The Observer®XT (version 9, Noldus Information Technology, Wageningen, The Netherlands) on a hand held computer (Psion Teklogix Workabout Pro G2, Teklogix Int. Inc, Mississauga, Canada). Observations were carried out: 1) around feeding time when most abnormal oral behaviours have been found to occur (Veissier et al., 1998), using continuous focal observations, and 2) throughout the day to investigate time budget, using instantaneous scan sampling. Continuous focal observations (“observing one individual [...] for a specific amount of time and recording all instances of its behaviour”, Martin and Bateson, 1993) were carried out 2 d a week, 1 h before and 1 h after morning

Table 3.1: Mean ( $\pm$  SEM) solid feed and milk replacer provision across treatments and months (g DM/calf/d)

Month	1	2	3	4
<i>Solid feed</i>				
A	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
B	242.6 $\pm$ 4.4	285.8 $\pm$ 5.6	347.2 $\pm$ 6.0	403.4 $\pm$ 8.9
C	492.2 $\pm$ 7.3	578.0 $\pm$ 12.2	696.0 $\pm$ 11.0	773.8 $\pm$ 35.4
D	742.9 $\pm$ 12.8	881.3 $\pm$ 18.8	1047.4 $\pm$ 16.7	1241.1 $\pm$ 20.1
<i>Milk replacer</i>				
A	1125.1 $\pm$ 18.9	1267.5 $\pm$ 13.9	1674.0 $\pm$ 21.0	1827.0 $\pm$ 15.3
B	1045.3 $\pm$ 16.5	1167.8 $\pm$ 10.9	1494.8 $\pm$ 20.5	1789.9 $\pm$ 13.3
C	961.6 $\pm$ 14.9	1080.0 $\pm$ 12.3	1310.9 $\pm$ 21.4	1722.2 $\pm$ 19.1
D	926.9 $\pm$ 12.5	1014.8 $\pm$ 13.9	1168.6 $\pm$ 24.7	1665.8 $\pm$ 28.6

and evening feeding, thus four sessions per day. During each session, four calves were observed, one from each treatment, continuously for 10 min. Treatments were observed in a random order in each session. This observation technique led to a total of 32 individual calves being observed each week. Once a week, instantaneous scan sampling (“a whole group of subjects is rapidly scanned [...] at regular intervals and the behaviour of each individual at that instant is recorded”, Martin and Bateson, 1993) was carried out at a 5 min interval every 2 h for 30 min between 06:30 and 19:00 h (i.e. 7 sessions per day). The same complete ethogram was used for both observation methods (Table 3.2). Certain behaviours were combined to create the following categories: abnormal oral, manipulate hair, comfort, non-nutritive sucking, and play behaviours (Table 3.2).

### Behavioural tests

Two 3-min behavioural tests were carried out to investigate motivation to orally manipulate two different novel objects. The general procedure for both tests was done according to a procedure for a novel object test previously used on commercial veal farms (Bokkers et al., 2009). In months 1 and 3, each pen was presented with a blue plastic ball (40 cm diameter) hanging off a pole at 1.5 m from the ground and the latency at which each calf made contact with the ball with their nose or tongue was recorded. Calves that failed to touch the ball at the end of the 3-min test were attributed the latency of 3 min. In month 3, each pen was presented with a dark-green overall placed over the fence of the pen and the frequency of oral contact with the overall was recorded for each individual animal using instantaneous scan sampling for

Table 3.2: Ethogram for continuous focal observation and instantaneous scan sampling. Certain behaviours were combined into one or two categories and the category and/or the individual behaviours were analysed

Behaviour	Description	Category
<i>Posture</i>		
Lie	Brisket in contact with floor.	
Stand	Body elevated from floor and weight supported by legs.	
<i>Activity</i>		
Chew	(sham) Chewing/ruminating. Any repetitive movements of lower jaw in lateral plane.	
Tongue play	Tongue playing/rolling. Turning, rolling and unrolling of tongue extended outside or inside mouth.	Abnormal oral
Manipulate objects	Oral manipulation of the pen structure and bucket.	Abnormal oral
Graze	Grazing pen mate hair situated on back. Mouth nibbles and pulls away from back, sometimes coming away with hair.	Abnormal oral, Manipulate hair
Self-groom	Tongue extending and shifted across any body part of self or nibbling of self.	Comfort, Manipulate hair
Rub	Rub any part of body against substrate.	Comfort
Lick another calf	Tongue extending and shifted across any body part of pen mate or nibbling of pen mate.	Manipulate hair
Urine drink	Preputial sucking of pen mates or drinking of urine flow.	Non-nutritive sucking
Cross-suck	Sucking any body part (ears, tail, face, body) of pen mate excluding prepuce.	Non-nutritive sucking, Manipulate hair
Leap/Jump/Buck/Turn	Forelegs lifted from ground, forepart of body elevated, with or without forward movement, kicking and turning.	Play
Head-shake	Head shaken or rotated.	Play
Head-butt	Butting of substrate or another calf.	Play
Mount	Mount other calf from any side.	Play
Run	Rapid movement forward with all four legs leaving the floor at one point in time.	Play
Sniff	Sniff at surroundings including calves.	
Other	Any other activity not mentioned above.	

3 min at a 10 s interval. All pens from all treatments were tested on the same day.

## 2.4 Statistical analysis

Data were expressed as a proportion of total scans for instantaneous scan sampling observations, proportion of total duration (i.e. 10 min) for behavioural states, and counts for behavioural events within the continuous focal observations. Latency data from the ball test were expressed as proportion of time before contact with the ball, of the total period (i.e. 3 min). All data were grouped by pen and month. Data were analysed using a generalised linear mixed model with a logit link function for proportions and a logarithmic link function for count data. The model comprised fixed main effects for months, blocks and treatments and interaction effects between months and treatments, and random pen effects to account for the repeated observations. The residual variance within pens was expressed as a multiple of the binomial (for proportions) or Poisson (for counts) variance respectively. Parameters were estimated by penalized quasi-likelihood (Schall, 1991; Breslow and Clayton, 1993; Engel and Keen, 1994). Significance tests were based on the Wald test (Cox and Hinkley, 1979), employing approximate denominator degrees of freedom (Kenward and Roger, 1997). Pairwise comparisons were done with Fisher's LSD method. All analyses were performed using the statistical programme SAS (SAS Institute Inc., 2008), employing routine PROC GLIMMIX.

Body weights were averaged per pen. The ADG was calculated for each pen across the entire fattening period. The effect of treatment was then estimated using a one-way ANOVA with treatment as the factor, employing routine PROC ANOVA.

## 3 Results

The exchange of milk replacer for solid feed successfully resulted in similar ADG (g/d) ( $\pm$  SEM) across the fattening period for each treatment: A =  $854.7 \pm 39.6$ , B =  $859.5 \pm 62.3$ , C =  $859.6 \pm 35.9$ , D =  $870.6 \pm 27.8$  ( $P = 0.995$ ). Play and non-nutritive sucking behaviours around feeding and throughout the day were too infrequently observed for analysis.

### 3.1 Continuous focal observations around feeding

The means ( $\pm$  SEM) and P-values for continuous focal observations around feeding are described in Table 3.3. Calves in treatment D spent less time displaying abnormal oral behaviours compared with calves in treatments A and C, and calves in all four



Table 3.3: Percentage of time spent (%) performing each behaviour (means  $\pm$  SEM), from continuous focal observations around feeding times, in veal calves fed the following amounts of solid feed (g DM/kg<sup>0.75</sup>/d): 0 (A), 9 (B), 18 (C), 27 (D), recorded during study months 1 ( $\sim$ 3months of age), 2 ( $\sim$ 4months of age), 3 ( $\sim$ 5months of age) and 4 ( $\sim$ 6months of age). P-values for main effects of treatment and month and the interaction are shown. Significant interactions are described in the main text. Means with different superscripts differ significantly ( $P < 0.05$ ). ‘Manip’ refers to ‘manipulate’.

	Abnormal oral	Tongue play	Manip object	Chew	Sniff	Comfort	Manip hair	Stand
A	41.8 $\pm$ 3.8 <sup>a</sup>	5.9 $\pm$ 1.2	34.3 $\pm$ 3.2	7.0 $\pm$ 0.9	3.1 $\pm$ 0.4	3.5 $\pm$ 0.2	8.4 $\pm$ 1.2	59.6 $\pm$ 2.9
B	31.7 $\pm$ 1.5 <sup>a,b</sup>	4.4 $\pm$ 2.1	26.7 $\pm$ 3.2	6.7 $\pm$ 1.8	4.8 $\pm$ 0.3	6.2 $\pm$ 1.3	10.9 $\pm$ 3.0	65.9 $\pm$ 6.4
C	36.3 $\pm$ 3.9 <sup>a</sup>	5.1 $\pm$ 3.5	30.4 $\pm$ 4.4	10.8 $\pm$ 2.2	4.0 $\pm$ 0.9	3.5 $\pm$ 1.1	8.7 $\pm$ 2.7	66.2 $\pm$ 2.5
D	24.0 $\pm$ 6.4 <sup>b</sup>	1.2 $\pm$ 0.7	22.7 $\pm$ 5.9	13.3 $\pm$ 3.1	5.3 $\pm$ 0.6	3.3 $\pm$ 0.5	5.4 $\pm$ 1.0	64.0 $\pm$ 5.9
<i>Treatment effect P-value</i>	0.019	0.255	0.202	0.357	0.056	0.349	0.453	0.643
Month 1	15.6 $\pm$ 2.3 <sup>a</sup>	1.1 $\pm$ 0.4 <sup>a</sup>	14.2 $\pm$ 2.2 <sup>a</sup>	14.7 $\pm$ 2.5	6.8 $\pm$ 0.8 <sup>a</sup>	3.9 $\pm$ 0.6 <sup>a</sup>	6.9 $\pm$ 1.1	52.8 $\pm$ 3.7 <sup>a</sup>
Month 2	39.5 $\pm$ 4.0 <sup>b</sup>	5.6 $\pm$ 1.9 <sup>b</sup>	32.3 $\pm$ 3.6 <sup>b</sup>	7.7 $\pm$ 2.2	4.4 $\pm$ 0.5 <sup>b</sup>	3.6 $\pm$ 0.5 <sup>a</sup>	8.8 $\pm$ 1.5	71.8 $\pm$ 4.9 <sup>b</sup>
Month 3	40.7 $\pm$ 4.7 <sup>b</sup>	4.8 $\pm$ 1.5 <sup>b</sup>	35.2 $\pm$ 5.0 <sup>b</sup>	7.6 $\pm$ 2.3	2.9 $\pm$ 0.3 <sup>c</sup>	4.7 $\pm$ 1.2 <sup>a,b</sup>	9.4 $\pm$ 1.9	67.4 $\pm$ 5.0 <sup>b</sup>
Month 4	41.1 $\pm$ 5.3 <sup>b</sup>	6.0 $\pm$ 2.0 <sup>b</sup>	34.8 $\pm$ 5.6 <sup>b</sup>	5.5 $\pm$ 1.7	2.2 $\pm$ 0.4 <sup>c</sup>	5.5 $\pm$ 0.9 <sup>b</sup>	8.3 $\pm$ 1.9	59.3 $\pm$ 5.0 <sup>a,b</sup>
<i>Month effect P-value</i>	<0.001	0.002	0.002	0.106	<0.001	0.036	0.380	0.042
<i>Treatment <math>\times</math> Month P-value</i>	0.845	0.124	0.856	0.892	0.820	0.323	0.006	0.916

treatments spent less time displaying abnormal oral behaviours in month 1 compared to all other months. No effect of treatment was found on tongue playing or manipulate objects, but these behaviours were displayed less in month 1 than in all other months. No effect of treatment or month was found on time spent chewing around feeding. There was an effect of month on time spent sniffing the environment, where calves spent more time sniffing in month 1 compared with months 2, 3, and 4, and in month 2 compared with months 3 and 4. An effect of month was also found on time spent performing comfort behaviours, with calves performing comfort behaviours for a higher proportion of observed time in month 4 compared with months 1 and 2. Observations around feeding also revealed an interaction between month and treatment for proportion of time spent manipulating hair, where calves in treatment A spent more time manipulating hair in month 2 (mean  $\pm$  SEM:  $13.5 \pm 3.1$ ) than months 3 ( $4.7 \pm 1.1$ ) and 4 ( $5.8 \pm 1.3$ ) ( $P < 0.05$ ), and calves in treatment C manipulated hair for longer in month 3 ( $16.9 \pm 4.6$ ) than in months 1 ( $3.8 \pm 1.0$ ), 2 ( $6.6 \pm 1.4$ ) and 4 ( $4.1 \pm 1.5$ ) ( $P < 0.05$ ). Finally, a month effect was found on standing time, where calves stood for a shorter period of time around feeding in month 1 compared with months 2 and 3.

### **Instantaneous scan sampling throughout the day**

The means ( $\pm$  SEM) and P-values for instantaneous scan sampling observations throughout the day are described in Table 3.4. Throughout the day, no differences between treatments were found regarding the display of abnormal oral behaviours. However, there was an effect of month, with calves performing abnormal oral behaviours less frequently in month 1 compared with all other months, in month 2 compared with months 3 and 4, and in month 3 compared with month 4. Calves in treatment A tongue played more frequently than calves in treatments B and D, and calves in treatment C tongue played more frequently than calves in treatment D. In addition, calves tongue played less frequently in month 1 compared with all other months. Calves manipulated objects more frequently every month, but no differences between treatments were observed. Results showed an interaction between month and treatment for chewing frequency. In month 1, calves in treatment D ( $30.1 \pm 2.0$ ) chewed more frequently than calves in treatments A ( $10.6 \pm 1.1$ ) and B ( $15.1 \pm 1.4$ ) ( $P < 0.01$ ), and calves in treatment C ( $20.6 \pm 1.7$ ) chewed more frequently than calves in treatment A ( $P = 0.020$ ). In month 2, calves in treatment D ( $18.1 \pm 2.0$ ) chewed more frequently than calves in treatment A ( $9.9 \pm 1.3$ ) ( $P = 0.023$ ). Moreover, calves in treatment C chewed more frequently in month 1 than months 2 ( $12.9 \pm 1.6$ ), 3 ( $10.2 \pm 1.4$ ) and 4 ( $11.3 \pm 2.3$ ) ( $P < 0.01$ ) and calves in treatment

D chewed more frequently in month 1 than in months 2, 3 ( $12.8 \pm 1.6$ ) and 4 ( $14.1 \pm 2.6$ ) ( $P < 0.001$ ), and in month 2 than in month 3 ( $P = 0.026$ ). Calves sniffed their environment more frequently in month 1 compared with months 2, 3 and 4, and less frequently in month 4 compared with months 2 and 3. In addition, calves stood more frequently in months 2, 3 and 4 compared with month 1. Finally, visual inspection of variation in standing frequency throughout the day revealed that calves in all four treatments were most active around the two feeding times (i.e. 07:00 and 16:00 h). Orally manipulating objects was also most frequent around feeding times, whereas tongue playing was relatively constant throughout the day in both months 1 and 4. Chewing behaviours, on the other hand showed a peak between feeding times in month 1. This peak was less obvious in month 4 (Fig.3.1).

### Behavioural tests

Results regarding the latency to touch the ball showed effects of both treatment ( $P = 0.002$ ) and month ( $P < 0.001$ ). Calves in treatment A touched the ball faster than calves in treatments B, C and D ( $P < 0.05$ ), and calves in treatment D were slower to touch the ball compared with calves in treatments B and C ( $P < 0.05$ ) (Fig.3.2). Moreover, the latency to touch the ball was longer in month 1 than in month 3 ( $P < 0.001$ ). There was also an effect of treatment on frequency of oral manipulation (i.e. chewing and licking) of the overall ( $P = 0.022$ ), with calves in treatments A and B being in contact with the overall more often than calves in treatments C and D ( $P < 0.05$ ) (Fig.3.3).

## 4 Discussion

The present study investigated the effect of increasing solid feed provision and adjusting milk replacer allowance for similar ADG on the behaviour and welfare of veal calves, and their variations over time.

Since calves in treatment A received no solid feed, the 10% average chewing observed in this treatment can be considered sham chewing, an abnormal behaviour described previously (Morisse et al., 1999). Our results support the previous proposition that calves have an innate drive to chew and ruminate, even in the absence of solid feed (Veissier et al., 1998; Morisse et al., 1999, 2000; Mattiello et al., 2002).

The feeding strategy used in treatment D, hence the highest solid feed provision and lowest milk replacer allowance, appears to have brought about the highest improvement in behaviour, with less abnormal oral behaviours around feeding and less tongue playing throughout the day, compared with the milk-only diet (i.e. treatment

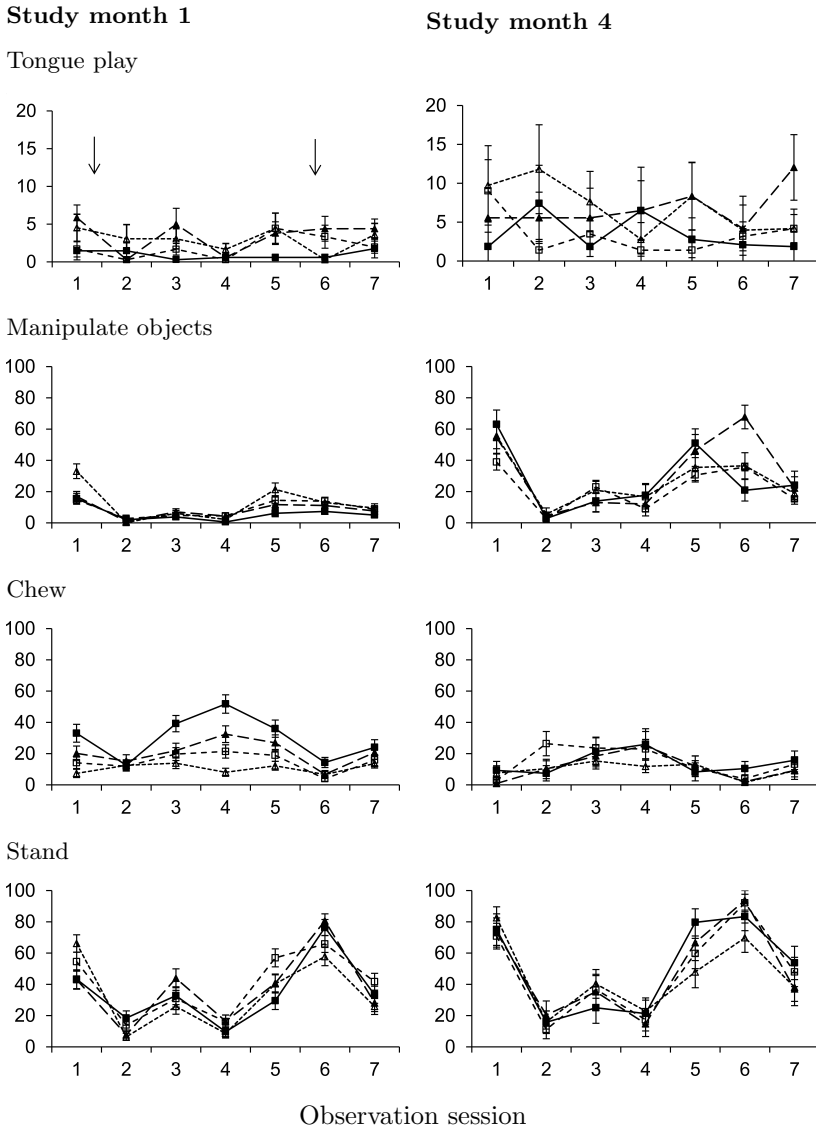


Figure 3.1: Time budget (mean  $\pm$  SEM, percentage total scans) of veal calves fed the following amounts of solid feed (g DM/kg<sup>0.75</sup>/d): 0 (A, open triangle), 9 (B, open square), 18 (C, closed triangle), 27 (D, closed square). Each session lasted a total of 30 min and calves were scanned at a 5 min interval at the following times of day: 06:30 (1), 08:30 (2), 10:30 (3), 12:30 (4), 14:30 (5), 16:30 (6), and 18:30 h (7). The arrows indicate feeding times (07:00 and 16:00 h).

Table 3.4: Frequency (%) of each behaviour (means  $\pm$  SEM) from instantaneous scan sampling observations throughout the day, in veal calves fed the following amounts of solid feed (g DM/kg<sup>0.75</sup>/d): 0 (A), 9 (B), 18 (C), 27 (D), recorded during study months 1 (~3months of age), 2 (~4months of age), 3 (~5months of age) and 4 (~6months of age). P-values for main effects of treatment and month and the interaction are shown. Significant interactions are described in the main text. Means with different superscripts differ significantly ( $P < 0.05$ ). ‘Manip’ refers to ‘manipulate’.

	Abnormal oral	Tongue play	Manip object	Chew	Sniff	Comfort	Manip. hair	Stand
A	26.8 $\pm$ 1.8	5.5 $\pm$ 2.1 <sup>a,c</sup>	20.1 $\pm$ 1.1	11.0 $\pm$ 1.7	3.1 $\pm$ 0.2	4.5 $\pm$ 0.3	9.0 $\pm$ 1.2	38.6 $\pm$ 2.4
B	19.4 $\pm$ 2.9	3.0 $\pm$ 1.9 <sup>b,d</sup>	15.9 $\pm$ 3.0	14.0 $\pm$ 2.7	4.0 $\pm$ 0.4	4.7 $\pm$ 0.6	8.5 $\pm$ 1.7	43.6 $\pm$ 1.8
C	24.2 $\pm$ 3.3	5.5 $\pm$ 2.9 <sup>b,c</sup>	18.2 $\pm$ 1.5	14.8 $\pm$ 1.7	3.4 $\pm$ 0.4	4.0 $\pm$ 0.4	7.6 $\pm$ 1.1	44.9 $\pm$ 2.8
D	17.6 $\pm$ 3.2	1.7 $\pm$ 0.8 <sup>d</sup>	15.6 $\pm$ 3.3	20.5 $\pm$ 2.2	3.6 $\pm$ 0.4	3.5 $\pm$ 0.5	6.6 $\pm$ 1.1	44.3 $\pm$ 2.5
<i>Treatment effect P-value</i>	0.273	0.030	0.496	0.222	0.088	0.220	0.602	0.377
Month 1	11.5 $\pm$ 1.3 <sup>a</sup>	2.3 $\pm$ 0.7 <sup>a</sup>	8.8 $\pm$ 0.9 <sup>a</sup>	19.1 $\pm$ 2.2 <sup>a</sup>	4.7 $\pm$ 0.4 <sup>a</sup>	4.4 $\pm$ 0.3	7.9 $\pm$ 0.6	36.1 $\pm$ 1.7 <sup>a</sup>
Month 2	24.4 $\pm$ 2.2 <sup>b</sup>	4.6 $\pm$ 1.2 <sup>b</sup>	19.2 $\pm$ 1.8 <sup>b</sup>	13.2 $\pm$ 1.3 <sup>b</sup>	2.9 $\pm$ 0.2 <sup>b</sup>	4.0 $\pm$ 0.3	7.9 $\pm$ 0.7	45.6 $\pm$ 1.5 <sup>b</sup>
Month 3	28.6 $\pm$ 2.1 <sup>c</sup>	4.9 $\pm$ 1.3 <sup>b</sup>	22.9 $\pm$ 1.7 <sup>c</sup>	12.2 $\pm$ 1.0 <sup>b</sup>	3.3 $\pm$ 0.2 <sup>b</sup>	4.1 $\pm$ 0.4	7.8 $\pm$ 0.8	47.3 $\pm$ 2.2 <sup>b</sup>
Month 4	32.9 $\pm$ 2.6 <sup>d</sup>	6.3 $\pm$ 1.7 <sup>b</sup>	25.8 $\pm$ 1.9 <sup>d</sup>	12.1 $\pm$ 1.7 <sup>b</sup>	1.8 $\pm$ 0.2 <sup>c</sup>	4.2 $\pm$ 0.5	8.0 $\pm$ 1.3	47.6 $\pm$ 1.3 <sup>b</sup>
<i>Month effect P-value</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.715	0.996	<0.001
<i>Treatment <math>\times</math> Month P-value</i>	0.097	0.413	0.165	0.002	0.216	0.065	0.626	0.325

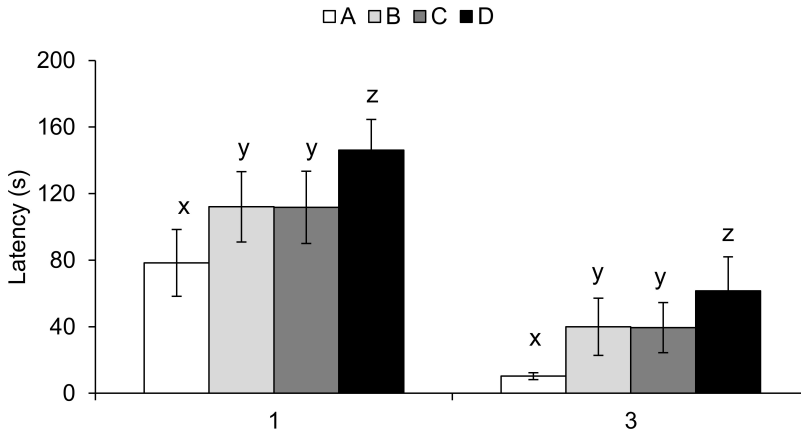


Figure 3.2: Latency (mean  $\pm$  SEM) to touch a ball with nose or tongue during a 3-min period in study months 1 ( $\sim$ 3 months of age) and 3 ( $\sim$ 5 months of age). The solid feed provision (g DM/kg<sup>0.75</sup>/d) for each treatment was as follows: 0 (A), 9 (B), 18 (C) and 27 (D). Means with different superscripts differ significantly from each other ( $P < 0.05$ ).

A). Moreover, compared with treatment A, treatment B resulted in lower tongue playing frequency throughout the day, but surprisingly, treatment C provided no benefit in terms of reducing abnormal oral behaviours. An inverse relationship was expected between the amount of solid feed provided and the frequency of abnormal oral behaviours displayed by veal calves. Treatment C, however, did not fit this expectation.

Although we are not sure why treatment C showed such high levels of abnormal oral behaviours, a similar pattern was shown in Mattiello et al. (2002). The latter study showed that calves fed 250 g of beat pulp, went from a chewing frequency of 12% in week 2 to only 4% in week 7, and showed similar levels of abnormal oral behaviours as milk-fed calves throughout the entire study. Latham and Mason's (2010) frustration hypothesis proposes that removing previously accessible enrichment from animals exacerbates stereotypic behaviours compared to animals kept in a barren environment throughout their life (Latham and Mason, 2010). Treatment C calves may have suffered a decrease in the reward they experienced from solid feed, when the time spent processing the feed, i.e. chewing and ruminating, decreased with what we assume was an age-related (or 'solid feed exposure time'-related) increase in chewing and ruminating efficiency (also shown in Mattiello et al., 2002). We suggest that a decrease, early in the fattening period, in time spent processing the amount of solid feed provided (as observed in treatment C), could actually be more deleterious to behaviour than maintaining a constant, even low, chewing frequency throughout the

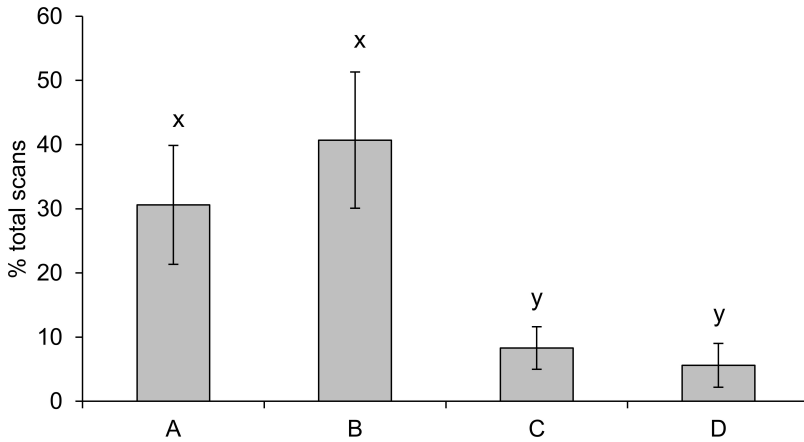


Figure 3.3: Percentage scans (mean  $\pm$  SEM) where veal calves were observed orally manipulating (i.e. chewing or licking) the overall during a 3-min period after presentation, in study month 3 ( $\sim$ 5 months of age). The solid feed provision (g DM/kg<sup>0.75</sup>/d) for each treatment was as follows: 0 (A), 9 (B), 18 (C) and 27 (D). Means with different superscripts differ significantly from each other ( $P < 0.05$ ).

entire fattening period (as observed in treatment B).

Treatment D experienced the largest decrease in chewing frequency and yet appears to have maintained a lower frequency of abnormal oral behaviours around feeding time compared with treatment A, as opposed to treatment C. Again, a similar finding is shown in Mattiello et al. (2002), where calves fed 250 g straw experienced a similar decrease in chewing frequency as the beat pulp treatment, but later on in the fattening period (week 13 instead of week 7), and yet maintained lower levels of abnormal oral behaviours compared to both the milk-fed and beat pulp treatments. A small increase in abnormal oral behaviours is observed at the end of the study in week 23 in the straw treatment. Since calves in treatment D in our study showed a reduction in chewing later on in the fattening period compared to Mattiello's straw treatment (i.e. week 20 instead of week 13), we hypothesise that our calves may also have shown an increase in abnormal oral behaviours later on. However, more research is needed to confirm this.

The two behavioural tests conducted in this study seemed to offer a reliable method to assess motivation to orally manipulate objects, since these tests distinguished between treatments as expected. The increased motivation to orally manipulate novel objects observed in calves fed smaller amounts of solid feed and larger amounts of milk replacer could be explained in three ways: 1) These calves were more curious/less fearful of novel objects (Van Reenen et al., 2004) maybe as a result of a more barren

environment due to low levels or a lack of solid feed; 2) They were less satiated as a result of a more empty rumen and hindgut; 3) They were frustrated from insufficient opportunities to chew on fibrous material from an early age. The first proposal is hard to prove or disprove in this study. Fear is generally accepted as an indicator of poor welfare (Boissy, 1995), as are abnormal behaviours (Bokkers et al., 2009), and the calves that displayed the lowest levels of abnormal behaviours in the current study (i.e. treatment D) interacted least with the objects. Interpreting the latency to interact with the novel object in terms of fear would mean that calves in treatment D were more fearful than those in the other treatment groups. However, at least from their behaviour, treatment D calves did not appear particularly frightened (kept lying and did not stand) of the novel objects (personal observation). Alternatively, satiety could have played a role here if, in calves, a full rumen leads to higher satiety than a full abomasum. Non-digestible fibre content of feed increases satiety through physical and chemical control (Allen, 2000), but no research could be found on comparisons between the effect of a full abomasum or rumen on satiety. Our third possible explanation of treatment differences in oral manipulation of novel objects would make sense on the assumption that the benefits of high chewing levels, in terms of reducing motivation to interact with novel objects, at the beginning of the fattening period, would carry over to the following months. Comparison of results from behavioural observations and behavioural tests seem contradicting. We suggest that both methods may have been measuring different aspects of behavioural state relating to feeding strategy.

No differences amongst treatments were found regarding the oral manipulation of hair, which is in line with previous research showing no relationship between solid feed provision and manipulation of hair (Morisse et al., 1999). Calves in treatment A showed a decrease in hair manipulation after month 2 whilst C showed an increase in this behaviour's frequency in month 3. Calves in treatment A may have redirected feed searching towards hair manipulation at the beginning of the study and thereafter decreased feed searching behaviours (Mattiello et al., 2002), when feed searching always failed to be rewarded. Calves in treatment C may have started to search for feed after their chewing frequency decreased, and, like A, they may have initially started to redirect feed searching behaviours towards hair manipulation.

Abnormal oral behaviours increased across the fattening period especially from month 1 to 2. Standing increased in a similar fashion pointing to an overall increase in activity with age (possibly due to cognitive development). Sniffing and chewing behaviours decreased over time and although comfort behaviours increased around feeding, this was by only 1% on average. Therefore, this increase in activity with age was mainly used by the calves in our study to increase time spent displaying abnormal



oral behaviours. Time was an important factor in this study. The prevention of the development of abnormal oral behaviours may depend on taking age into account when devising new feeding strategies for veal calves. In practice, calves receive an increasing amount of solid feed until around 6 wk before slaughter, when this amount is reduced to stimulate further milk replacer intake. This study supports the idea that calves have an increasing behavioural need for solid feed as they grow older (Mattiello et al., 2002), and that increasing solid feed provision relative to metabolic weight appears insufficient.

No treatment differences were observed for standing both around feeding time and throughout the day, thus calves that spent less time ruminating did not stand more and thus level of activity was not affected by the feeding treatments. Similar findings were reported in the past (Morisse et al., 1999; Mattiello et al., 2002). Most activity in all four treatments occurred around feeding times, also shown in previous research (Veissier et al., 1998; Bokkers and Koene, 2001). Although most oral manipulation of objects also occurred during the most active periods around feeding, tongue playing was relatively constantly displayed throughout the day in both months 1 and 4. This could explain why a treatment effect was only found for tongue playing for the observations throughout the day.

It is difficult to get to the underlying drives behind the present findings without conducting further research. These findings provide however a caution regarding the increase of solid feed provision to veal calves in practice. It may indeed be important to ensure a high level of chewing throughout the fattening period, and especially at the beginning of the fattening period, that does not decline rapidly early on. Such a decline could potentially lead to greater frustration in calves.

## 5 Conclusions

Supplying veal calves with 27 g DM/kg<sup>0.75</sup>/d (roughly over 6 times the EU minimum requirement for solid feed at 250 kg body weight) of a mixed solid feed (50% concentrate, 25% maize silage, and 25% wheat straw), while reducing the milk replacer ration, resulted in lower levels of abnormal oral behaviours and lower motivation to orally manipulate novel objects compared to calves fed milk replacer only, and, therefore, potentially improved calf welfare. Time had an important impact on abnormal oral behaviours. It appears that high levels of chewing have to be maintained long enough at the beginning of the fattening period to have beneficial effects on behaviour until slaughter. Moreover, the same amount of solid feed, relative to metabolic weight, may be sufficient at a young age but no longer be enough to stimulate high levels of chewing when calves get older, and chewing/ruminating efficiency increases.



# Chapter 4

## Dietary preferences of Holstein-Friesian calves: What do calves choose to eat and how do preferences affect behaviour?

Laura Webb, Bas Engel, Harma Berends, Kees van Reenen, Walter Gerrits, Imke de Boer, Eddie Bokkers

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(revised form)

*“Dis-moi ce que tu manges, je te dirai qui tu es.”* (Brillat-Savarin, 1826)

### Abstract

Calves raised for milk or meat are fed diets that differ from feral-herd calf diets and are based on the nutritional requirements of the ‘average calf’. These diets may not meet the dietary needs or preferences of each individual calf. This study explored diet preferences in calves with free dietary choice, and the effect of these preferences on behaviour. Group-housed Holstein-Friesian bull calves ( $N = 24$ ) were given unlimited access to five diet components (i.e. milk replacer [MR], concentrate, maize silage, hay and barley straw). At 3 and 6 months of age, calves were moved for 7 d to an automated test pen in groups of four, where intake, time spent eating, and visit frequency to each diet component was recorded to assess preferences. Behaviour was recorded using instantaneous scan sampling at a 2 min interval in the test pen, every 2.5 h for 30 min from 07:30 to 18:00 h, for 2 d. Solid feed intake at 6 months averaged  $3201.7 \pm 174.8$  g DM/d. At 3 months, calves selected the following proportion of MR, concentrate and roughage in relation to total g DM intake:  $51.6 \pm 5.0\%$ ,  $25.0 \pm 4.7\%$  and  $23.4 \pm 2.8\%$ . At 6 months, the calves conserved the roughage proportion ( $23.3 \pm 1.6\%$ ), but increased concentrate intake ( $47.1 \pm 2.1\%$ ) at the expense of MR ( $29.6 \pm 1.9\%$ ). Order of preference for the five diet components varied according to whether intake, time spent eating each component, or visit frequency was considered. On the whole, MR was preferred followed by concentrate and hay at both ages. Offering a dietary choice led to large individual variation in intake, suggesting diets based on the ‘average calf’ would meet only few calves’ dietary preferences. Different variables showed different preference rankings and studies in the future should consider the relative importance of these variables in assessing animal preferences.

## 1 Introduction

Calves raised for milk or meat are fed diets that differ from feral-herd calf diets and are based on nutritional requirements of the ‘average calf’. These diets may not meet the needs of each individual (Manteca et al., 2008). Prolonged prevention of behavioural needs leads to the development of abnormal behaviours (Jensen and Toates, 1993), implying chronic stress and consequently poor welfare (Broom and Fraser, 2007). Here, the term ‘behavioural needs’ refers to behaviours that if prevented would result in signs of suffering (Jensen and Toates, 1993). Veal calves, for example, develop abnormal oral behaviours due to restricted rumination (Veissier et al., 1998). Before developing novel calf diets it is important to understand their dietary needs.

When investigating needs, one commonly used tool is assessing preference for resources in choice tests: animals can choose from various resources (Broom and Fraser,

2007), and time spent with each resource or the frequency of choosing each resource is recorded (Petherick et al., 1993; Veillette and Reeb, 2011). Ruminants choose an array of different diet components to meet their nutritional requirements (Provenza, 1995; Atwood et al., 2001). Therefore, providing a range of components potentially enables individuals to select an adequate diet. Ruminants also display different dietary preferences at different times of day (Atwood et al., 2001; Manteca et al., 2008), and post-ingestive cues are important in the establishment of dietary preferences (Favreau et al., 2010). Rumen development is a long term process, which in young animals may result in different preferences being observed at different ages (Rushen et al., 2008). This is the reason why preferences of calves should be investigated over several months.

This study investigated diet choices and behaviour of calves with unlimited access to five diet components (milk replacer, concentrate, maize silage, hay and barley straw) up to 27 wk of age.

## 2 Materials and methods

The study was carried out at Wageningen University, Wageningen, Netherlands, within the experimental facilities of the Animal Science Department. All procedures met the terms of the Dutch law for animal experiments, which complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive). These procedures were further approved by Wageningen University's Committee on Animal Care and Use. The experiment ran from January to August 2011. This paper focused on intake, diet preferences and behaviour. Measurements for *in vivo* and post-mortem health and performance are described in a separate manuscript (Berends et al., unpublished).

### 2.1 Animals and husbandry

#### General

Two-wk-old Holstein-Friesian bull calves ( $N = 40$ ) were purchased from a Dutch dairy calf trader. For practical reasons (i.e. limited use of one test pen), two batches of calves 6 wk apart in age were acquired. The calves were housed in home pens with wooden-slatted floors and  $1.9 \text{ m}^2/\text{calf}$  floor area. These calves were fed one of two feeding strategies, both comprising of milk replacer (**MR**) and solid feed (see below). MR and concentrate composition were selected to meet beef cattle requirements in terms of minerals and vitamins (NRC, 2000). Feeding of solid feed was done at 08:00 and 16:00 h. MR (120 g DM milk powder per L) was fed using an automated milk dis-

penser (AMD) (TAP5-VH1-50-F2, Förster Technik®, Egen, Germany). The calves received transponders (FDX Cattle, Nedap N.V., Groenlo, Netherlands) in their right ear tag upon arrival. Water supply was unrestricted and offered in drinking bowls. The barn was mechanically ventilated and lit by TL-lamps switched on between 06:00 and 22:00 h. Minimum and maximum temperature and humidity in the stable were recorded daily: minimum =  $17.2 \pm 0.1^\circ\text{C}$  and  $57.5 \pm 0.5\%$ ; maximum =  $21.0 \pm 0.1^\circ\text{C}$  and  $73.0 \pm 0.5\%$ . Calves were familiarised to the environment, feeding strategy and observers for the first 3 wk after arrival. Plasma haemoglobin levels were examined at 3 and 6 months of age and calves below 5.5 mmol/L were injected with extra Fe. Calves were treated with antibiotics as and when required, according to a standard veterinarian protocol. The experiment ended when calves reached 27 wk of age.

### Dietary preferences

Twenty-four calves were used to assess dietary preferences (batch 1: N = 12,  $47.0 \pm 0.6$  kg,  $17.3 \pm 0.9$  d; batch 2: N = 12,  $45.1 \pm 0.6$  kg,  $17.9 \pm 1.2$  d). Calves from different batches were housed in two separate home pens. Calves could select their own diet from a choice of five diet components, offered simultaneously and ad libitum: MR, pelleted concentrate, maize silage, long hay, and long barley straw (composition shown in Table 4.1). The choice of the four solid feed components was based on the need to provide components varying in both structure, which may affect chewing and rumination, and fermentable fibre, which may affect intake. These two characteristics of the feed will additionally affect gastrointestinal health differently (Greenwood et al., 1997; Beharka et al., 1998; Mattiello et al., 2002). Solid feed components were fed in separate troughs and the location of each component varied daily to avoid positional preference. In the first week, the MR allowance was kept constant at 5 L/d, then gradually increased to ad libitum provision in the following 2 wk. The maximum drinking speed was set at 0.3 L/min in an attempt to enable calves to fulfil their need to suck and minimise cross-sucking (Haley et al., 1998; Margerison et al., 2003). Weekly intakes of solid feed and MR in the home pens are shown in Fig.4.1.

### Reference

Sixteen calves were used as a reference in terms of feeding strategy (batch 1: N = 8,  $46.2 \pm 0.7$  kg,  $17.1 \pm 0.5$  d; batch 2: N = 8,  $45.4 \pm 0.8$  kg,  $16.5 \pm 1.0$  d). These calves were fed according to a milk-fed calf production system based on the principles of veal farming: i.e. large quantities of MR fed alongside relatively small amounts of a solid feed mixture. Reference calves received 3.2 L/d MR from the day after arrival, gradually increased to 22.0 L/d at 27 wk of age. The provision of solid feed

Table 4.1: Nutrient composition of diet components (g/kg DM) (diet components were sampled every 8 wk, subsequently bulked and poolsampled for analysis).

Nutrient <sup>1</sup>	Milk replacer <sup>2</sup>	Concentrate <sup>3</sup>	Long barley straw	Hay	Maize silage
Dry Matter, g/kg	965	881	922	908	303
Crude Protein	210	179	31	92	84
Crude fat	173	40	8	15	36
Crude ash	80	54	61	104	37
NDF	-	209	787	590	399
Sugars	-	43	51	132	2
Starch	31	380	1	2	352
Fe, mg/kg DM	49	119	41	1002	97

<sup>1</sup> Presented as g/kg DM unless specified otherwise.

<sup>2</sup> Ingredient composition of milk replacer: 22.2% whey protein concentrate, 38.1% whey powder, 14.7% palm oil, 8.8% low lactose whey powder, 4.6% soy concentrate, 3.7% coconut oil, 3.0% wheat protein, 2.0% starch, 2.9% premix.

<sup>3</sup> Ingredient composition of concentrate: 26% maize, 25% barley, 25% lupines (CF < 70, CP < 335), 20% wheat middlings, 4% premix.

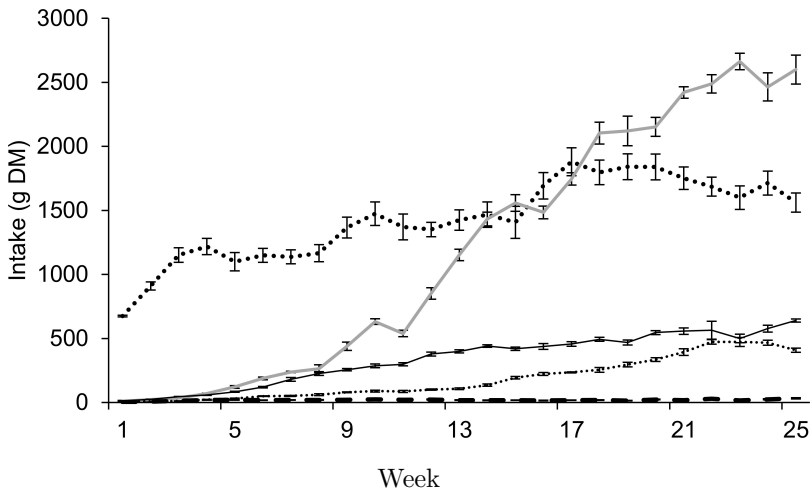


Figure 4.1: Average intake of each diet component per wk of study, recorded in the home pens (averaged across batch). Continuous grey line: concentrate; Thick dotted line: milk replacer; Continuous black line: long hay; Thin dotted line: maize silage; Tick dashed line: long barley straw.

increased gradually from 50 g DM/d at 2 wk to 500 g DM at 22 wk. After 22 wk, solid feed provision decreased gradually to 411 g DM/d at 27 wk. The solid feed comprised of 65% maize silage, 30% pelleted concentrate and 5% chopped barley straw (on DM basis). Reference calves were fed MR during three 7 h periods during the day. Speed of drinking increased in the first few weeks from 0.4 L/min to 0.7 L/min. Descriptive data for reference calves are given for comparison with calves with a dietary preference. A descriptive comparison of solid feed and MR intake in the home pen between preference and reference calves is shown in Appendix A.

## 2.2 Recording individual dietary preferences

All calves visited a test pen at 3 and 6 months of age in groups of four (6 groups in total), except for one group of three as one calf died. These groups are subsequently referred to as 'test groups' (N.B. reference calves also visited the test pen but were given no dietary choice). Calves in one test group were familiar with each other, as they came from the same home pen. Calves remained in the test pen for 7 d. Each group of calves was habituated once to the test pen for 2 to 4 d prior to the first visit. In addition, the first 3 d in the test pen at 3 and 6 months were treated as additional habituation time.

The test pen (3 m × 4 m) was situated in a room adjacent to the home pen and was equipped with an AMD, four solid feed troughs and one water bowl, each accessible by one calf at a time due to narrow walk-ways. Via the AMD, the following data were recorded for individual calves using the Institute software (Förster Technik®, Egen, Germany) on a PC: MR intake, daily number of visits to the AMD, and duration of each visit. The solid feed and water intake of individual calves was recorded using a transponder-antenna (SAT-A4-LR-P-125kHz Scemtec, EasyLogic, Moordrecht, the Netherlands) system connected to a reader (SIL-1400 Scemtec, EasyLogic, Moordrecht, the Netherlands), sending data to a PC. For solid feed intake, four scales (WPI-T, All Scales Europe, Veen, the Netherlands) weighed each trough. For water intake, a water flow meter (FTB601B, Omega Engineering, INC., Stamford, USA) connected to a totaliser (DPF702, Omega Engineering, INC., Stamford, USA) was used. The program used to collect the data was developed for this study (TUPOLA, Wageningen, the Netherlands) and used Purebasic V4.60. Solid feed intake, daily number of visits to the troughs, and duration of each visit was recorded for each solid feed component.

Calves were walked to the test pen one after the other before fresh solid feed was provided in the morning. The calves were weighed when they entered and exited the test pen. Each solid feed trough was filled with one of the four types of solid feed (in



random order for each test group). Solid feed refusals were weighed daily to validate computer-recorded solid feed intake data at test group level in the test pen. MR was fed to calves in the test pen in exactly the same way as it was fed in the home pens. The test pen stable was mechanically ventilated and lit using TL lamps from 06:00 to 22:00 h. A small light was on at night. Temperature and relative humidity were recorded daily: minimum =  $16.7 \pm 0.1^\circ\text{C}$  and  $59.0 \pm 0.7\%$ ; maximum =  $21.0 \pm 0.1^\circ\text{C}$  and  $75.6 \pm 0.6\%$ .

## 2.3 Behavioural observations

### Direct observations

Direct observations were done in the test pen by two observers. Inter-observer reliability over two 10 min instantaneous scan sampling sessions at 1 min interval were carried out. The first scan resulted in 74% agreement, therefore, behaviours were re-discussed. The second scan resulted in 97% agreement. Calf behaviour was recorded using instantaneous scan sampling at a 2 min interval for 30 min every 2.5 h from 07:30 to 18:00 h, on the 4<sup>th</sup> and 5<sup>th</sup> d (out of 7 d) of visit to the test pen, at both 3 and 6 months. Instantaneous scan sampling is described as follows: “whole group of subjects is rapidly scanned. . . at regular intervals and the behaviour of each individual at that instant is recorded.” (Martin and Bateson, 1993). Individual behaviours were combined to form the following categories: oral behaviours, tongue play, cross-suck, rumination, play, and stand (Table 4.2).

Direct observations were also performed in the home pen, using instantaneous scan sampling at a 5 min interval for 30 min every 2.5 h from 07:00 to 17:30 h. Due to the small number of home pens these data could not be statistically analysed. Instead, oral and ruminating behaviour data are reported in Appendix B.

One-zero sampling was carried out during instantaneous scan sampling observations to record play behaviour in the test pen. One-zero sampling is described as follows: “on the instant of each sample point. . . , the observer records whether or not the behaviour pattern has occurred during the preceding sample interval” (Martin and Bateson, 1993). Play behaviours in veal calves during a similar time of day were too infrequent and too short to be reliably recorded using instantaneous scan sampling (Webb et al., 2012).

### Indirect observations

The percentage of calves standing in the home pens was recorded from videos when lights were on: from 06:00 to 22:00 h. For these observations instantaneous scan

Table 4.2: Behavioural categories.

Behaviours	Description
Oral	Oral manipulation of the pen structure, including feed troughs, water trough and automated milk dispenser, tongue playing and rolling, and grazing of the coat of other calves.
Tongue play	Rolling and unrolling of tongue inside or outside of the mouth.
Cross-suck	Sucking any body part of another calf, including ears, nose, tail, and preputium, and drinking from urine flow.
Rumination	Repetitive movements of lower jaw in lateral plane. No notion of what the calf has in its mouth.
Play	Head butting calf or object, any form of jumping, running, and mounting.
Stand	Body elevated from floor and weight supported by two or more limbs.

sampling at a 10 min interval was used.

## 2.4 Statistical analysis

### General

Body weights at entry and exit of the test pen were averaged per calf at 3 and 6 months. Metabolic weights (**MBW**,  $\text{kg}^{0.75}$ ) at 3 and 6 months were calculated from body weights. Intake data were expressed as  $\text{g DM/kg}^{0.75}/\text{d}$ . Behavioural data from instantaneous scan sampling were expressed as proportions of total scans and one-zero sampling data were expressed as proportions of the number of intervals. Data were either analysed with a linear mixed model (**LMM**) or a generalised linear mixed model (**GLMM**) (McCulloch, 2006). Continuous data, i.e. intake and visit duration to feeding stations, were analysed with a LMM. Proportion data were analysed with a GLMM with a logit link, specifying the variance as a multiple of the binomial variance function. Count data, i.e. number of visits to the AMD, were analysed with a GLMM with a logarithmic link, specifying the variance as a multiple of the Poisson variance function.

All models comprised fixed main effects and interactions between ages and batches, and random effects for test groups, the interaction between test groups and ages, and

animals within test groups. The random effects accounted for the dependence between measurements from animals in the same test group and between repeated measurements on the same animals (within test groups). In the LMM additional random residual terms were included (i.e. random interaction between animals within test groups and ages) with an associated residual variance. The equivalent of this residual variance in the GLMM is the multiplicative (over)dispersion parameter included in the binomial and Poisson variance functions. For LMMs, an analysis with restricted maximum likelihood (REML) was performed (McCulloch, 2006), whereas for GLMMs, an analysis with penalised quasi-likelihood was used (Schall, 1991; Breslow and Clayton, 1993; Engel and Keen, 1994). For the fixed effects, approximate F-tests were constructed (Kenward and Roger, 1997). All analyses were done in SAS (SAS Institute Inc., 2008) using the GLIMMIX procedure.

### Validation of computer-recorded data

For MR and water intake, no validation was performed as intake was only computer-recorded. Manually recorded feed intake data in the test pen was averaged per test group per age and divided by test-group-averaged MBW. Computer-recorded intake data in the test pen was averaged across the last 4 d in the test pen per calf per age, and divided by individual MBW, then averaged per test group. The difference between computer-recorded and manually recorded data was calculated, and analysed with a LMM. This LMM comprised fixed main effects and interaction for ages, batches and diet component, and random effects for test groups and the interaction of test group and age.

### Feed preferences

Diet components were ranked based on intake, duration and visit frequency, in order to describe what the ‘average’ calf in the present study preferred. To compare two diet components in terms of intake level, a new variable was created, which expressed the intake proportion of one component with respect to the total intake for both components, e.g. comparison of intakes for concentrate ( $c$ ) and hay ( $h$ ):  $a = c/(c+h)$ . Variable was created for each pair of diet components. For each pair, the corresponding variable  $a$  was analysed with a GLMM, and the main effects of age, batch and the interaction between age and batch were tested. When e.g. neither interaction effects, nor main effects for age and batch were significant, they were removed from the model. In that case, the fixed part of the model for  $a$  represented just a single mean, and a Wald test was performed to check whether this mean was different from zero (zero on the logit scale corresponding to a proportion of 0.5 on the original scale,

i.e. no preference).

When e.g. only age showed a significant main effect, it was concluded that there was a preference, at least at one of the two ages. In that case, each of the two separate means for the two ages (again on the logit scale) was compared to zero. When an estimated mean was significantly different from zero, the two diet components were considered to be significantly different in the direction indicated by the sign of the estimated mean. The same procedure was used to find differences between total daily duration (min/d) with each diet component, and differences in daily visit frequency (visit/d).

### Relationship between behaviour and intake

Potential relationships between behaviour and intake variables were investigated to see whether differences in individual calves' intake resulted in predictable differences in behaviour (e.g. higher roughage intakes might lead to less abnormal oral behaviours). To relate behaviour to intake, the intake data were introduced by a covariate (explanatory variable) in the GLMM for the behavioural data (response). The size of the coefficient of the covariate reflects the strength of the relationship. Interactions between the covariate and age were introduced and tested to check whether the coefficient of the covariate could be assumed to be constant across ages.

## 3 Results

### 3.1 Validation of computer-recorded data

Analysis of the difference between computer- and manually-recorded intake data sets showed no effect of age ( $P = 0.766$ ), diet component ( $P = 0.431$ ) or batch ( $P = 0.234$ ), or any interaction effects. Moreover, the constant was not significantly different from zero ( $P > 0.05$ ), demonstrating that the two test pen data sets for intake (manual and computer) did not differ from one another.

### 3.2 Intake and behaviour

Solid feed, MR and water intake of preference and reference calves, as well as visitation duration of the troughs and AMD are shown in Table 4.3. Behaviours of preference and reference calves are described in Table 4.4. Activity, i.e. percentage of calves standing, observed from 06:00 to 22:00 h in the home pens from videos is shown in Fig.4.2.

Table 4.3: Means ( $\pm$  SEM) for body weight, intake and visitations to feeding stations in the test pen for preference ( $N = 23$ ) and reference ( $N = 16$ ) calves at 3 and 6 months of age.

	Preference		Reference <sup>1</sup>	
	3 months	6 months	3 months	6 months
Weight (kg)	110.6 $\pm$ 3.0	232.2 $\pm$ 6.5	104.7 $\pm$ 5.2	200. $\pm$ 2 8.0
<i>Intake</i>				
Solid feed (g DM/d)	986.6 $\pm$ 100.0	3205.5 $\pm$ 174.6	246.8 $\pm$ 61.5	351 $\pm$ 50.1
Milk replacer (g DM/d)	905.1 $\pm$ 83.6	1249.6 $\pm$ 80.0	1325.1 $\pm$ 84.8	2078.8 $\pm$ 120.3
Water (L/d)	2.5 $\pm$ 0.5	8.3 $\pm$ 1.0	1.8 $\pm$ 0.3	4.5 $\pm$ 0.8
<i>Solid feed troughs</i>				
Total duration (min/d)	105.1 $\pm$ 6.2	95.2 $\pm$ 3.9	57.8 $\pm$ 7.8	46.8 $\pm$ 5.6
Duration per visit (min)	21.2 $\pm$ 2.4	19.7 $\pm$ 1.8	29.0 $\pm$ 6.3	23.5 $\pm$ 4.7
Frequency (visits/d)	27.0 $\pm$ 1.5	29.0 $\pm$ 1.2	19.4 $\pm$ 2.1	18.3 $\pm$ 1.5
<i>AMD<sup>2</sup></i>				
Total duration (min/d)	55.7 $\pm$ 5.2	61.3 $\pm$ 2.6	80.2 $\pm$ 13.7	74.2 $\pm$ 8.1
Duration per visit (min)	7.4 $\pm$ 0.5	8.0 $\pm$ 0.4	3.1 $\pm$ 0.3	3.4 $\pm$ 0.4
Frequency (visits/d)	6.5 $\pm$ 0.5	8.5 $\pm$ 0.5	15.2 $\pm$ 2.8	22.1 $\pm$ 3.6

<sup>1</sup> No statistical analysis was carried out to compare preference and reference calves, because the number of home pens in each treatment was too low (i.e. 2).

<sup>2</sup> AMD = automated milk dispenser.

Table 4.4: Mean ( $\pm$  SEM) percentage of observed scans in which behaviours were displayed in the test pen in preference ( $N = 23$ ) and reference ( $N = 16$ ) calves at 3 and 6 months. Behaviours were recorded using instantaneous scan sampling.

	Preference		Reference <sup>1</sup>	
	3 months	6 months	3 months	6 months
Oral	4.7 $\pm$ 0.7	5.6 $\pm$ 0.7	12.6 $\pm$ 1.9	13.5 $\pm$ 1.6
Tongue play	0.17 $\pm$ 0.08	0.61 $\pm$ 0.25	1.00 $\pm$ 0.45	1.69 $\pm$ 0.77
Rumination	23.1 $\pm$ 1.9	19.0 $\pm$ 2.1	15.0 $\pm$ 1.9	13.7 $\pm$ 1.9
Play <sup>2</sup>	5.2 $\pm$ 0.5	2.9 $\pm$ 0.4	5.3 $\pm$ 0.7	2.6 $\pm$ 0.4
Stand	27.8 $\pm$ 2.5	32.9 $\pm$ 2.2	32.8 $\pm$ 3.3	43.4 $\pm$ 2.6

<sup>1</sup> No statistical analysis was carried out to compare preference and reference calves, because the number of home pens in each treatment was too low (i.e. 2). Means of reference calves are provided for guidance and speculation only.

<sup>2</sup> Play behaviours were recorded using zero-one sampling.

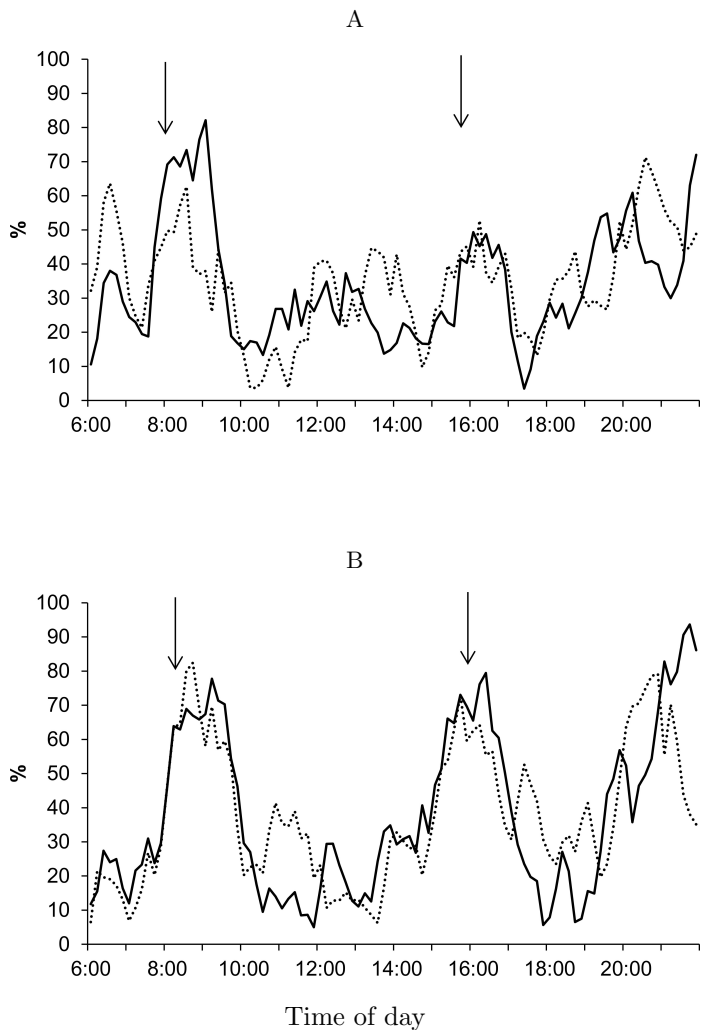


Figure 4.2: Percentage of calves standing in the home pens from 06:00 to 22:00 h in the preference (continuous line) and reference (non-continuous line) groups. Percentages are shown as an average for months 0-3 (A) and 4-6 (B). The arrows indicate when solid feed was renewed.

Table 4.5: Preference ranking for the five diet components provided ad libitum to the preference calves (N = 23). Preference was quantified using three variables: i.e. total intake (g DM/kg<sup>0.75</sup>/d), total duration with each diet component (min/d), and frequency of visits (visits/d). This ranking is based on statistical analysis of differences between components ( $P < 0.05$ ). The percentage of calves with the same ranking as the average ranking is also shown (only based on comparison of numbers, and with '=' not taken into account).

Age	Variable	Ranking	%
3 months	Intake	MR <sup>1</sup> > concentrate = hay > straw > maize	47
	Duration	MR > hay > concentrate = straw > maize	80
	Frequency	MR = concentrate = hay > straw = maize	53
6 months	Intake	concentrate > MR > hay = maize > straw	74
	Duration	MR > hay > concentrate > maize > straw	57
	Frequency	MR = concentrate = hay = maize > straw	65

<sup>1</sup> MR = milk replacer.

### 3.3 Feed preferences

Diet components differed in terms of intake level, daily duration, and visit frequency (Fig.4.3). Looking at visit frequency, diet components were more similar to one another and more difficult to order compared to intake level and daily duration, which both showed more variation between diet components. The rankings of diet components based on statistical differences between components are shown in Table 4.5. Preference rankings are given for intake, duration, and frequency. These rankings give an overview of preferences of calves in this study for MR, concentrate, hay, maize silage and barley straw. Different rankings were observed for the different variables. In general, calves preferred MR, followed by concentrate and hay. For each of these rankings, the percentage of calves that showed the same ranking as the average ranking is also given in Table 4.5. Individual intakes of all five diet components and water at 3 and 6 months varied greatly and are shown in Fig.4.4 and Fig.4.5.

At 3 months, individual variation in the proportions of MR, concentrate and roughage selected by the calves was: MR =  $51.6 \pm 5.0\%$ ; concentrate =  $25.0 \pm 4.7\%$ ; roughage =  $23.4 \pm 2.8\%$  (based on g DM). At 6 months, this was: MR =  $29.6 \pm 1.9\%$ ; concentrate =  $47.1 \pm 2.1\%$ ; roughage =  $23.3 \pm 1.6\%$  (based on g DM). At 6 months calves replaced MR by concentrate, but the proportion of roughage in the diet remained constant. The concentrate to roughage ratios were 51:49 at 3 months and 67:33 at 6 months in the preference calves.

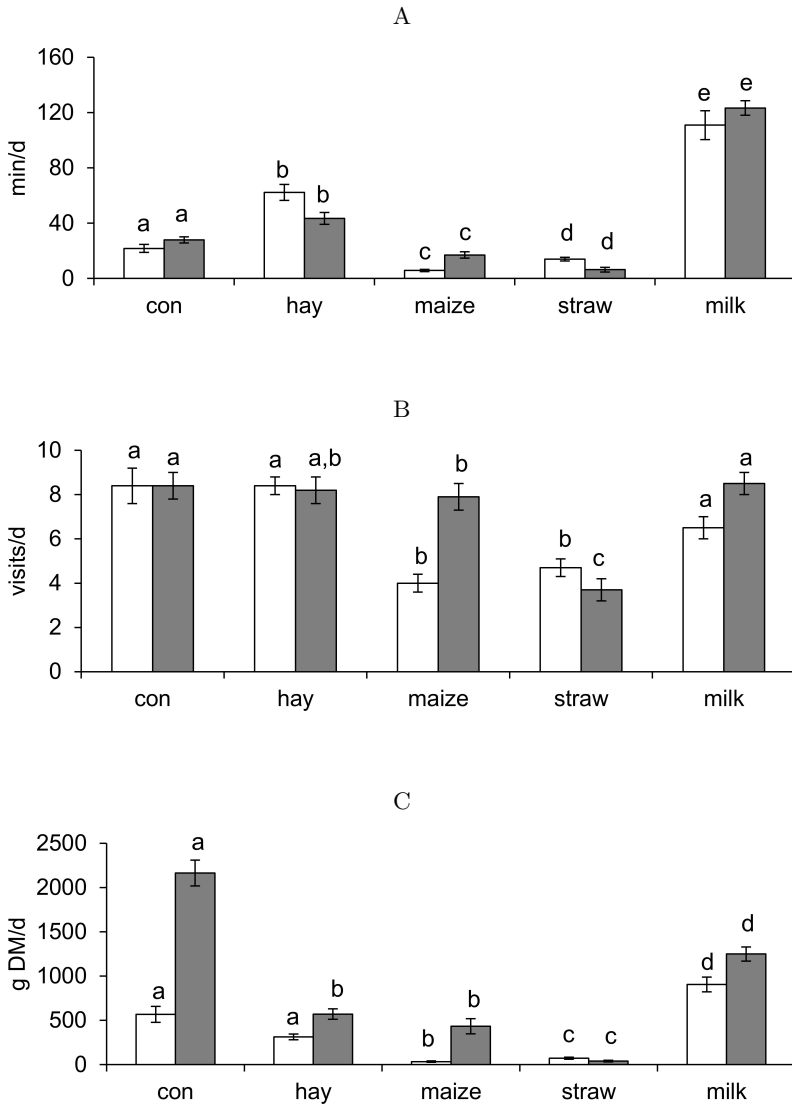


Figure 4.3: Intake (A), total daily duration with each diet component (B) and daily visit frequency (C) at 3 (no fill) and 6 (dark fill) months. a-f Bars without a common superscript differ ( $P < 0.05$ ) within one age. ‘Con’ refers to concentrate. Intake is shown in g DM/d but statistics were performed on intakes corrected for metabolic weight.



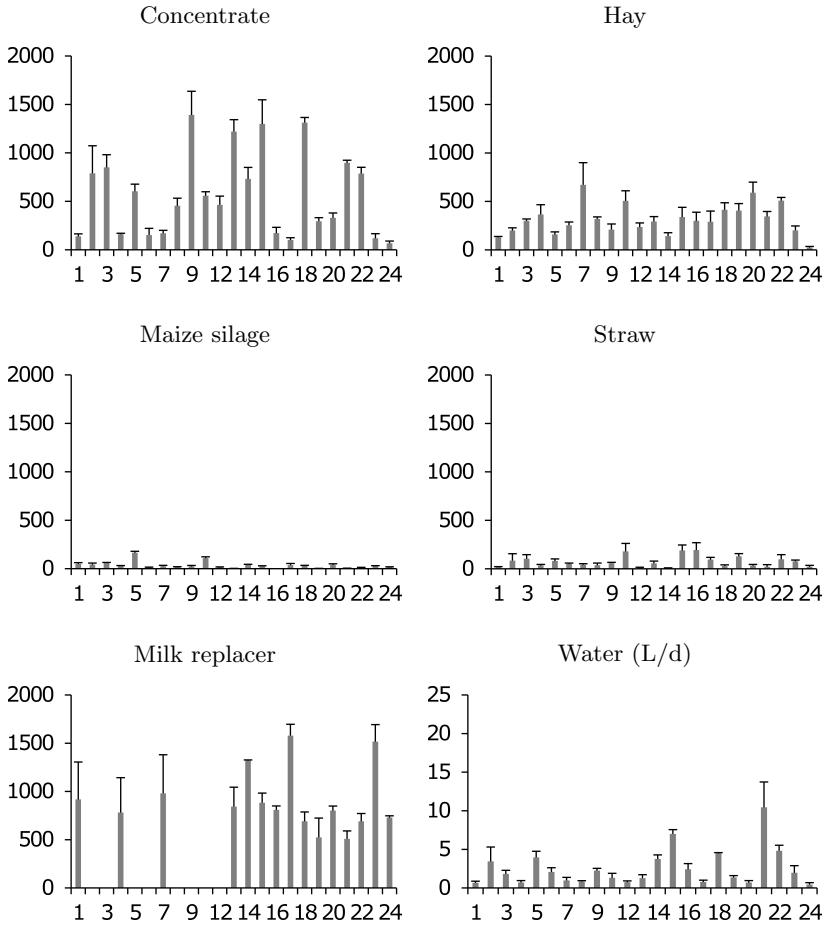


Figure 4.4: Individual calves' intake (mean  $\pm$  SEM; g DM/d) of concentrate, hay, maize silage, straw, milk replacer and water (L/d) at 3 months of age. Each bar represents one calf. NB: Milk replacer intake data is missing for 8 calves.

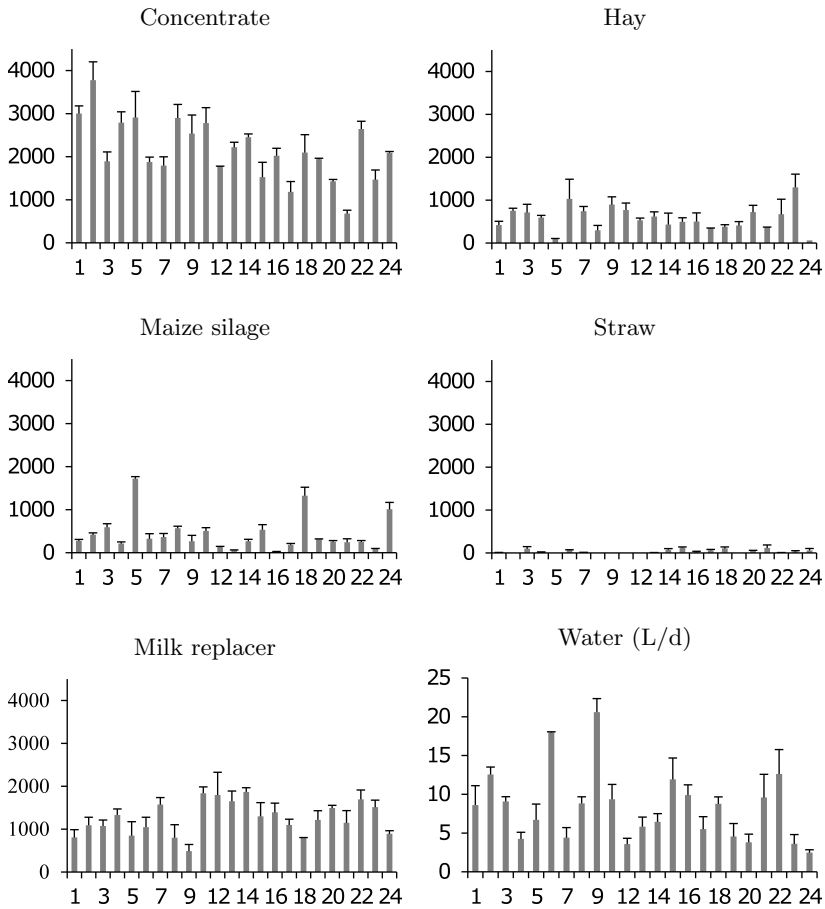


Figure 4.5: Individual calves' intake (mean  $\pm$  SEM; g DM/d) of concentrate, hay, maize silage, straw, milk replacer and water (L/d) at 6 months of age. Each bar represents one calf. NB: The scale of these graphs is larger than for Fig.4.4, except for water.

### 3.4 Relationship between behaviour and intake

All behaviour categories (Table 4.2) were tested for a relationship with MR, concentrate, hay, maize silage, all roughages or all solid feed intake (corrected or not for metabolic weight). No significant linear relationship was found between any of the behaviour and intake variables ( $P > 0.05$ ).

## 4 Discussion

This study offered calves ad libitum access to MR and four different solid feed types (differing in structure and fermentable fibre) over the first 6 months of age. To the best of our knowledge, this is the first study offering the choice of MR and four solid feed components ad libitum to young ruminants over several months.

### 4.1 Total intake

The average DMI for solid feed was comparable to previous studies in similar-aged calves (Estermann et al., 2003; Boga et al., 2009; De Vargas et al., 2011; Faleiro et al., 2011; Castells et al., 2012). The average duration spent at the solid feed troughs was 105 min/d at 3 months. Previous research found that dairy calves fed 4.5 L/d (500 g/d) MR consumed concentrate and straw for 135 min/d and concentrate and grass for 240 min/d, on average (Phillips, 2004). These differences in time spent eating solid feed between dairy calves and the calves in the present study are likely explained by differences in MR intake and subsequent differences in solid feed intake. Castells et al. (2012) found higher concentrate proportions relative to roughage, i.e. from 86 to 96% depending on the type of roughage fed, in dairy calves fed 4 L/d MR (500 g DM/d). This discrepancy with the present study most likely stems from the current study providing three types of roughage (instead of one) and the opportunity to consume more MR. Because of a higher MR intake, our calves would have needed less energy from concentrate (see also Miller-Cushon et al., 2013). The concentrate to roughage ratios found in the present study are closer to some of those presented by Suárez et al. (2007), who showed that a 70:30 concentrate-to-roughage ratio fed to veal calves minimised gastrointestinal health problems, compared with only providing concentrate with MR. Based on this, we can speculate that the calves in our study made choices at least partly aimed at avoiding impaired rumen function and abomasal health.

The average intake for MR was within the range of those found in previous work in ad libitum fed dairy calves or suckling calves of similar ages (Le Neindre and Petit,

1975; Appleby et al., 2001; Jasper and Weary, 2002; Borderas et al., 2009; Todd et al., 2010; De Vargas et al., 2011). No previous research could be found on MR to solid feed ratios in calves given free choice, but the present study showed that, over time, calves shift their diet in such a way that the proportion of MR in their diet is reduced (from 52% at 3 months to 30% at 6 months of age, based on DM). This indicates a shift from pre-ruminant towards ruminant digestion, with a higher proportion of the diet relying on ruminal fermentation and digestion at 6 compared to 3 months of age. Calves with the dam at pasture have been found to wean around 7 to 14 months of age (Reinhardt and Reinhardt, 1981; Rushen et al., 2008). Despite differences in the type of feed available between calves at pasture and the calves in the present study, we did not expect to see calves weaning themselves in the current study. The average AMD visit duration (7-8 min) was close to sucking durations in suckling calves with regards to visits to the dam (11-12 min: Le Neindre and Petit, 1975; 8-9 min: Reinhardt and Reinhardt, 1981). Total duration and number of visits at the AMD were similar to total sucking duration and frequency in suckling calves of 3 but not 6 months (Le Neindre and Petit, 1975; Reinhardt and Reinhardt, 1981). Differences between the present study and studies looking at suckling calves at 6 months of age are probably a result of cows increasingly rejecting sucking bouts as calves grow older: an element that was absent in the current study.

Regarding water intake, a study looking at Holstein heifers (143 to 367 kg body weight) fed ad libitum barley straw and concentrate, found an average water intake of 23 L/d (Faleiro et al., 2011). When we added water intake to MR intake in the present study similar quantities were found.

## 4.2 Behaviour

Low levels of oral behaviours, and especially tongue rolling, were observed in calves with a dietary choice. In earlier studies, we found 13% and 24% oral behaviours in veal calves fed ad libitum hay and up to 1240 g DM/d of a solid feed mixture, respectively (Webb et al., 2012, 2013). In addition, based on means comparison, preference calves showed lower levels of oral behaviours compared with reference calves. Oral behaviours in calves are considered abnormal if displayed in excess (e.g. orally manipulating the pen structure) or in a repetitive, unvarying fashion (e.g. tongue playing) (Veissier et al., 1998; Broom and Fraser, 2007). Abnormal oral behaviours in veal calves are thought to originate from frustration due to limited chewing and rumination opportunities (Veissier et al., 1998). Our findings, therefore, suggest that preference calves were able to select a diet that led to satisfying levels of chewing and ruminating.

As expected, rumination levels were high and averaged around 20% of total scans at 3 and 6 months in calves with a dietary choice. Means comparison showed that reference calves displayed lower levels of rumination (14-15%). In earlier studies, we found around 16 to 20% rumination in calves fed high amounts of solid feed (Webb et al., 2012, 2013). This may be considered as a good basis for a rumination time budget in calves that could be aimed for when developing novel feeding strategies. However, it is important to keep in mind that time required to chew and ruminate a given amount of feed may be reduced in older/more experienced calves (Mattiello et al., 2002; Webb et al., 2012). Improved foraging skills with more experience of roughage in ruminants was initially proposed by Provenza and Balph (1987).

Based on means comparison, it seems that calves with free choice drank less MR, visited the AMD less often, but for longer durations compared to reference calves. A higher frequency of visits and lower duration at the AMD in reference calves can be explained by the fact that some visits were not rewarded with MR if they had finished their allowance, and this is consistent with research on dairy calves before weaning (Borderas et al., 2009). Moreover, preference calves seemed to visit the solid feed troughs more than reference calves, which is expected with higher solid feed allowances. Tongue playing/rolling was not observed as frequently as was expected in the reference calves, based on previous findings in veal calves (Webb et al., 2012, 2013). This suggests that tongue playing may have origins other than limited feed allowance, e.g. lack of sucking on a teat. Future studies could investigate whether providing MR via an AMD could reduce tongue playing. Play behaviour levels in preference calves were comparable with reference calves. Play behaviours are suggested as an indicator of good welfare, as they have been termed “luxury activities” (Lawrence, 1987). However, being short lasting, infrequent behaviours, they can be difficult to monitor accurately, and maybe studies that stimulate play (e.g. introduction of head-butting device) could find differences between calves with different diets. The present results showed activity peaks around times at which solid feed was refreshed, despite ad libitum feeding (Veissier et al., 1998; Bokkers and Koene, 2001; Webb et al., 2012), and just before the lights went out. An activity peak at dusk was also observed in calves at pasture (Reinhardt and Reinhardt, 1981).

The reference calves were used in the present study as a baseline. They provided data for a milk-fed calf system, based on the principles of veal farming. We use the word ‘system’ because the reference differed from the preference calves, not only in terms of the amount and type of feed provided, but also in the way the components were provided. Differences included: chopped straw instead of long, no hay versus hay provision, mixed feed instead of separate components, MR provision restricted in three daily periods, and high versus low MR drinking speed. This system comparison

enables the pointing out of potential differences between calves with a choice and calves fed according to a reference production system, but does not enable the pinpointing of particular reasons why the differences occurred. The mechanisms behind the differences will need to be investigated in further studies using control treatments and a wider range of reference systems. Given the small number of home pens, no statistics could be performed to formally compare preference and reference calves. We used the comparison between preference and reference calves to speculate about effects of free choice versus restricted feeding on behaviour, and further welfare, but differences described here should be treated with care.

### 4.3 Feed preferences

All diet components were eaten by the calves in this study. When it comes to diet selection, ruminants tend to select a range of diet components even if one component on its own could meet their nutritional needs (Provenza et al., 1996), potentially because of aversion developed within a meal to single foods (Provenza et al., 1996). Alternatively, the physical properties of the diet components may also be important in diet selection (Baumont, 1996) in terms of rumen development (Harrison et al., 1960; Tamate et al., 1962), and for stimulating chewing and ruminating (Webb et al., 2013).

The preference of calves was assessed using three variables based on previous research investigating preferences in animals: intake (e.g. Provenza et al., 1996), duration (e.g. Ngwa et al., 2000), and frequency (e.g. Manninen et al. (2002)). These three variables showed slightly different orders of diet components. Because of these differences, future studies using choice tests should consider using all three of these variables in order to get a complete picture of animal preferences. MR was always preferred, at both 3 and 6 months of age. Preference for hay or concentrate varied according to the variable considered, with concentrate being generally more or equally preferred compared to hay, except when duration was considered. Concentrate enables rapid intake of energy, whereas long hay takes more time to chew and ruminate. Rapidly ingested and digested feeds are more palatable to ruminants (Kenney and Black, 1984; Baumont, 1996). In our study, however, calves were willing to devote a large proportion of their time budget to feed on hay. This points to their need for coarser, more abrasive feed types, which help rumen development and nutrient yield from rumen fermentation, prevent the development of hairballs, and enable high levels of chewing and rumination (Tamate et al., 1962; Cooper et al., 1995, 1996; Morisse et al., 1999; Mattiello et al., 2002; Suárez et al., 2007; Webb et al., 2013). Improved early rumen development may also protect to some extent against abomasal damage

(Berends et al., 2012b). Maize silage and straw appear to have been the least preferred feed components, though maize silage was more preferred at 6 than 3 months of age, and straw was equally preferred as concentrate at 3 months of age when duration was taken into account. Early in the fattening period, calves selected low levels of maize silage (Fig.4.1 and 4.2). We suggest that, in our study, young calves were reluctant to ingest maize silage. This was suggested in the past as a result of silage fermentation products potentially reducing intake (van Soest 1982 in Castells et al., 2012). It is worth noting that the present preference rankings are a result of the specific choice of diet components offered to calves in this study and that preferences are relative.

DMI, diet component ratios, and preferences in the present study varied much between individuals. Previous studies showed that up to 50% of the animals differed from the average in terms of diet preferences (Provenza et al., 1996; Scott and Provenza, 1999). Similarly, in the present study 53% of the calves at 3 months and 26% of the calves at 6 months differed from the average ranking for intake. Given that the calves showed high levels of chewing and low levels of oral behaviours compared to previous research (Webb et al., 2012, 2013), the authors speculate that a diet for the ‘average calf’ would mean poorer welfare on average. It is possible that free choice, instead of mixed rations, is the only way to ensure that the dietary preferences of all animals are met (Manteca et al., 2008; Villalba et al., 2010), given that ‘appropriate’ diet choices are offered; i.e. calves should be provided with an array of diet components that include variation in fermentable fibre and structure. Offering only one monotonous mixed ration can result in nutrient imbalances and food aversions: two processes which compromise welfare (reviewed in Villalba et al., 2010). In addition, calves seemed to agree more in their preferences at an older age. Early on, ruminants must learn about post-ingestive consequences of feed through trial and error (Provenza and Balph, 1987), and this learning phase may lead to greater inter-individual variation at an early age. Additionally, health issues, more prominent in the first months of age, may lead to such differences (Berends et al., unpublished).

#### **4.4 Relationship between behaviour and intake**

No relationship was found between the intakes of the different dietary components and behaviour, implying that intake did not affect behaviour in a linear way. In other words, differences in intake of a particular diet component between calves did not have any obvious negative or positive effect on any of the behaviours considered in this study. As mentioned above, dietary choice should enable ruminants to select an adequate diet, namely a diet that better meets each individual’s behavioural and physiological needs compared to when fed a mixed ration, and should, therefore, result

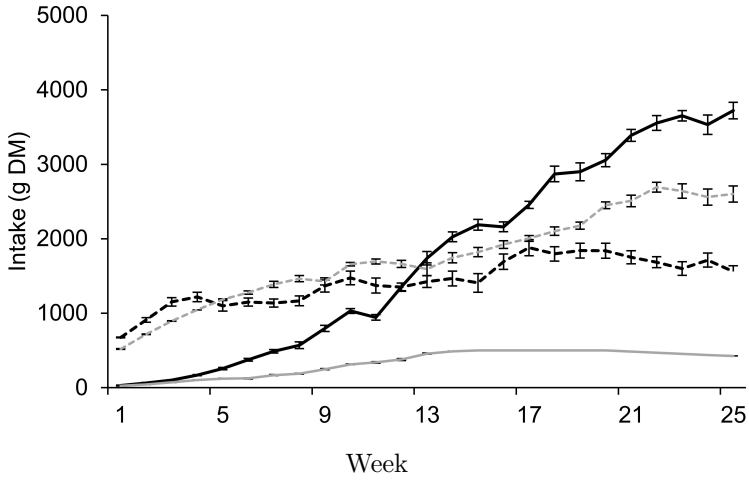
in improved welfare of each and every individual (Atwood et al., 2001; Manteca et al., 2008). This is why we could expect all calves to show behavioural signs of good welfare (i.e. high levels of chewing and low levels of abnormal oral behaviours) regardless of their dietary choices.

## 5 Conclusions

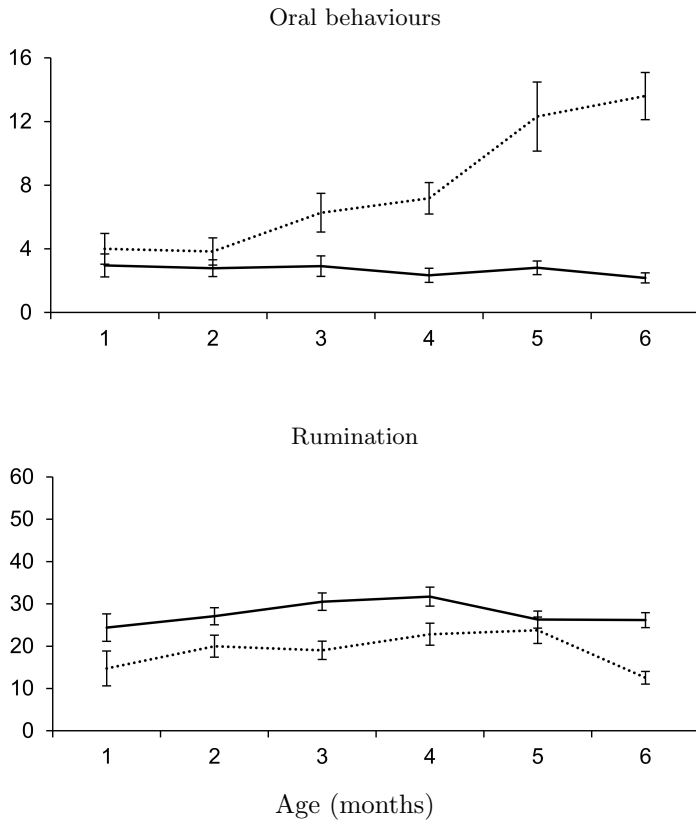
The 1997 EU Directive stipulates that calves should receive a minimum of 250 g fibrous feed at 20 wk of age. Calves with free choice from five diet components, including MR and four solid feed sources consumed almost thirteen times this amount (on DM basis). On average, calves preferred MR, concentrate and hay, over straw and maize silage. The different variables used in this study to investigate preferences gave a different pattern of preference. Therefore, future preference tests related to food should also consider all three variables rather than a single one, if a complete picture is to be drawn. Moreover, the present study indicated that young calves were able to select a diet that led to high levels of chewing and ruminating and low levels of abnormal oral behaviours. However, marked individual variation was observed in dietary choices. Diets based on the 'average animal' may not address every individual's particular behavioural needs, and future diets should take individual differences into account, potentially by providing a choice.



## Appendix



Appendix A: Average solid feed (continuous lines) and milk replacer (non-continuous lines) intake ( $\pm$  SEM) (g DM/d) in preference (black lines) and reference (grey lines) calves per week of study, recorded in the home pens (averaged across batch).



Appendix B: Average frequency (% total scans) of oral and ruminating behaviours observed in the home pens in the preference (continuous line) and reference (non-continuous line) calves ( $\pm$  SEM).

# Chapter 5

## Methods for cross point analysis of double-demand functions in assessing animal preferences

Bas Engel, Laura Webb, Margit Bak Jensen, Kees van Reenen, Eddie Bokkers

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*“While people can express their preferences in abstract form, animals can only demonstrate which food they prefer by which food they eat. . .”* (Forbes and Kyriazakis, 1995)

### Abstract

Cross point analysis of double demand functions provides a compelling way to quantify the strength of animal preferences for two simultaneously presented resources. During daily sessions, animals have to work to gain access to (a portion of) either resource, e.g. by pressing one of two panels a required number of times (the workload). Each panel is linked to one of the simultaneously presented resources. Workloads are varied over sessions and resources. Per session, for each resource the number of times that an animal is rewarded by access to the resource is observed. Four statistical approaches for analysis of these observations, including two novel approaches, are presented and discussed. Data from an experiment investigating preferences of Holstein-Friesian bull calves for two types of roughage (chopped and long hay) will be used to illustrate the calculations. The rationale of the four statistical approaches is given, and their pros and cons are discussed, including considerations of accuracy (efficiency), sensitivity to model assumptions (robustness), and computational aspects. The two novel approaches will be recommended for future practical use.

## 1 Introduction

Developing methods to assess behavioural needs and preferences, in terms of environmental resources, is central in the assessment of animal welfare. The double demand approach, where two substitutable resources are presented simultaneously and the cross point of two demand functions is calculated, is currently the most comprehensive method for assessing preferences (e.g. Sørensen et al., 2004; Jensen and Pedersen, 2007, 2008). In particular, double demand techniques using the two alternating lever procedure (where the price varies on both panels instead of only one), with each resource being presented on both sides (instead of always presenting the more aversive resource on the same side) minimise the risk of animals developing a preference for one particular panel (Holm et al., 2007). Statistical models and inference for the double demand approach, however, require further thought and development.

The aim of this paper is to give the rationale of four statistical approaches, illustrate the calculations, compare the approaches, discuss their pros and cons, and ultimately come to a recommendation as to which of these approaches is most appropriate for practical use, considering accuracy, sensitivity to model assumptions, and computational aspects. Two of the approaches discussed are based on an assumption of approximate normality of the (log transformed) counts of numbers of rewards for the two resources, and have been previously applied (e.g. Sørensen et al., 2001, 2004). The other two approaches discussed are novel approaches based on an analysis

of relative proportions of rewards for the two resources. The latter two approaches focus upon the fact that resources are offered simultaneously and consequently animals have to choose between them. This leads to a substantial reduction in the number of model parameters, and a more straightforward, and numerically more stable analysis. Data from an experiment investigating preferences of calves for chopped or long hay using cross point analysis of double demand functions (Webb et al., 2014b) were used to illustrate the four statistical methods. Technical details are given in appendices. This paper provides a basis for cross point analysis of double demand functions, with statistical programmes for GenStat (VSN-International, 2012) and SAS (SAS Institute Inc., 2008) for the two novel approaches made available on request via the first author.

## 2 Experimental design

The data used as an illustration in the present paper was collected from nine Holstein-Friesian bull calves working for two different types of roughage (chopped and long hay) on concurrent schedules of reinforcement. Details on animals and management, as well as training and testing procedures are described in Webb et al. (2014b). Briefly, roughage rewards of 5 g were made accessible by pressing one of two panels in a test pen, one panel for each of the two resources. By pressing a panel a certain number of times (the workload, also referred to in the literature as fixed ratio or **FR**) a calf was immediately rewarded by a portion (5 g) of the associated resource, in a bucket next to the panel. Each calf visited the test pen in two series of five consecutive days. In the first series, the following pairs of workloads (for chopped and long hay respectively) were assigned randomly to the five test days: (7, 35), (14, 28), (21, 21), (28, 14), or (35, 7). So, pair (7, 35) implies that 7 presses on one panel were required for 5 g of chopped hay and 35 presses on the other panel were required for 5 g of long hay. The second series of 5 d was a repeat of the first series, except that the resources were switched between panels and pairs of workloads were presented in a new random order. Note that the workloads for the two resources always summed up to 42. The number 42 was chosen on the basis of observations from three calves, obtained during a pilot study. Similar to previous research in pigs (Pedersen et al., 2005; Jensen and Pedersen, 2007) the workloads were chosen as a multiple of the lowest workload.

In Fig.5.1, the data from calf no.3 are depicted as an example. Along the horizontal axis, from left to right, workload  $x$  for chopped hay increases from 7 to 35, and simultaneously workload  $(42 - x)$  for long hay decreases from 35 to 7. Consequently, the number of rewards along the vertical axis tends to decrease for chopped hay and increase for long hay. In the next section, smooth curves will be fitted through

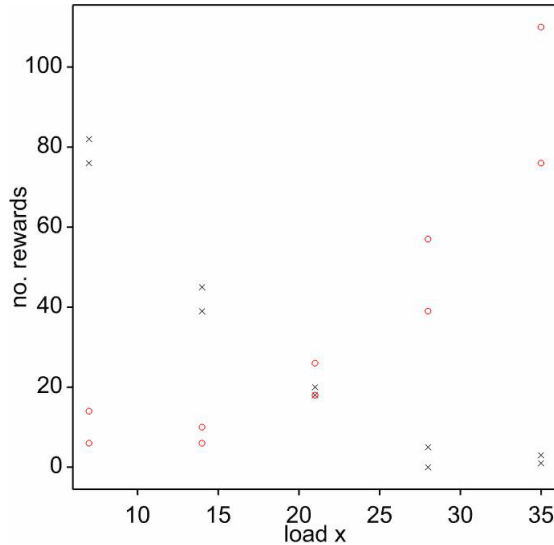


Figure 5.1: Number of rewards for chopped hay (crosses) and long hay (circles) against the workload  $x$  of chopped hay, for calf no.3.

the points for chopped and long hay. The point where these curves intersect is the estimated cross point for calf no.3. The position of the cross point relative to the midpoint  $x = 21$  (where the workloads on the two resources are equal) indicates whether the calf shows a preference or not.

### 3 Four statistical approaches

The four statistical methods for analysis will be referred to as count/1s, count/2s, prop/1s and prop/2s. Count/2s and prop/2s are two-step (2s) approaches that are applied to (log transformed) counts or proportions respectively. In the first step, individual cross points are calculated in separate analyses per animal. In the second step, an overall cross point is derived from the individual cross points, and a hypothesis test is introduced for comparing the overall cross point and the midpoint. A significant difference shows that there is a preference in the population, e.g. for long hay. Note that in that case, the majority of the animals in the target population will have a preference for e.g. long hay, but there may be individual variation in preference. Count/1s and prop/1s are one-step (1s) approaches applied to (log transformed) counts or proportions respectively. With these approaches, the data are analysed with a single model, rather than aggregating data per animal into individual

cross points first.

In the one-step approaches the dependence structure between data from the same animal needs to be properly modelled. The need to do so is circumvented in the two-step approaches by the aggregation into individual cross points in the first step. Aggregation into individual cross points simplifies the analysis in the second step, but implies some loss of information. Therefore, a one-step approach compared with the associated two-step approach will be more accurate (more efficient in statistical terms), but will also be more vulnerable to departures from model assumptions (less robust in statistical terms) and computationally more demanding. We start with a description of method count/2s, because this method is most convenient to introduce the notion of individual cross points and overall cross point. Next, we will argue that an analysis of proportions is more appropriate than an analysis of (log transformed) counts and move to prop/2s. Finally, prop/1s and count/1s will be discussed.

### 3.1 Counts/2s, a two-step approach for counts

#### Count/2s – 1st step – aggregation into individual cross points

In order to make the response data for the two resources scatter more closely around two straight lines (straight lines are easy to fit), in Fig.5.2, the number of rewards along the vertical axis is log transformed. Zero counts are problematic when a log transformation is used and one zero value (from another calf than calf no.3) was replaced by 0.5, prior to log transformation.

The two lines in Fig.5.2 are fitted separately for chopped and long hay by the method of least squares, i.e. linear regression per resource of log transformed counts of rewards ( $y$ ) upon workload  $x$  (of chopped hay). The equations for the two fitted lines for chopped ( $y_1$ ) and long ( $y_2$ ) hay are:

$$y_1 = 5.686 - 0.1559x,$$

$$y_2 = 1.219 + 0.0915x.$$

The point where the two lines cross is solved from the equation  $5.686 - 0.1559 x = 1.219 + 0.0915 x$ . The solution  $x = 18.0$  is the (estimated) individual cross point for calf no.3, expressed in terms of the workload for chopped hay. Because the lines cross before the midpoint 21, where the workloads on the two resources are equal, and  $x$  is the workload for chopped hay, calf no.3 expressed a preference for long hay.

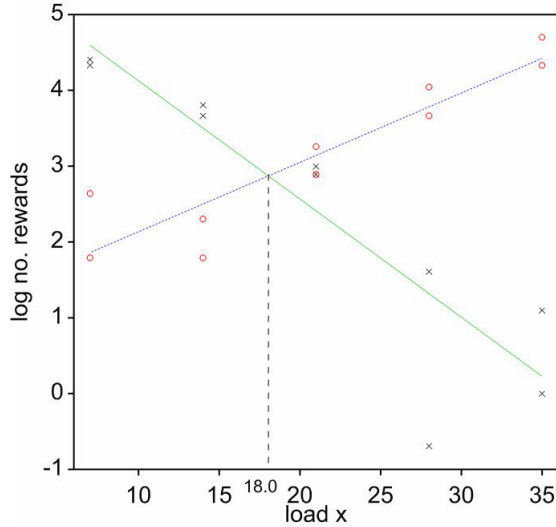


Figure 5.2: Number of log transformed rewards  $y_1$  for chopped hay (crosses) and  $y_2$  for long hay (circles) against the workload  $x$  of chopped hay, for calf no.3. Separate regression lines are shown as well. The estimated individual cross point of 18.0 of calf no.3 with count/2s is indicated.

### Count/2s – 2nd step – introduction of the overall cross point

The animals in an experiment are regarded as a random sample from a target population of animals and we want to draw general conclusions from the data about preferences in the population. Therefore, we will introduce the notion of an overall cross point  $cp$  for the population, as was done in previous studies (Sørensen et al., 2001, 2004; Jensen and Pedersen, 2007). The overall cross point  $cp$  is defined as the median of the individual cross points of all animals in the population, i.e. 50% of the animals have individual cross points below  $cp$ , and the other 50% above. The median is chosen because the distribution of individual cross points may be markedly skewed. The obvious estimate of the overall cross point  $cp$ , denoted by  $\hat{cp}$ , is the median of the estimated individual cross points of the animals in the experiment.

Table 5.1 lists the individual cross points for count/2s for the nine animals in the experiment for the comparison between chopped and long hay. All animals expressed a preference for long hay, because all individual cross points were below midpoint 21. The median of the nine individual cross points was the estimate  $\hat{cp} = 14.5$  for the overall cross point.



Table 5.1: Estimates of individual cross points and overall cross point (and 95% confidence interval) for the two-step approaches based on log transformed counts and proportions.

Calf	Individual cross point	
	Count/2s	Prop/2s
1	13.2	14.2
2	17.3	17.1
3	18.0	18.9
4	12.5	13.8
5	11.5	12.2
6	17.8	17.9
7	14.2	14.5
8	14.5	14.3
9	19.1	19.3
Overall	14.5	14.5
95% confidence interval	(13.2,17.5)	(13.9, 17.8)

### Count/2s – 2nd step – statistical inference for the overall cross point

Are the results for the nine animals in Table 5.1 strong enough to conclude that there is a preference in the population, i.e. to conclude that  $cp < 21$  and the majority of the animals in the population prefer long hay? To find out, estimate  $\hat{cp}$  was compared with the midpoint 21 with a significance test. A 95% confidence interval for  $cp$  was constructed as well. Should value 21 be in the interval, implying that  $cp$  and midpoint 21 do not significantly differ (P-value  $> 0.05$ ), it cannot be concluded that there is a preference. Then, when either the lower bound of the interval is well below 21 or the upper bound is well above 21, we may conclude that possibly there is a preference, but that variation within and/or between animals was too large for this to show up clearly. In that case we may consider collecting more data, e.g. by repeating the experiment with new animals.

To compare the overall cross point  $cp$  with the midpoint, value 21 was subtracted from all estimates of individual cross points and Wilcoxon's signed rank test (Conover, 1980) was applied to these differences. With a sizeable number of animals, a proper check for normality (and symmetry in particular) of the differences could be carried out, and the t-test could be used instead of Wilcoxon's signed rank test. Wilcoxon's signed rank test yielded an exact P-value of 0.004. Therefore, there is a preference in the target population of calves. The preference is for long hay over chopped hay, because  $\hat{cp} = 14.5$ , which is expressed in terms of the workload for chopped hay, is

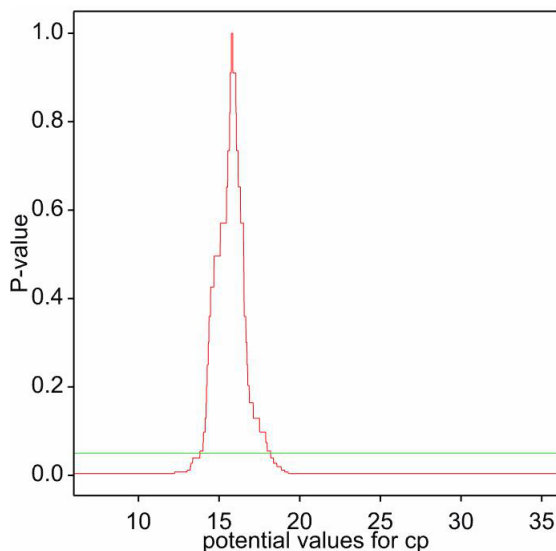


Figure 5.3: P-values from Wilcoxon's signed rank test corresponding to different values for overall  $cp$  being tested for count/2s. The horizontal line corresponds to a significance level of 0.05. All values for overall cross point  $cp$  with P-value larger than 0.05 are not rejected by Wilcoxon's signed rank test and constitute the 95% confidence interval for the overall cross point  $cp$ .

below midpoint 21.

By subtracting other values than 21 from the individual cross points and applying Wilcoxon's test again, a number of times, we can find out which values for overall cross point  $cp$  are not rejected on the basis of the data. Together, all values that are not rejected make up the confidence interval for  $cp$ . In Fig.5.3, P-values are plotted against a range of values for  $cp$  that are tested and a curve is drawn through the points. Where the curve cuts through the horizontal line at value 0.05, we can read the lower and upper confidence bound. This simple, but adequate approach has been implemented in GenStat and SAS, and the interval is determined automatically. The 95% confidence interval was (13.0, 17.8).

### 3.2 Prop/2s, a two-step approach for proportions

#### From counts to proportions

Calf no.3, in a session with workloads 7 and 35 for chopped and long hay respectively, showed a total count of 88 rewards for both resources together. The total 88 reflects

the animal's motivation, and depends both on internal motivating factors, characteristics of the resources, and the associated workloads. The total 88, however, does not tell us about the animal's appreciation of one resource relative to another in this session. For the relative preference we need to know the proportions for chopped and long hay, which were 82/88 and 6/88 respectively. These proportions will be used next as the basic building bricks of method prop/2s.

Because the two resources were offered simultaneously, there was a certain element of competition involved between the resources. This is an essential element of a double demand experiment, and we may expect this to affect the choice of model used for analysis. An animal has a choice of two resources, but can only work for and access one of the resources at any time during a session. The basic data are binary: when an animal completes the required number of presses on a panel, it has chosen either chopped or long hay. The observed proportions reflect the probabilities for an animal to choose either chopped or long hay. We will focus upon the proportions, say  $y$ , for chopped hay. The proportions for long hay are not required, because these are  $(1 - y)$ . Proportion  $y$  reflects the probability, say  $p$ , that an animal chooses chopped hay, given work load  $x$  (and workload  $(42 - x)$  for long hay). We will construct a model that relates  $p$  to  $x$ . From this relationship the individual cross point will be estimated as the value for  $x$  where there is no preference, i.e. where  $p = 0.5$ . Given the individual cross points, we will introduce overall cross point  $cp$  as a median and proceed with Wilcoxon's signed rank test to compare  $cp$  with midpoint 21 and construct a 95% confidence interval, analogous to count/2s.

### Prop/2s – modelling proportions

Because probabilities  $p$  are in between 0 and 1, which is a nuisance for modelling, they are 'stretched' into numbers from minus to plus infinity. The common way to do this is by using a logit function (the logarithm of the odds):

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right).$$

Workload  $x$  is introduced as an explanatory variable:

$$\text{logit}(p) = a + bx.$$

Here,  $a + bx$  is a line with intercept  $a$  and slope  $b$ , but on the ‘logit scale’. This line corresponds to a sigmoid curve of  $p$  against  $x$  on the original scale for proportions:

$$p = \frac{1}{1 + \exp(-a - bx)}.$$

Intercept  $a$  and slope  $b$  were estimated by a technique called maximum quasi-likelihood (McCullagh and Nelder, 1989), employing standard software for logistic regression that is available in e.g. GenStat and SAS. Estimates for calf no.3 for intercept  $a$  and slope  $b$  were 4.257 and -0.2258. The fitted line on the logit scale and the associated sigmoid curve for proportions on the original scale for calf no.3 for chopped hay are shown in Fig.5.4. Note that the sigmoid curve for long hay, also shown in Fig.5.4A, is 1 minus the curve for chopped hay, so these sigmoid curves intersect at the individual cross point where  $p = 0.5$ . The logit of  $p = 0.5$  is:

$$\ln\left(\frac{0.5}{1 - 0.5}\right) = \ln(1) = 0,$$

and the individual cross point for calf no.3 was solved from:

$$4.257 - 0.2258x = 0.$$

The solution was  $4.257/0.2258 = 18.9$ . Estimated individual cross points for prop/2s for all nine animals are listed in Table 5.1. The overall cross point  $cp$  was estimated by the median 14.5. Using Wilcoxon’s signed rank test to compare overall cross point  $cp$  to midpoint 21, the P-value was 0.004. Therefore, there is a preference in the population for long over chopped hay. The 95% confidence interval for the overall cross point, also derived with Wilcoxon’s signed rank test, was (14.0, 18.0).

### 3.3 A two-step or a one-step approach?

A two-step approach has several advantages: (1) it provides estimates for individual cross points, (2) it aggregates information over animals into a simple estimate for an overall cross point, (3) it is robust (not vulnerable to critical model assumptions), and (4) it only requires stable standard software. Nevertheless, it can be argued that the first aggregation step at individual level into individual cross points is less efficient (less accurate), and that an analysis of all data together, i.e. a one-step approach, is more efficient. A one-step approach is more efficient in the sense that it can produce a more powerful test and a narrower 95% confidence interval for the overall cross point. This increased efficiency is achieved at a price: the next approaches prop/1s

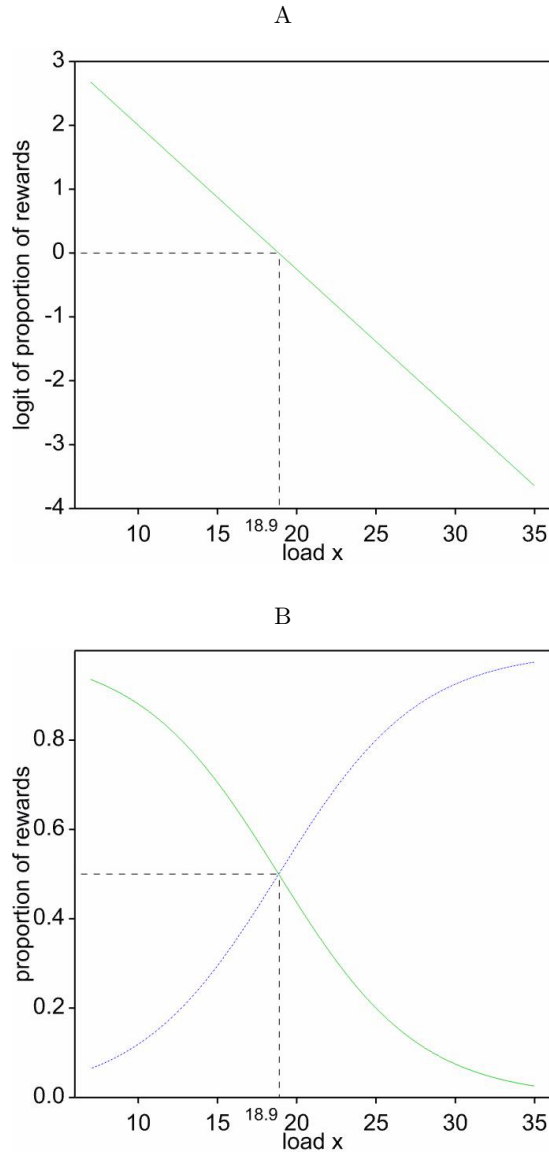


Figure 5.4: The line fitted with  $\text{prop}/2s$  for chopped hay on the logit scale (A) and the fitted sigmoid curve for chopped hay (downward) and long hay (upward) for the proportions of rewards (B), for calf no.3. The estimated individual cross point 18.9 of calf no.3 is indicated. In (B) observed proportions for chopped hay (crosses) are indicated as well.

and count/1s require more model assumptions. Consequently, they are less robust, and numerically more demanding. Prop/1s will be discussed first, because it is the natural contender for prop/2s that we have just discussed, and because the model can be seen as a step-up to the more complex count/1s.

### 3.4 Prop/1s, a one-step approach for proportions

Without the first aggregation step into individual cross points, the fact that we have repeated measures per animal must be taken into account. The animals in the experiment are assumed representative for the target population. That means that we analysed the data as if the animals were a random sample from the target population. Each time we ‘sampled’ an animal from the population, we ‘sampled’ an individual intercept and slope from respective distributions. The intercept and slope, say  $a_i$  and  $b_i$  for the the  $i$ -th animal, can be denoted by:

$$a_i = a + e_{a,i} \quad \text{and} \quad b_i = b + e_{b,i}.$$

Here,  $a$  and  $b$  are an overall intercept and slope and  $e_{a,i}$  and  $e_{b,i}$  are animal specific random departures. These random departures are assumed to be normally distributed around 0, with variances  $\sigma_a^2$  and  $\sigma_b^2$ , and correlation  $r_{ab}$  between them. This is a so-called random coefficient model on the logit scale (technical details are in Appendix A) for repeated measures and an instance of a general class of models referred to as generalised linear mixed models (**GLMMs**) (e.g. McCulloch, 2006). The model should contain a so-called (over)dispersion parameter. This dispersion parameter allows for dependence between choices of the same animal. For instance, an animal may successfully work at the same panel for a number of choices in a row, for no obvious reason perhaps, sticking to that side of the pen for a while, which inflates variation. The estimation procedure that is used in this paper is referred to as penalised quasi-likelihood (**PQL**) (Breslow and Clayton, 1993), iterative re-weighted restricted maximum likelihood (**IRREML**) (Engel and Keen, 1994), or pseudo-likelihood (Wolfinger and O’connell, 1993). Although motivated in different ways, PQL, IRREML and pseudo-likelihood are equivalent and yield the same results. Software in e.g. GenStat or SAS can be used. In the experiment with bull calves from Webb et al. (2014b), estimates for overall intercept  $a$  and slope  $b$  were 2.745 and -0.1729. The overall cross point, solved from  $(a + bx) = 0$ , is  $cp = -a / b$  (technical details are in Appendix B) and was estimated by  $\hat{cp} = -2.745 / -0.1729 = 15.9$ . The P-value for comparison of overall cross point  $cp$  with midpoint 21, obtained from a t-test, was 0.003, and the 95% confidence interval, based on the same t-test, was (14.0, 17.7) (technical details are in Appendix C).

### 3.5 Count/1s, a one-step approach for (log transformed) counts

Method count/1s, the counterpart of count/2s, is most commonly used in published studies on cross point analysis (Sørensen et al., 2001, 2004; Pedersen et al., 2005; Holm et al., 2007; Jensen and Pedersen, 2007). We return to the two lines per animal for the (log transformed) counts. It will be assumed that the distributions of the (log transformed) counts can be approximated by normal distributions. To account for the repeated measurements per animal for each resource, animal specific random effects were introduced into the model for each resource. The intercepts and slopes, say  $a_{i1}$  and  $b_{i1}$  for resource 1, and  $a_{i2}$  and  $b_{i2}$  for resource 2, for the  $i$ -th animal, can be denoted by:

$$\begin{aligned} a_{i1} &= a_1 + e_{a,i,1} & \text{and} & & b_{i1} &= b_1 + e_{b,i,1}, \\ a_{i2} &= a_2 + e_{a,i,2} & \text{and} & & b_{i2} &= b_2 + e_{b,i,2}. \end{aligned}$$

Here,  $a_1$ ,  $a_2$  and  $b_1$ ,  $b_2$  are the overall intercepts and slopes for the two resources, and  $e_{a,i,1}$ ,  $e_{a,i,2}$ ,  $e_{b,i,1}$  and  $e_{b,i,2}$  are animal specific random departures from normal distributions. This is a random coefficient model for (log transformed) counts for each resource, with separate variances and correlations for  $e_{a,i,1}$ ,  $e_{b,i,1}$  and  $e_{a,i,2}$ ,  $e_{b,i,2}$ . In addition, the model comprises correlations between animal specific random effects of the different resources, e.g. correlation between animal specific departures  $e_{b,i,1}$  and  $e_{b,i,2}$  from overall slopes  $b_1$  and  $b_2$ . A common residual variance was assumed for the scatter of the data points around the two lines of the individual animals.

Count/1s is an instance of a linear mixed model and can be fitted by restricted maximum likelihood (REML), which is a standard estimation procedure for linear mixed models (e.g. McCulloch, 2006). Standard software from e.g. GenStat or SAS can be used. The estimated overall intercept and slope were  $\hat{a}_1 = 4.2455$  and  $\hat{b}_1 = -0.1018$  for chopped hay, and  $\hat{a}_2 = 1.5634$  and  $\hat{b}_2 = 0.0719$  for long hay. The overall cross point, as solved from  $a_1 + b_1x = a_2 + b_2x$  is  $cp = -\Delta a / \Delta b$ , where  $\Delta a = a_1 - a_2$  and  $\Delta b = b_1 - b_2$  are the differences between the overall intercepts and slopes of the two resources respectively. The estimate for the overall cross point was  $\hat{cp} = -(4.2455 - 1.5634) / (-0.1018 - 0.0719) = -2.6820 / -0.1737 = 15.7$ . The P-value for comparing overall cross point  $cp$  with midpoint 21 was 0.002, and the 95% confidence interval for overall cross point  $cp$  was (13.5, 17.3). Both were based on a t-test. Details for inference about the overall cross point  $cp$  are basically the same as for prop/1s, replacing  $a$  and  $b$  in Appendix C by  $\Delta a$  and  $\Delta b$ .

## 4 Comparison of the four methods and discussion

### 4.1 Counts versus proportions

Two aspects of double demand data are of particular importance: (1) animals are tested repeatedly where they have access to the resources, but at different combinations of workloads, and (2) resources are offered simultaneously. The first aspect means that we have repeated measurements on the same animal. The second aspect means that an element of competition is involved between resources: at a given moment within a session an animal can only work for one resource at a time. The choice reflects the animal's preference and the workloads associated with the two resources. We have argued that the total number of rewards acquired by an animal in a session reflects its motivation and the joint appeal of the two resources (including associated workloads), but not the animal's preference for one resource relative to the other resource. Given the total number of rewards, preference is reflected by the proportions of rewards for the two resources. These proportions reflect the probabilities that an animal chooses one resource or the other at any given moment when it is motivated to work for a resource during the test session. Therefore, the concurrent schedule for the two resources is explicitly represented in the methods based on proportions, but only implicitly covered in the methods based upon (log transformed) counts. In count/1s competition between resources is implicitly covered in the model by correlations between animal specific random effects of the two resources, e.g. correlation between the two slopes of the same animal for the two resources. Should we have offered the resources separately, the model for count/1s would have been the same, although the estimates for the population parameters would have been different. In that sense there is a stronger mechanistic model element in the approaches based on proportions than in the approaches based on (log transformed) counts.

Count/1s comprises considerably more population parameters that have to be estimated than prop/1s. In prop/1s, by focussing upon proportions, we only need to model a single line per animal on the logit scale. By contrast, in count/1s we need to model two lines per animal for log transformed counts. With count/1s also the total number of rewards of the two resources per session is modelled, while these totals do not reflect an animal's preference for one resource relative to the other resource. Count/1s comprises 15 unknown population parameters (two overall intercepts, two overall slopes, two variances for intercepts, two variances for slopes, six correlations among all intercepts and slopes, and a common residual variance around the lines), while prop/1s comprises only six unknown population parameters (one overall intercept, one overall slope, one variance for intercepts, one variance for slopes, one



correlation between intercepts and slopes, and an overdispersion parameter).

Because of the relatively large number of population parameters, fitting count/1s to data may require some effort. Good starting values and proper control of the numerical iteration process for the estimation procedure (restricted maximum likelihood or REML) are important with a modest number of animals in the experiment. For instance, good starting values for variances of animal specific random effects for intercepts and slopes may be obtained by initially omitting all correlations from the model. In the iteration process, the difference between subsequent values of a population parameter (e.g. the slope of one of the lines) may be reduced.

## 4.2 Prop/1s versus prop/2s

With prop/2s, first individual cross points are calculated per animal, thus avoiding the need to model the repeated measurements per animal, and second, from the estimated individual cross points, an estimated overall cross point is derived with an associated confidence interval, and a significance test for preference in the target population. With prop/1s, repeated measurements per animal are modelled. In the second step of prop/2s, all individual cross points are on an equal footing, i.e. they carry the same weight in the subsequent analysis. By contrast, prop/1s that takes account of both between and within animal variation, implicitly covers for individual cross points that are estimated with different accuracy (see Appendix A). Therefore prop/1s is more efficient (more accurate) than prop/2s. Prop/1s is more efficient than prop/2s, but also more sensitive (less robust) to departures from model assumptions, such as normality and equal variance on the logit scale, and more computationally intensive. The greater efficiency of prop/1s may be outweighed by the greater simplicity and robustness of prop/2s, especially for a small number of animals. When individual cross points are accurately determined, i.e. fitted sigmoid curves closely follow observed proportions, we recommend the use of prop/2s, particularly when the experiment involves a modest number of animals, like the nine animals in the study of Webb et al. (2014b). Prop/2s offers direct insight into variation between animals, it is based on stable numerical procedures (logistic regression and Wilcoxon's signed rank test), and it is robust. When individual cross points are less accurately determined, but a sizeable number of animals are involved in the experiment, say 20 or more, prop/1s may be preferred, because of its greater efficiency. Note that when individual cross points are less accurately determined, it can be argued that the number of combinations of workloads per animal or the sum of workloads should be reconsidered.

### 4.3 Final remarks

The method for estimation that was used for prop/1s was PQL/IRREML/pseudo-likelihood (Schall, 1991; Breslow and Clayton, 1993; Wolfinger and O’connell, 1993; Engel and Keen, 1994). For certain data configurations, PQL/IRREML/pseudo-likelihood is known to underestimate components of variance for (overdispersed) binomial data (Engel, 1998). However, this is no problem for cross point double-demand data, as long as the total numbers of rewards for the two resources together are sizeable: generally larger than 10 and preferably larger than 20.

When there are additional experimental factors for animals that are of interest, such as gender, age or breed, these extra factors can be included in the model. For prop/2s, in the second step, Wilcoxon’s signed rank test or the t-test may be replaced by the Kruskal-Wallis test (Conover, 1980) or analysis of variance. Obviously, larger numbers of animals will be required for a reliable analysis. Additional effects may be included in prop/1s as well. For instance, for the calf data from Webb et al. (2014), we included main effects for the two series of sessions (with a resource either on the left or right hand side of the pen) and interaction with workload  $x$  (representing different slopes for the two series of sessions) in the model. Neither interactions, nor main effects, were significant (technical details are in Appendix A). Therefore effects for the two types of sessions were omitted from the analyses shown in the present paper.

Occasionally it may happen with prop/2s (and count/2s) that an estimated individual cross point is out of range, e.g. for the bull calf data from Webb et al. (2014b) an estimated individual cross point below 0 or above 42 may be problematic. This can occur when an animal consistently preferred one resource over the other within the experimental range of workloads. It may mean that the range of workloads or the training require reconsideration. Note that Wilcoxon’s test is quite robust in this respect: the difference with e.g. 21 corresponding to a deviant individual cross point simply gets the highest or lowest rank, but the actual difference itself is not used. The t-test is less robust, because it uses the actual value of the deviant individual cross point.

As outlined, both for theoretical and practical reasons, from the four approaches for analysis described in this paper, we recommend the use of the two approaches based on proportions: prop/1s and prop/2s. In particular, for a modest number of animals, and well determined individual cross points, we recommend prop/2s. For the data from Webb et al. (2014b) the four approaches yielded similar results. There are some technical arguments in Appendix D that suggest when this can be expected to happen. Programs for prop/1s and prop/2s, written in GenStat and SAS, are

available from the first author.

## Appendix: Technical details

### A. Details of the statistical model for prop/1s

In a session with workloads  $x$  and  $(42 - x)$  for chopped and long hay probability  $p_i$  for the  $i$ -th animal to work successfully for chopped hay follows from:

$$\text{logit}(p_i) = (a + e_{a,i}) + (b + e_{b,i})x.$$

Pairs  $e_{a,i}$ ,  $e_{b,i}$  for different individuals are independently sampled from normal distributions around 0, with variances  $\sigma_a^2$  and  $\sigma_b^2$ , and some correlation  $r_{ab}$  between them. Proportion  $y_i$  of rewards for chopped hay for workload  $x$  (and workload  $(42 - x)$  for resource 2) will have (conditional) mean  $p_i$  and variance  $\sigma^2 p_i (1 - p_i)/n_i$ . Here,  $n_i$  is the total number of rewards for the resources together, and  $\sigma^2$  is a dispersion parameter. This is a logistic random coefficients model for overdispersed binomial data. In this generalised linear mixed model  $\sigma^2 p_i (1 - p_i)/n_i$  replaces the residual variance of a conventional random coefficients regression model for normally distributed data. Note that a larger total  $n_i$  reduces the ‘residual variance’, i.e. the variation within animals, but not the variation between animals, as represented by random effects  $e_{a,i}$  and  $e_{b,i}$  for individual intercepts and slopes. More effects, e.g. for the two series of sessions with chopped hay either on the left or right hand side of the pen, may be added to the model. P-values for interactions and main effects were derived from an approximate F-test from Kenward and Roger (1997) (applied to the adjusted dependent variate of the last iteration of the iterated re-weighted REML algorithm) that is available in e.g. GenStat and SAS.

### B. Details of overall $cp$ for prop/1s and prop/2s

In prop/1s, an animal’s individual cross point is solved from:

$$\text{logit}(0.5) = 0 = (a + e_a) + (b + e_b)x \quad \text{as} \quad x = -(a + e_a)/(b + e_b).$$

Overall cross point  $cp$  is the median of individual cross points in the target population, so:

$$P(-(a + e_a)/(b + e_b) \leq cp) = 0.5.$$

This is the same as  $P(a + b \ cp + e_a + cp \ e_b \leq 0) = 0.5,$   
 implying that the median of  $(a + b \ cp + e_a + cp \ e_b)$  is 0.

However, assuming normality, or at least symmetry, for random effects  $e_a$  and  $e_b$ , this median is also  $a + b \ cp$ . So,  $a + b \ cp = 0$  or  $cp = -a/b$ , estimated by  $\hat{cp} = -\hat{a}/\hat{b}$ . For prop/2s, first individual cross points are estimated, and second, overall cross point  $cp$  is estimated by the median of these individual estimates. Although estimates for overall cross point  $cp$  differ between prop/1s and prop/2s, clearly both are estimates of the same population parameter.

### C. Details of the test and confidence interval for $cp$ for prop/1s

The estimated standard error of the estimator  $\hat{cp}$  for overall cross point  $cp$  follows from:

$$\sqrt{\frac{\hat{a}^2}{\hat{b}^4} s_b^2 + \frac{1}{\hat{b}^2} s_a^2 - 2 \frac{\alpha}{\hat{b}^3} s_{ab}},$$

where standard errors  $s_a$ ,  $s_b$  and covariance  $s_{ab}$  ( $= s_a s_b r_{ab}$ ) corresponding to estimated intercept  $\hat{a}$  and slope  $\hat{b}$  can be read from the output of e.g. GenStat or SAS. The distribution of  $\hat{cp}$  may be markedly skewed, and this standard error may not be very useful for testing or construction of a confidence interval. For a difference between overall cross point  $cp$  and midpoint 21, we test whether  $a + 21b = 0$ , rather than  $-a/b = 21$ , because a normal approximation can be expected to perform better for estimator  $\hat{a} + 21 \hat{b}$  than for  $-\hat{a}/\hat{b}$ . The test statistic is the ratio  $t$  of  $\hat{a} + 21\hat{b}$  and its standard error:

$$\frac{\hat{a} + 21\hat{b}}{\sqrt{s_a^2 + 441s_b^2 + 42s_{ab}}}.$$

When  $\text{abs}(t) > 1.96$  it is concluded (normal approximation, P-value  $< 0.05$ ) that cross point and midpoint 21 are significantly different. The test will be more conservative when 1.96 is replaced by the 97.5 percentile point of a t-distribution with  $(K - 1)$  degrees of freedom, where  $K$  is the number of animals in the experiment. For instance, with  $K = 9$  animals, 1.96 would be replaced by 2.31. When other values than 21 are

tested for  $cp$ , the non-rejected values (P-value < 0.05) constitute a 0.95 confidence region. The lower and upper confidence bound can be solved from:

$$\{(1.96)^2 s_b^2 - \hat{b}^2\}cp^2 + \{2(1.96)^2 s_{ab} - 2\hat{a}\hat{b}\}cp + \{(1.96)^2 s_a^2 - \hat{a}^2\} = 0.$$

In this paper, confidence intervals were calculated using 2.31 instead of 1.96. Writing the equation as  $A cp^2 + B cp + C = 0$ , lower and upper bound are:

$$cp_{low} = \frac{-B + \sqrt{(B^2 - 4AC)}}{2A} \quad \text{and} \quad cp_{up} = \frac{-B - \sqrt{(B^2 - 4AC)}}{2A}.$$

We need  $abs\left(\frac{\hat{b}}{s_b}\right) > 1.96$  (or 2.31) and  $(B^2 - 4AC) > 0$ , otherwise the confidence region will not be an interval. This may happen when information is sparse, i.e. too few animals and too much variation.

### D. Comparing count/1s and prop/1s

When counts  $y_1$  and  $y_2$  for the resources are assumed to follow Poisson distributions with means  $\mu_i$  ( $i = 1, 2$ ) given by  $\log(\mu_i) = a_i + b_i x$ , and (rather unrealistically) independence is assumed as well, conditioning upon the total count  $n = (y_1 + y_2)$  will lead to a logistic regression model for  $y_1$ , with probability  $p$  following from  $logit(p) = (a_2 - a_1) + (b_2 - b_1)x$ . Approximating  $\log(y_1)$  and  $\log(y_2)$  by normal distributions, the means as a function of  $x$  are approximately following two straight lines, but the variances are not stable but inversely proportional to the means  $\mu_i$ . It is perhaps a weakness of count/1s that the use of a conventional regression model with random coefficients requires (approximate) linearity, normality and homogeneity of variances. Although the initial independence assumption is unrealistic and (consequently) prop/1s may involve an additional dispersion parameter, this line of argument illustrates that count/1s and prop/1s are not completely equivalent. However, it also suggests that, as long as the assumption of equal variances is not markedly violated, the two approaches are likely to produce similar results for the overall cross point and associated confidence interval.



# Chapter 6

## Chopped or long roughage: what do calves prefer? Using cross point analysis of double demand functions

Laura Webb, Margit Bak Jensen, Bas Engel, Kees van Reenen, Walter Gerrits, Imke de Boer, Eddie Bokkers

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*“We identify a need as a state, which if not attained causes suffering to an animal as indexed by disturbed behaviour, an increased risk of pathology and/or a hormonal profile consistent with stress.” (Jensen and Toates, 1993)*

### Abstract

The present study aimed to quantify calves' (*Bos taurus*) preference for long versus chopped hay and straw, and hay versus straw, using cross point analysis of double demand functions, in a context where energy intake was not a limiting factor. Nine calves, fed milk replacer and concentrate, were trained to work for roughage rewards from two simultaneously available panels. The cost (number of muzzle presses) required on the panels varied in each session (left panel/right panel): 7/35, 14/28, 21/21, 28/14, 35/7. Demand functions were estimated from the proportion of rewards achieved on one panel relative to the total number of rewards achieved in one session. Cross points (*cp*) were calculated as the cost at which an equal number of rewards was achieved from both panels. The deviation of the *cp* from the midpoint (here 21) indicates the strength of the preference. Calves showed a preference for long versus chopped hay (*cp* = 14.5; *P* = 0.004), and for hay versus straw (*cp* = 38.9; *P* = 0.004), both of which improve rumen function. Long hay may stimulate chewing more than chopped hay, and the preference for hay versus straw could be related to hedonic characteristics. No preference was found for chopped versus long straw (*cp* = 20.8; *P* = 0.910). These results could be used to improve the welfare of calves in production systems; for example, in systems where calves are fed hay along with high energy concentrate, providing long hay instead of chopped could promote roughage intake, rumen development, and rumination.

## 1 Introduction

Foraging animals gather information about available resources at the expense of optimising immediate rate of energy gain (Forkman, 1991; Inglis et al., 2001). Ruminants have been found to trade-off between optimising rate of energy gain and minimising disadvantages to rumen function caused by the intake of high energy food, by including in their diets roughage high in fibre and low in energy (Cooper et al., 1995, 1996). This requires prior association between the sensory characteristics of feed and their post-ingestive consequences (Provenza, 1995). Ruminants spend extensive time feeding and ruminating. Mastication and rumination promote salivation, an important buffering agent in the rumen, and reduce feed particle size to enable passage of feed into the abomasum (Welch, 1982; González et al., 2012). As a consequence, ruminants have a high incentive to chew and ruminate (Redbo, 1990; Redbo and Nordblad, 1997), and they may sometimes show a preference for roughages that require long chewing times (Hughes and Duncan, 1988b). The latter is especially relevant in farmed ruminants fed high energy diets with little fibre as these animals develop abnormal oral behaviours due to limited opportunity to chew and ruminate (Veissier



et al., 1998; Webb et al., 2012, 2013). Abnormal behaviours occur in sub-optimal environments and are a sign of poor welfare in captive animals (Mason and Latham, 2004).

A method for investigating foraging behaviour in ruminants is to quantify the preferences for two simultaneously available feeds. Manipulating the particle length of roughage is an easy way to control the rate of energy gain, without affecting taste and smell. Compared to longer ones, smaller particles of roughage are ingested at a higher rate (Kenney et al., 1984; De Boever et al., 1990; Krause et al., 2002; Al-Saiady et al., 2010; Kammes and Allen, 2012), and pass faster/more easily through the reticulorumen (Wilson and Kennedy, 1996), resulting in an increased rate of energy gain. However, feeding only small amounts of small particles of roughage, as opposed to longer roughage particles, on top of a high concentrate diet, may lower ruminal pH in the long term, increasing the chances of developing acidosis (reviewed in González et al., 2012). These diets may also lead to ruminal plaque formation, i.e. a sticky mass of hairs and small feed particles between the papillae (Suárez et al., 2006), and ruminal hairball development (Webb et al., 2013). In addition, small roughage particles lead to less chewing and rumination than longer particles. Less chewing and rumination increases energy intake rate by decreasing ingestion and digestion effort, but these behaviours also stimulate saliva secretion, which is an important buffering agent in the rumen (reviewed in González et al., 2012). Ruminants were capable of making foraging choices that favour good rumen function by selecting a large portion of chopped roughage particles (30%) in their total diet, when chopped and ground roughages were offered together (Cooper et al., 1995, 1996). In previous studies, however, animals had to balance energy intake and good rumen function, because no other feed was provided besides roughage. If energy intake was taken out of the equation, by, for example, feeding high energy concentrate, ruminants are expected to prefer longer particles of roughage, as the need for good rumen function would then become more important than rate of energy gain.

Previous research investigating preferences for different particle lengths of roughage in ruminants used short-term (Kenney and Black, 1984; Kenney et al., 1984) or long-term (Cooper et al., 1995, 1996) choice tests. Providing freely available alternative resources and imposing no cost on preference, however, does not reflect foraging environments in the wild and does not quantify the strength of a given preference. Cross point analysis of demand functions, where two substitutable resources are presented simultaneously and the workload for each resource is varied relative to the other, includes a 'cost' on the choice and is suggested as a more accurate and biologically relevant method for quantifying preferences (Sørensen et al., 2004; Jensen and Pedersen, 2007). In this method, demand function refers to the linear regression between

rewards achieved and resource costs (Hursh, 1993). The cross point designates the combination of costs (one for each resource) at which an equal number of rewards is achieved for both resources. The cross point analysis of double demand functions enables quantification of preferences, and may be viewed as reflecting the natural foraging situation where food availability (cost) varies.

The present study aimed to quantify calves' preference for long versus chopped hay and straw, using double demand operant conditioning, in a context where energy intake was no limiting factor (i.e. feeding large quantities of milk replacer and concentrate). We hypothesised that calves would prefer long roughage particles over chopped because they value long chewing time and good rumen function. This presupposes that calves previously learnt post-ingestive consequences of different roughage types. Hay is associated with increased energy intake rate and better rumen function (Suárez et al., 2007), but decreased chewing time (Kenney and Black, 1984), compared to straw. Moreover, sensory characteristics, such as smell, taste or texture, may also affect the relative preference of hay and straw. The preference for hay and straw was also quantified in the present study.

## 2 Materials and methods

This study was carried out at Wageningen University's Animal Science Department experimental facilities, The Netherlands. The experiment ran from April to August 2012.

### 2.1 Ethics statement

All procedures met the terms of the Dutch law for animal experiments, which complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive), and were approved by Wageningen University's Committee on Animal Care and Use (DEC no.2012006).

### 2.2 Animals and husbandry

Nine 7-wk-old Holstein-Friesian bull calves (body weight mean  $\pm$  SEM:  $84.6 \pm 1.3$  kg) were purchased from one Dutch veal farm. Calves were individually housed for the first 4 wk after arrival at the veal farm (from 2 to 6 wk of age), and thereafter, housed in a large group of 95 male calves. On the veal farm, calves had access to brushes (for grooming), bouncy balls (for head butting), and rubber teats (for sucking and chewing). The calves were fed milk replacer, concentrate (400 g per calf) and a

small amount of chopped wheat straw (10 g per calf). The calves for the experiment were selected based on two criteria: similar size and no previous health treatment. At arrival at the experimental facilities, the nine calves were housed together in one 9.40 m × 2.45 m home pen with a slatted wooden floor. The home pen was fitted with two brushes (for grooming) and one plastic ball hanging from a chain for enrichment.

The calves received commercial milk replacer (18% crude protein and 18% crude fat) twice a day at 07:30 and 16:30 h in buckets with floating teats. Calves were also fed pelleted concentrate (17.5% crude protein, 37% starch, 24% NDF, based on 71% cereal and cereal by-products and 25% lupins as the main ingredients), which were provided once a day in the milk buckets immediately after the milk was consumed during the afternoon feeding. All calves finished their milk meal within 10 min. Calves were restrained during milk feeding to prevent them from ingesting other calves' milk. The daily allowance of milk replacer and concentrate corresponded to ad libitum intakes of these feeds in similar age calves in a previous study, where milk replacer, concentrate, maize silage, hay and barley straw were offered ad libitum (Webb et al., 2014a). The allowance of milk replacer ranged from 10.0 L/d at 7 wk of age to 15.6 L/d (122 g DM/L) at 5 months of age, while the allowance of concentrate ranged from 0.3 kg/d at 7 wk of age to 2.7 kg/d at 5 months of age (Fig.6.1). The choice of the feeding strategy (milk fed twice a day and concentrate fed only at night) enabled control of intake before testing

In the home pen, calves were offered one of five roughages: chopped barley straw, long barley straw, chopped grass hay, long grass hay (straw: 3.1% crude protein and 79% NDF; hay: 9.2% crude protein and 59% NDF), and chopped Lucerne hay mixed with 8% cane molasses and linseed oil (molashine, Gedizo Trading Int.). Chopped roughage particles were 2-3 cm, while long particles were unprocessed and around 20-30 cm. These particle lengths were chosen as providing the largest possible variation in length, with the smaller length reflecting what is commonly fed to fattening calves. The five roughages were offered one after the other in order to familiarise the calves with sensory and post-ingestive information associated with each roughage type. This familiarisation was done for three consecutive days per roughage type (i.e. 15 d of familiarisation in total starting the day after arrival), offered ad libitum. After this initial familiarisation period, calves only received roughage (i.e. long and chopped hay and straw) in the home pen during days with no training or days with no testing. During the training period, which lasted a total of 6 wk, calves were not brought into the operant pen during the weekend, i.e. there were 2 d/wk without training. During the testing period, which also lasted 6 wk, the Sundays were used for habituation to the new roughage types on a low workload, i.e. there was 1 d/wk without testing (see subsection 'Testing calves' below). All test-roughages (i.e. all except Lucerne hay)

were offered in the home pen each weekend. Roughage intake in the home pen during familiarisation and during days without training or testing was recorded.

Milk and concentrate refusals in the home pen were weighed daily. Milk refusals only occurred once (on the day of arrival at the experimental facilities). Concentrate refusals were less than 5% of provision, on average, throughout the study. The calves received water ad libitum via two drinking nipples. Lights were on between 07:00 and 22:00 h. Temperature was regulated with a heater and mechanical ventilation, and ranged from 14.4 to 26.1°C. Relative humidity ranged from 50.6 to 97.1%. A radio was turned on during the day in an attempt to maintain constant ambient background noise. In the week after arrival, calves were blood sampled for haemoglobin (Hb) and serum iron (SeFe) analysis in order to ensure that they were not anaemic: (mean  $\pm$  SEM) Hb = 6.8  $\pm$  0.1 mmol/L and SeFe = 36.3  $\pm$  3.2 mol/L. Given these values, calves were not given extra iron.

In order to test the equipment and develop a training protocol for the calves in this study, a pilot study was conducted using three calves prior to the present study.

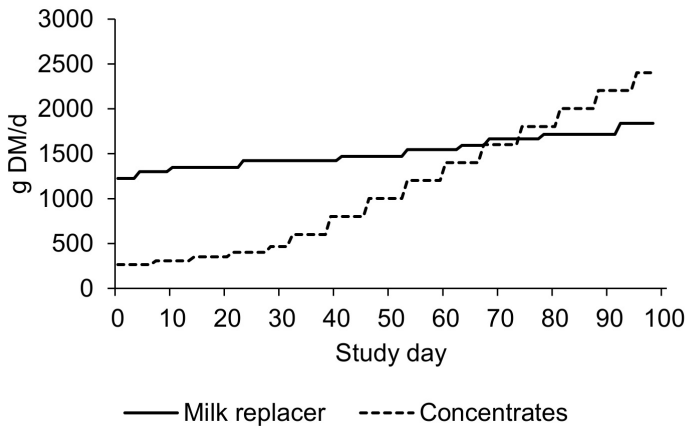


Figure 6.1: Milk replacer and concentrate feeding. Feeding schedule for milk replacer and concentrate in g DM per day per calf. Milk replacer was fed in two meals per day at 07:30 and 16:30 h, whereas concentrate were fed only at 16:30 h. Milk replacer and concentrate were fed in buckets, with floating teats for the milk.

### 2.3 Training calves on double demand operant conditioning

The test pen (2.35 m × 2.45 m) was immediately adjacent to the home pen, fitted with a wooden slatted floor and black plastic walls (1.45 m high), and accessible from the home pen through a door. Calves could, therefore, be walked from the home pen, through the door, into the test pen. On the wall opposite the door were two panels (24 cm × 20 cm) and two buckets (33 cm diameter). The two buckets were located between the two panels. Each bucket was 17 cm away from the corresponding panel, and the distance between the two buckets was 53 cm. The panels were raised 60 cm above the floor and the bottom of the buckets were raised 46 cm above the floor. Above the buckets were cylindrical automated feed delivery systems with a flap that opened to release roughage rewards into the buckets, via a computer that recorded the number of successful presses made on the panels. The left panel and bucket were associated to each other, in such a way that the correct number of presses on the left panel would result in the delivery of a roughage reward into the left bucket. The same applied to the right panel and right bucket. When panels were active, that is when the computer system was switched on, panels were lit with white led lamps. Each successful press made to an active panel was rewarded with a bell sound. When a reward was delivered, an alarm sound was played and the lights in both panels went off for 500 ms.

The nine calves were randomly assigned to groups of three, and randomly assigned to a working order within each group. During the entire experiment, including habituation, shaping, training and testing, calves were always placed in the test pen in the same order so that they could form expectations as to when they would be given the opportunity to work for roughage. One section of the home pen, adjacent to the test pen, could be closed off and formed a 'waiting room' (2.35 m × 2.45 m). To avoid disturbing all calves every time a new calf was collected for testing, calves were placed in the waiting room in their groups of three and remained there until all three calves had visited the test pen. Calves were first habituated to the test pen in their groups of three for 10 and 30 min. They were then habituated to the test pen individually for 10 and 20 min. Each calf visited the test pen once per day. During all habituation sessions, except the last two, the panels were inactive, meaning that the lights in the panels were off and a muzzle press resulted in neither sound nor reward. In the last two habituation sessions, the panels were active in order for calves to habituate to the lights in the panels. One muzzle press resulted in reward delivery.

During shaping and training, the reward was 10 g of Lucerne hay. During shaping, one panel and corresponding bucket were blocked off with a barrier, and calves could only access one panel and its corresponding bucket. Calves were rewarded for the

following behaviours in the following sequence: approach the panel, sniff the panel from any angle, sniff the top of the panel, touch the top of the panel with the muzzle, and press the panel. When calves successfully learnt to press the panel to gain access to a reward, they were shaped on the other side. The side made accessible first was balanced for each group of calves.

Once calves were shaped on both panels, the fixed ratio (**FR**), i.e. number of presses required for one reward, was increased to two (FR2). After this, the barrier was removed and calves were trained on both panels, which were accessible simultaneously, on FR2. Subsequently, the FR on both panels was gradually increased, maintaining the same FR on both panels, until FR10. Finally, the difference in FR between the two panels was gradually increased until calves could be trained on the five FR pairs used during testing: (Left/right panel) 7/35, 14/28, 21/21, 28/14, 35/7. Training ended when all calves worked economically, i.e. accessed over 60% of rewards from the panel with the lowest FR. At this stage, calves were 15 wk old. Training sessions lasted a minimum of 30 min, but no maximum duration was imposed on the calves. This was done to enable all calves to work at their own individual speed and to access the number of rewards that they were motivated to get. Training sessions were ended when the calves had received no rewards for 3 min, after the initial 30 min. Training sessions lasted 39 min on average. For testing sessions, the minimum session time was reduced to 20 min, but again no maximum session time was imposed. When calves did not receive a reward for 3 min between 20 and 40 min in the test pen, or when calves walked away from the panels after 40 min in the test pen, the session was ended. Testing sessions lasted 39 min on average. Therefore, changing the criteria used during training did not affect average session duration.

## 2.4 Testing calves

Calves' preference for three combinations of roughage types was tested, each combination was tested for 2 wk: 1) chopped hay versus long hay, 2) chopped straw versus long straw, 3) chopped hay versus chopped straw. Each week comprised of one day of habituation with FR7 on both panels (to allow calves to familiarise themselves with the two roughage types and the location of each type) and five testing days (one day per FR pair: (Left/right panel) 7/35, 14/28, 21/21, 28/14, 35/7 presented in a random order). The 2 wk with the same combination were repetitions of each other, but the location of the two roughage types was switched in order to control for any pre-existing side bias. The first two combinations of roughage types, which both investigated preference for different particle lengths, were presented in a cross-over design, with half the calves starting with chopped versus long hay and the other half

starting with chopped versus long straw. After this, calves' preference for hay versus straw (both chopped) was tested in all calves. During testing of chopped versus long roughage, the reward size was 5 g, whereas during the testing of hay versus straw, the reward size was 8 g. The reward size was increased in an attempt to reduce test session duration and to take into account the increased age of the calves. If calves did not consume all rewards, refusals were weighed at the end of the session and noted for each roughage type. The number of rewards used in the analysis was based on consumed rewards (number of rewards delivered minus number of rewards not consumed).

## 2.5 Post-mortem measurements

In order to check for any underlying health issues that may have affected the preferences of calves for different types of roughage, post-mortem health measurements were collected. At 6 months, all calves were slaughtered in a small slaughter house and routine Welfare Quality® post-mortem measurements were carried out (Welfare Quality, 2009). Respiratory and gastrointestinal health measurements were made on all calves. Pneumonia was scored from 0 to 3 based on damaged area on the lungs, and presence of pleuritis was noted. Plaque and hyperkeratosis in the rumen, as well as lesions in the torus pylorus and pylorus areas of the abomasum were noted as present or absent. Rumen development was scored from 1: low to 4: full. A rumen score was calculated as the median of the rumen scores on the 9 rumens. Damage from abomasal lesions of  $< 0.5 \text{ cm}^2$  (category 1),  $0.5\text{-}1.0 \text{ cm}^2$  (category 2), and  $> 1.0 \text{ cm}^2$  (category 3), were scored from 0 (absent) to 4 based on the number present. An abomasal lesion score was calculated for each calf as the sum of the lesion number, multiplied by the lesion category. The median of these scores was then calculated.

## 2.6 Data analysis

The response variable was the proportion of rewards of one resource over the total number of rewards achieved for both resources within a session. This choice for a response variable differs from previous studies using cross point analysis of double demand functions, which generally used (logarithms of) reward counts (Sørensen et al., 2001, 2004; Pedersen et al., 2005; Holm et al., 2007; Jensen and Pedersen, 2007). We suggest that using proportions is more appropriate, as it takes into account the dependence between two simultaneously presented resources. A two-step approach was followed where (1) a model was fitted to the data of each individual animal and individual cross points were estimated, and (2) these individual cross points were

compared to the midpoint. The midpoint in the present study was 21, i.e. the point where the FR values for the two resources were the same.

The two-step approach circumvented the need for modelling a dependence structure between proportions of the same animal over different sessions (resulting from repeated measures design). The model fitted to the data per animal was a generalised linear model (**GLM**) (McCullagh and Nelder, 1989) with a logit link, the variance was specified as a multiple of the binomial variance function, and FR (of the chopped reward or of the hay reward, depending on whether particle lengths or roughage sources were compared) was introduced as an explanatory variable. Individual cross points corresponded to the values of FR where the expected proportion  $p = 0.5$  and differed across animals. Individual cross points were calculated as:  $cp = -\alpha/\beta$ , where  $\alpha$  and  $\beta$  are an animal's estimated intercept and slope on the logit scale. The overall cross point was defined as the median of the cross points of all animals in the target population and estimated by the median of the individual cross point of the animals in the experiment. The overall cross point was compared to the midpoint (i.e. 21) using Wilcoxon's signed rank test, applied to the differences between the individual cross points and the midpoint, and an associated 0.95-confidence interval for the overall cross point was constructed.

In order to demonstrate the meaning of 'cross point' when using proportions instead of counts, a graphical representation, plotting predicted proportions of chopped hay rewards against FR for chopped hay, is shown for calf no.2 (Fig.6.2). The curves fitted by proportions are sigmoid, and the curve for long hay is the opposite ( $1 - p$ ) of the curve for chopped hay ( $p$ ). The cross point corresponds to the point where  $p = 0.5$ , which in this figure is illustrated by the intersection between the two curves (Fig.6.2).

P-values lower than 0.05 were considered significant. Calculations were conducted using SAS version 9.2 (SAS Institute Inc., 2008) and Genstat version 15 (VSN-International, 2012).

### 3 Results

At the end of the study, calves weighed  $248.4 \pm 5.9$  kg on average, with an average daily gain of  $1.5 \pm 0.1$  kg/d. Roughage intake in the home pen during the weekend is shown in Table 6.1.



Table 6.1: Roughage intake in the home pen (mean  $\pm$  SEM g/d).

Period	Age (wk)	Chopped hay	Long hay	Chopped straw	Long straw	Lucerne hay
Start <sup>1</sup>	7-9	106 $\pm$ 22	216 $\pm$ 12	83 $\pm$ 12	93 $\pm$ 9	366 $\pm$ 41
Training <sup>2</sup>	9-15	362 $\pm$ 49	355 $\pm$ 55	266 $\pm$ 32	142 $\pm$ 17	
Testing <sup>3</sup>	15-21	505 $\pm$ 55	423 $\pm$ 56	238 $\pm$ 84	316 $\pm$ 30	

<sup>1</sup> Roughage was provided ad libitum during the habituation period, one roughage type at a time.

<sup>2</sup> Roughage was provided ad libitum, one roughage type at a time (2 d per week without training).

<sup>3</sup> Roughage was provided ad libitum, two roughage types at a time (1 d per week without testing). The two types of roughage provided were from the same source but had different particle lengths.

Table 6.2: Cross points of individual calves for each comparison, including training.

Calf	Training	Chopped vs. long hay	Chopped vs. long straw	Hay vs. straw
1	18.5	14.2	6.7	30.8
2	25.1	17.1	22.2	33.8
3	22.7	18.9	22.5	27.5
4	23.2	13.8	21.6	42.3
5	18.6	12.2	19.9	33.5
6	20.8	17.9	20.8	38.9
7	25.9	14.5	6.8	41.4
8	17.0	14.3	30.9	117.4
9	21.7	19.3	20.7	46.1
Median	21.7	14.5	20.8	38.9
Confidence interval	18.9-23.9	14.0-18.0	13.8-25.4 <sup>1</sup>	32.3-42.0 <sup>2</sup>
P-value	0.734	0.004	0.910	0.004

<sup>1</sup> Note that the confidence interval here includes 21.0 and is wide, indicating a large variation between individual calves and a difficulty in drawing conclusions on this particular comparison.

<sup>2</sup> Note that 42.0 is the largest value that the upper bound can take, since large values would correspond to negative values for 42.0 -  $x$  for the other resource.

Table 6.3: Total median number of rewards achieved (and total grams).

Comparison	FR	Median	Q1 <sup>3</sup>	Q3 <sup>3</sup>
Chopped vs. long hay <sup>1</sup>	7-35	57.0 (285)	45.0	86.5
	14-28	27.5 (138)	22.0	45.0
	21-21	26.0 (130)	15.0	42.0
	28-14	49.0 (245)	25.0	58.0
	35-7	81.5 (408)	45.0	100.8
Chopped vs. long straw <sup>1</sup>	7-35	22.0 (110)	11.0	43.0
	14-28	19.5 (98)	12.0	25.0
	21-21	17.0 (85)	10.0	24.0
	28-14	15.5 (78)	10.0	24.0
	35-7	31.0 (155)	17.0	52.0
Hay vs. straw <sup>2</sup>	7-35	79.7 (638)	60.6	105.8
	14-28	46.0 (368)	31.7	78.0
	21-21	28.1 (225)	19.0	36.0
	28-14	24.0 (192)	16.4	33.8
	35-7	18.7 (150)	14.6	31.6

<sup>1</sup> Reward size was 5 g.<sup>2</sup> Reward size was 8 g.<sup>3</sup> 1<sup>st</sup> and 3<sup>rd</sup> quartile for the median.

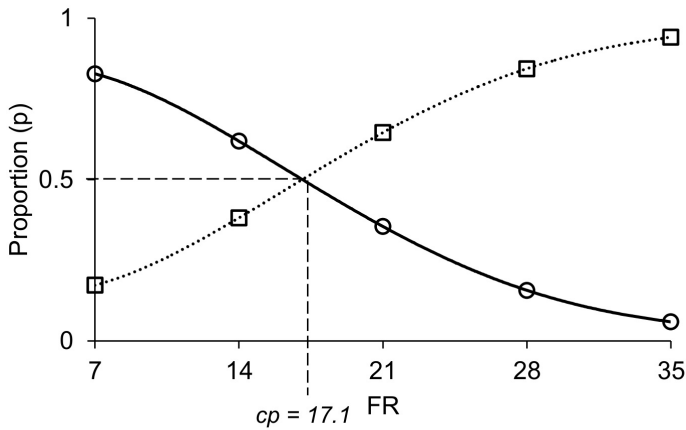


Figure 6.2: Cross point analysis illustrated. Graphical representation of the cross point ( $cp$ ) of calf no.2 for the comparison chopped hay (circles) versus long hay (squares) using proportions ( $p$ ) of chopped hay rewards over total number of rewards. The proportions for long hay rewards were calculated as  $1 - p$ . The x-axis shows fixed ratio (FR) values for the chopped hay (the long hay fixed ratio values are  $42 - FR$ ). The lines connecting the points are 4th order polynomials.

### 3.1 Double demand and cross points

The nine calves used in the present study were successfully trained to work economically on two panels delivering the same roughage reward (i.e. Lucerne hay), in that they consistently chose the panel with the lowest workload more often than the other panel (Table 6.2). Moreover, all calves were motivated to work for both hay and straw rewards throughout the study, despite high milk replacer and concentrate provision in the home pen (Table 6.3).

Calves showed a preference for long hay over chopped hay, indicated by an overall median cross point below the midpoint 21 and different from the midpoint (Table 6.2). The overall cross point for the comparison chopped straw versus long straw was not different from the midpoint (Table 6.2). However, the confidence interval was wide, indicating large variation between individuals, and three calves seemed to have expressed a preference for chopped straw (calves no.1 and 7) or long straw (calf no.8) (Table 6.2). Calves showed a preference for chopped hay over chopped straw, indicated by an overall cross point higher than the midpoint, and different from the midpoint (Table 6.2). The cross point, i.e. 38.9, is higher than 35, which is the highest

FR that was imposed in the present study, indicating that calves always achieved more hay rewards than straw rewards regardless of the cost. Median number of rewards consumed during one session was highest for the comparisons including hay rewards, and higher when the preferred resource was available at a low price for the comparison chopped versus long hay, and hay versus straw, i.e. comparisons where one resource was preferred over the other (Table 6.2).

### 3.2 Post-mortem results

The calves in the present study had no overt health problems during the experiment. The results of the post-mortem gastrointestinal and respiratory health measurements showed no severe pneumonia, no rumen hyperkeratinisation, and relatively good rumen development (rumen development score [median] = 3.0). The median abomasal lesion score was 4.0 and was close to that found in European veal farms with large numbers of animals (Brscic et al., 2011).

## 4 Discussion

The main aim of this study was to investigate the preferences of calves for different roughage particle lengths. Relative preference was quantified using a double demand operant conditioning paradigm. Double demand operant conditioning has previously been applied to rats (Sørensen et al., 2001, 2004; Holm et al., 2007), chickens (McAdie et al., 1993), pigs (Pedersen et al., 2005; Jensen and Pedersen, 2007), and adult cattle (Matthews and Temple, 1979), but we could not find a study applying the double demand approach to calves. The methodology used to train the calves in the present study took 6 wk in total, starting with 9 wk-old calves (training started 2 wk after the arrival of the calves, the first 2 wk being used to familiarise calves to the roughages used during training and testing). The results showed that calves fed a high energy diet were willing to work for extra roughage rewards, including Lucerne hay, good quality hay and barley straw. The calves adjusted their efforts on the two panels according to their respective price such that when the two panels yielded the same roughage (Lucerne hay), they obtained more rewards from the panel with the lowest cost in all sessions. Calves expressed their preferences when two different rewards were available. It was possible to quantify the strength of preferences via the deviation of the cross point from the midpoint. This is clearly seen when comparing the deviations found for the preference of long hay over chopped hay (deviation of 6.5 from the midpoint) and the preference of hay over straw (deviation of 17.9 from the midpoint). This suggests that the preference of hay over straw is stronger than

that of long hay over chopped hay in calves. Hay differs from straw in a number of ways apart from structure, as it contains more energy (Kenney and Black, 1984), has a different flavour (Provenza et al., 1996) and is thought to have a beneficial influence on rumen function (Suárez et al., 2007). Due to increased fermentation, hay should lead to better papillae development (Suárez et al., 2007). However, this effect may be minimal in this study because of the high level of concentrate fed. The cross point for the comparison of hay versus straw is above 35, which is the highest cost imposed on resources in the present study. This indicates that for this comparison, the range of costs did not include a large enough difference in values. However, the results obtained do seem to confirm the hypothesis that hay is a preferred roughage compared to straw, even when energy is no limiting factor.

The statistical method used in this paper for cross point analysis of double demands, differs from methods used in previous studies (Sørensen et al., 2001, 2004; Pedersen et al., 2005; Holm et al., 2007; Jensen and Pedersen, 2007). The presently applied method considers three aspects in the analysis of double demand functions. First, the dependence between data for the two resources offered simultaneously is included by using proportions as a response variable. Second, individual variation is expressed in an accessible and clear manner, and looking at individual cross points offers a clear picture of variation in preferences across animals (also shown in Sørensen et al., 2001, 2004). Third, the analysis is robust, that is, not critically dependent upon complex model assumptions, and the use of Wilcoxon's signed rank test offers a conceptually and computationally straightforward statistical method.

Calves did not consistently prefer the roughage associated to the shortest ingestion and digestion time, i.e. chopped roughage; they did show a preference for long hay over chopped hay, but no preference was apparent for either long straw or chopped straw. Calves in this study were fed a high energy diet, consisting of milk replacer and concentrate, between testing sessions. It was, therefore, expected that these calves would not necessarily show a preference for the roughage permitting the best rate of energy gain. Furthermore, calves did not 'abandon' the panel with the highest workload. This was the case when both panels provided the same reward, as well as when the 'cheap' panel delivered the preferred reward. Contrafreeloading describes the concept that animals work for food when the same food is simultaneously freely available (Osborne, 1977; Inglis et al., 1997; Inglis, 2000; Inglis et al., 2001). Although the food in the present study was never 'free', it was sometimes very 'cheap'. Therefore, the animals displayed something very close to contrafreeloading, that we could term contracheaploading, and which most likely stems from the same motivations. Previous studies using double demand also observed this behaviour in their animals (Pedersen et al., 2005; Holm et al., 2007). Contracheaploading in double demand

operant set-ups most likely signals information gathering from various available resources, just like contrafreeloading (Information primacy theory, e.g. Inglis, 2000) and could be an indication of animals' adaptation to a changing environment, e.g. the depletion of the highest quality food patch (Inglis et al., 1997; Inglis, 2000; Inglis et al., 2001). In nature, food patches used by animals will deplete over time, and gathering information about alternative patches may increase survival over the long term. In the present set-up the relative cost of the two resources were alternated between daily sessions and thus there was a high level of uncertainty, which is hypothesised to increase contrafreeloading (Inglis et al., 2001). In other contexts, contrafreeloading could be an indication of animals' need to express appetitive behaviour (Hughes and Duncan, 1988b). However, since calves had to work for all roughage resources, this is an unlikely explanation in the present set-up.

The preference for long hay found in the present study could be explained in two non-mutually-exclusive manners. First, calves may have preferred long hay because it required more chewing, and calves may have a high motivation for performing this behaviour (Hughes and Duncan, 1988b). The calves may have perceived the long hay portion as being larger than the chopped hay portion, through increased eating time (De Boever et al., 1990), increased rumen fill (suggested in Kammes and Allen, 2012), and slower clearance rate of the reticulorumen (Wilson and Kennedy, 1996). Long hay also most likely increased rumination as a post-ingestive consequence (Heinrichs, 2005; Webb et al., 2013). During the habituation period and in the home pens on days without training or testing, calves were fed each roughage type on separate occasions, which is assumed to have been sufficient for calves to learn post-ingestive consequences of all roughage types, including consequences for rumination (e.g. Kyriazakis et al., 1998).

Second, calves may have preferred long hay because it resulted in improved rumen function compared to the chopped hay, given that calves were indeed aware of post-ingestive consequences of each particle length. Longer particles of roughage take longer to chew and ruminate before the particle length is sufficiently reduced to move from the reticulorumen to the abomasum, and increased rumination increases salivation (e.g. De Boever et al., 1990; González et al., 2012). Saliva secretion increases the buffering capacity of rumen fluid (e.g. De Boever et al., 1990; González et al., 2012), and prolonged presence of roughage particles in the rumen improves rumen motility and stimulates the removal of ingested hair and small feed particles from the rumen papillae (Morisse et al., 1999). This is especially important in calves fed large quantities of concentrate, and for which access to roughage is restricted. Therefore, longer roughage particles improve rumen muscularisation, papillae development, and rumen osmolality and pH (e.g. Krause et al., 2002; Al-Saiady et al., 2010), while

preventing hairball and plaque development (Morisse et al., 1999; Suárez et al., 2006; Webb et al., 2013).

Interestingly, calves showed a preference for long over chopped roughage for hay but not for straw. Given the large variation between calves found in the comparison of chopped versus long straw (illustrated by the 95% confidence interval), it is difficult to conclude on this particular result. It is possible that with a larger sample of animals, a preference for one of the straws would have been observed. Straw is a coarse and low quality roughage with low energy and high fibre content, resulting in a low rate of energy gain (Kenney and Black, 1984). Preference for shorter particles of straw was found to be stronger compared to preference for shorter particles of high quality roughage (such as hay) in sheep (Kenney and Black, 1984). Therefore, ruminants may show preferences for different structures, even with low quality roughages. In our study, given the high energy feeding strategy provided outside of testing, calves were expected to show a preference for longer particles. Since this preference was not found for straw, we can only speculate that long straw was associated with some sort of cost that outweighed the benefits, and that this cost was not present, or present to a lesser extent in long hay. A possible cost could be worse abomasal damage (Mattiello et al., 2002). Abomasal damage, i.e. lesions on the abomasal wall, could result from a combination of three factors: a) overfilling of the abomasum because of large milk meals causing local loss of blood supply of the abomasal wall (ischaemia), b) exacerbation of this damage from poorly digested feed particles coming from a poorly developed rumen, and c) exacerbation of this damage by coarse feed stuffs (Breukink et al., 1991; Berends et al., 2012b; Webb et al., 2013).

The post-mortem health measurements were carried out in the present study to check whether calves were healthy, and whether any underlying health problems could have explained any of the preferences. The feeding strategy combined with possibility to work for roughage in the operant pen aimed to permit a good growth, and this was successfully achieved. Looking at the numbers, rumen development seemed better than that found in European veal calves, but abomasal damage appeared comparable (Brscic et al., 2011). Similar abomasal damage could indicate that milk feeding was an important factor in causing abomasal damage (Breukink et al., 1991), or that the improvement in rumen development was insufficient to minimise abomasal damage in the current study (Berends et al., 2012b). The infrequent feeding of large amounts of milk replacer in the present study may have caused the observed abomasal damage (Breukink et al., 1991) (and could have further caused other physiological problems, such as for example insulin resistance (Vicari et al., 2008; Bach et al., 2013), although this is not thought to have affected the results in any way). It is not known how abomasal damage may affect the preference for long or chopped particles of roughage.

Despite these potential health issues, this feeding strategy was chosen to enable good control of milk intake (in terms of amount and time) before testing, in order to reduce inter- and intra-calf variation.

## 5 Conclusions

The present findings showed that 2-5 month old calves can learn a double demand operant set-up and are motivated to work for roughage in addition to a high energy diet comprising of milk replacer and concentrate. Overall, calves preferred long particles of hay, but not straw, compared to chopped, and calves had a strong preference for chopped hay over chopped straw. These findings support the idea that ruminants are able to make choices based on rumen function and possibly also based on their motivation to chew and ruminate. These findings could be used to improve the welfare of calves in production systems: farmed calves fed high energy diets alongside hay might benefit (e.g. in terms of rumen function) from being offered long hay instead of chopped hay.



# Chapter 7

## Does temperament affect learning in calves?

Laura Webb, Kees van Reenen, Margit Bak Jensen, Océane Schmitt, Eddie Bokkers

Submitted

*“The study of individual variation has an illustrious history, forming one of the cornerstones of Darwin’s theory of evolution by natural selection.”* (Hayes and Jenkins, 1997)

### Abstract

Understanding how temperamental traits affect learning ability in animals can help to shape training schedules to individual requirements and minimise drop outs (so-called ‘non-performers’). This is of particular importance when training and subsequent learning is required of captive animals to assess aspects of their preferences, and when these preferences are used to devise novel management procedures to improve their welfare. The relationship between temperament and learning has, to our knowledge, never been studied in calves. Two-month-old Holstein-Friesian bull calves ( $N = 9$ ) were used in this study. Hypothesised temperament variables were recorded in four challenge tests: novel object (NOT), novel environment (NET), social isolation, (SIT), and social isolation with a novel environmental cue (SI/E). Hypothesised learning variables were recorded during training on a double demand operant conditioning set-up, where two panels with varying workloads (i.e. number of presses: (left/right panel) 7/35, 14/28, 21/21, 28/14, 35/7) delivered Lucerne hay (1 delivery = 10 g). Principal Component Analysis (PCA) was conducted on temperament variables on the one hand, and learning variables on the other hand. Principal Components hypothesised to reflect underlying temperamental traits (T) and learning ability (L) were extracted from these two PCAs. Spearman’s rank correlations were carried out to find relationships between Ps and Ls. For four of the Ps and two of the Ls the explained variance was more than 10%. The four Ps were hypothesised to reflect fearfulness, activity, exploration, and attention towards the environment, and these were consistent with previous studies using larger numbers of calves. The two Ls were hypothesised to reflect feed motivation and working speed. Three correlations were found between Ps and Ls suggesting a relationship between hypothesised temperamental traits and learning ability in calves.

## 1 Introduction

Darwin’s theory of evolution by natural selection was founded on individual variation: “... differences ... in the individuals of the same species inhabiting the same confined locality” (Darwin, 1859). Differences between individuals in terms of their behavioural response to challenging situations have been especially studied, not only in humans but also in various non-human animal species (e.g. Kagan et al., 1988; Fujita et al., 1994; Van Reenen et al., 2004; Bolhuis et al., 2005). These behavioural responses have been aggregated into so-called ‘temperamental traits’; namely, stable, consistent underlying phenotypes, or causal factors, mediating distinct behavioural reactions (Boissy, 1995; Jensen, 1995; Koolhaas et al., 2007; Van Reenen et al., 2013). Research in rodents, pigs and cattle has exposed the multidimensional nature of these

underlying traits, with responses being characterised along two axes (or more): fearfulness (or emotional reactivity, or timidity) and coping style (Courvoisier et al., 1996; Ramos et al., 1997; Andersen et al., 2000; Van Reenen et al., 2004; Koolhaas et al., 2007). Fearfulness is described by Boissy (1995) as “a personality or temperament trait defining the general susceptibility of an individual to react to a variety of potentially threatening situations”. Coping styles are described as “alternative response patterns in reaction to a stressor” (Koolhaas et al., 2007) and as the two extremities of a continuum related to flexibility in behaviour (Benus et al., 1991). Behavioural flexibility is defined by Coppens et al. (2010) as “the ability of an individual to directly respond and adjust its behaviour to environmental stimuli”. Coping styles seem mediated by central nervous system, neuroendocrine and physiological mechanisms (e.g. Hessing et al., 1994; Koolhaas et al., 2007). These different traits can further be explained by their ultimate function in wild animals: ensuring the existence of alternative strategies in, for example, foraging (e.g. specialists versus generalists), social (e.g. dominance or dispersion) and reproductive (e.g. male-male aggression) behaviours, and the success of different strategies may depend on the stability of spatial and temporal variation in environmental conditions (Bekoff, 1977; Searle et al., 2010).

The effect of temperamental traits on learning have mainly been studied in rodents (Benus et al., 1987, 1990; Fujita et al., 1994; Teskey et al., 1998) and horses (Haag et al., 1980; Heird et al., 1981, 1986; Marinier and Alexander, 1994; Le Scolan et al., 1997; Visser et al., 2003a). These studies suggest that underlying fearfulness or coping style may affect learning (Benus et al., 1987, 1990; Teskey et al., 1998; Bolhuis et al., 2004). In this paper we consider how individual differences (or temperament) among animals relate to their learning ability in a double demand operant set-up. While studying animal preferences, usually in the context of improving captive animal welfare, cross point analysis of double demand function offers the most adequate method (Sørensen et al., 2004; Pedersen et al., 2005; Webb et al., 2014b). Improving calf welfare via the development of novel feeding strategies necessitates the assessment of feed preferences, hence the use of double demand operant set-ups. However, this test is rather complex and ‘non-performers’, i.e. animals that seem to fail to learn a task (Teskey et al., 1998; Visser et al., 2003a), with potentially specific preferences, require additional training time (and passed studies may have excluded these animals altogether). It is, therefore, important to understand how individual differences affect learning ability in this type of test. This study evaluated the effects of temperament on learning ability in calves being trained on a double demand paradigm.

## 2 Materials and methods

### 2.1 Animals

A detailed description of training calves on a double demand operant set-up is given in Webb et al. (2014b). In brief, 7-wk-old Holstein-Friesian bull calves ( $N = 9$ ;  $84.6 \pm 1.3$  kg) were obtained from a Dutch veal farm and housed together in one pen ( $9.40 \text{ m} \times 2.45 \text{ m}$ ) with wooden slatted floors, two brushes (for grooming) and a plastic ball hanging from a chain (for head-butting). The calves received milk replacer (MR) in buckets with floating teats, twice a day (07:30 and 16:30 h), and concentrate once a day (16:45 h). MR allowance per calf gradually increased from 1225 at 7 wk to 1544 g DM at 15 wk of age. Concentrate allowance per calf gradually increased from 300 at 7 wk to 1363 g DM at 15 wk of age. MR and concentrate provisions were based on calf consumption in a study with ad libitum access to different feed components (Webb et al., 2014a). Feeding times and methods were chosen to enable control over feed intake before calves were tested. The first 2 wk after arrival and in the weekend, calves were offered ad libitum access to roughage (one type at a time), either chopped Lucerne hay mixed with 8% cane molasses and linseed oil (molashine, Gedizo Trading Int.), barley straw or hay. Water was offered ad libitum via drinking nipples. Artificial lighting was switched on between 07:00 and 22:00 h. Temperature and relative humidity ranges were  $16.5\text{-}23.9^\circ\text{C}$  and  $50.6\text{-}97.1\%$ . Constant background noise was sustained via a radio, switched on during the day between 07:30 and 17:00 h.

### 2.2 Test pen

A test pen ( $2.35 \text{ m} \times 2.45 \text{ m}$ , wooden slatted floor, black opaque walls) was directly adjacent to the home pen, with a single door through which calves could be gently directed from the home pen into the test pen. On the far wall of the test pen were two panels and two corresponding buckets. The buckets were situated between the panels. Cylindrical feed delivery systems with claps were positioned over the buckets. The panels and feed delivery systems were connected to a computer. When the test pen system was switched on, the correct number of nose presses to a panel (signalled by a bell sound) would result in the automatic delivery of a feed reward into the corresponding bucket (signalled by an alarm sound).

### 2.3 Assessing temperamental traits

‘Challenge’ tests were conducted to unravel consistent individual variation between calves in their behavioural response to novelty and stress: novel object test (**NOT**), novel environment test (**NET**), social isolation test (**SIT**), and social isolation test with a change in the environment (**SI/E**). These tests were performed in the aforementioned order for all calves. Moreover, all tests were video recorded and behaviours were observed from the videos using focal animal sampling and continuous recording (The Observer XT version 10.1, Noldus Information Technology, Wageningen, the Netherlands).

The NOT was conducted in a section of the home pen that could be closed off from the rest of the pen (henceforth referred to as ‘waiting pen’). During NOT, calves that were not being tested were held at the opposite end of the home pen. This test was, therefore, performed in a familiar environment, whilst visual contact with pen mates was possible, in order to minimise potential effects of environment novelty and social isolation. Calves were randomly selected to enter the waiting pen and tested for a duration of 5 min. The novel object, an orange cone (height = 22.5 cm, diameter = 13.5 cm) hanging from the fence, was thrown into the pen after an initial period of 1 min.

The NET, SIT and SI/E were performed in the test pen. The calves were randomly allocated to a group of three calves and randomly given a testing order within each group. This order was maintained throughout the experiment for NET, SIT, SI/E and training on a double demand operant conditioning set-up. One after the other, each group of calves was put into the waiting pen until all calves within the group had been tested. This was done to avoid disturbing all calves every time a new calf was walked to the test pen. NET was carried out with all calves within a group entering the pen together, to remove any effect of social isolation. As a consequence, animals within a group could not be considered independent for NET. NET lasted a total of 10 min. For SIT and SI/E, individual calves were walked to the test pen and left there for 10 min and 20 min respectively. For SI/E, a familiar barrier was placed in the test pen, blocking one panel and corresponding bucket (side randomised for each group). All calves initially walked to this barrier to explore it and subsequently moved to the accessible bucket, where a free reward was available. The latency to move away from the barrier was recorded. During SIT and SI/E, the test pen system was switched on, and each press on any of the panels resulted in the delivery of 10 g of Lucerne hay.

Behavioural variables recorded during these challenge tests are described in Table 7.1. These behavioural variables were selected based on previous research investigating temperament in calves (Van Reenen et al., 2004, 2005, 2009), and were hypoth-

Table 7.1: Medians, minimums and maximums for behavioural variables hypothesised to relate to temperament and learning.

Variables	Median	Min	Max
<b>Hypothesised temperament variables</b>			
<i>Novel object test</i>			
Latency to touch object (s)	34.0	7.0	300.0
Walk (% of time)	13.3	10.4	33.0
In contact with object (% of time)	5.9	0.0	81.5
<i>Novel environment test</i>			
Walk (% of time)	12.1	5.1	17.5
Explore (% of time)	59.5	10.4	74.7
<i>Social isolation test</i>			
Walk (% of time)	10.8	5.5	17.3
Explore (% of time)	42.4	11.5	48.0
<i>Social isolation with change in environment</i>			
Walk (% of time)	11.6	4.6	21.3
Explore (% of time)	56.9	34.3	67.4
Latency to move away from barrier (s)	20.0	4.0	93.0
<b>Hypothesised learning variables</b>			
Deviation from midpoint	2.4	0.2	4.9
Latency to eat first reward (s)	46.4	22.8	62.0
Presses per minute	9.1	6.7	13.4
Panel switch proportion	0.40	0.22	0.50
No. of rewards	24.8	13.6	54.2
Session duration (s)	2358.2	1877.4	3097.2
Latency to do forced choice (s)	53.0	17.0	76.0

esised to reflect aspects of temperament. Therefore, behavioural variables recorded in challenge test are hereafter referred to as ‘temperament variables’. The latency to touch the novel object and time in contact with the novel object are variables thought to be related to fearfulness, whereas duration of walk in all tests was thought to reflect activity, and possibly underlying coping style (Van Reenen et al., 2005). Reactive copers are believed to explore the environment more and be more aware of environmental cues (Benus et al., 1987, 1990), therefore, variables relating to exploration and latency to move away from a novel environmental aspect (a barrier in SI/E) were also expected to be associated to coping style.

## 2.4 Assessing learning ability

After the challenge tests were performed, calves were trained for 6 wk on a double demand operant conditioning set-up, and their learning ability was assessed. Each calf was placed inside the test pen once a day. During shaping, there was a barrier blocking one of the panels and associated bucket. During shaping, calves were rewarded for the following behaviours in the given order: approach panel, sniff panel, sniff top of panel, touch top of panel with muzzle, press panel with muzzle. When calves could press the panel, the barrier was moved to the familiar panel, and calves were shaped on the other panel. After this, the number of presses required for reward delivery (i.e. the fixed ratio of presses to reward, **FR**) was increased to two (FR2). Then the barrier was removed and FR2 was imposed on both panels. At this stage, a 'forced choice' was imposed on the calves: after the delivery of the first reward, the corresponding panel was rendered inactive and calves were forced to obtain a reward from the other panel before both panels were active once more. When calves consistently pressed the panel for a reward on FR2, the number of presses on both panels was gradually increased to FR10. Finally, the number of presses required on both panels was varied until the following FR pairs were achieved: (Left/right panel) 7/35, 14/28, 21/21, 28/14, 35/7. Training was concluded when calves could achieve over 60% of their rewards from the panel with the lowest number of presses, reflecting that they worked 'economically'. Training sessions lasted at least 30 min, with no imposed maximum duration, enabling calves to continue working as long as they were motivated to do so. This resulted in training sessions lasting on average 39 min.

To evaluate how well calves learned the double demand operant conditioning task, cross point analysis (Sørensen et al., 2001, 2004; Holm et al., 2007; Jensen and Pedersen, 2007) was used to calculate calves' individual cross point, i.e. workload at which 50% of the total number of rewards achieved within one session were acquired from each of the two panels (Webb et al., 2014b). The midpoint is the number of presses for which both panels have an equal workload, here 21. Because the rewards on both panels are the same, i.e. 10 g Lucerne hay, perfect economic working (i.e. consistently accessing most rewards from the panel with the lowest workload, and accessing an equal number of rewards from both panels when workload is the same on both panel) should result in the cross point being equal to the midpoint, i.e. calves obtained an equal number of rewards from both panels when both panels had the same workload. The extent to which individual calves' cross points deviated from the midpoint was calculated, as described in Webb et al. (2014b).

Deviation from the midpoint and a number of other variables collected during training were selected for analysis of learning ability. Because these behavioural vari-

ables were hypothesised to reflect learning ability they are henceforth referred to as ‘learning variables’. Learning variables included: response rate (presses per min, from first press to last press for first 10 rewards), total number of rewards acquired during a session, total session time, and panel switch proportion (proportion of rewards within first 10 rewards that were not from the same panel as the first reward acquired). These four variables were assessed in the fourth week of training. The first three of these variables likely reflect motivation, i.e. how motivated calves were to access feed rewards. The fourth variable likely reflects behavioural flexibility (some calves would return to the panel which had the lowest workload on the previous day and stay there for a long period of time; personal observation). Additionally, two learning variables were derived from behaviour during the learning of the ‘forced choice’ task: latency between first and second reward (excluding eating time), and duration of eating the first reward. The first of these variables likely reflects a further measure of behavioural flexibility (i.e. how fast would calves learn to move to the other panel, when the panel they were originally working on became ‘inactive’), while the second was included as an extra measure of feeding motivation.

## 2.5 Statistical analysis

Duration (latency) data collected during challenge tests were expressed as a proportion of total testing time (e.g. proportion of test time gone by before calf touched novel object). To achieve approximate normality, latency data and count data were log transformed and proportion data were logit transformed. Behavioural variables recorded during challenge tests (i.e. temperament variables,  $N = 10$ ) and behavioural variables recorded during training (i.e. learning variables,  $N = 7$ ) were first separately analysed by Principal Component Analysis (**PCA**), to summarise correlated variables into principal components (**PC**). PCs were subjected to varimax rotation. High loadings were considered to be loadings higher or equal to 0.5. PCs derived from the PCAs were then analysed for correlations between temperament and learning ‘traits’ (PCs are hypothesised to reflect underlying factors mediating behavioural variables) using Spearman’s rank correlations. PCAs were carried out in GenStat (VSN-International, 2012), whilst correlations were carried out in SAS (SAS Institute Inc., 2008) using the PROC CORR routine.

## 3 Results

Summary statistics for the temperament and learning variables are described in Table 7.1. Results of the PCA on temperament variables showed that the first 4 PCs



Table 7.2: Loadings for behavioural variables recorded during challenge tests, or hypothesised ‘temperament variables’, on the first five principal components (T), and eigenvalues and percentages of explained variation for each T. High loadings (i.e. > 0.50) are in bold.

Variables	T1	T2	T3	T4	T5
Latency to touch object in NOT <sup>1</sup> (s)	<b>0.98</b>	0.04	0.00	-0.07	-0.17
Walk during NOT (% of time)	0.31	0.01	0.20	-0.19	<b>-0.89</b>
In contact with object in NOT (% of time)	<b>-0.93</b>	0.09	0.03	0.08	0.21
Walk during NET <sup>1</sup> (% of time)	-0.21	-0.33	<b>0.90</b>	0.09	-0.09
Explore during NET (% of time)	-0.47	<b>0.74</b>	-0.01	0.34	-0.23
Walk during SIT <sup>1</sup> (% of time)	<b>0.73</b>	0.18	<b>0.65</b>	-0.04	0.00
Explore during SIT (% of time)	0.20	<b>0.91</b>	-0.13	-0.27	0.13
Walk during SI/E <sup>1</sup> (% of time)	0.31	-0.04	<b>0.79</b>	-0.41	-0.30
Explore during SI/E (% of time)	-0.01	<b>0.66</b>	-0.39	<b>0.60</b>	-0.07
Latency to move away from barrier in SI/E (s)	-0.09	-0.06	-0.03	<b>0.96</b>	0.20
Eigenvalues	3.88	2.39	1.42	1.25	0.75
Variance explained (%)	39	24	14	13	7

<sup>1</sup> NOT = novel object test; NET = novel environment test; SIT = social isolation test; SI/E = social isolation with change in environment.

(referred to as T, for temperament) had an eigenvalue above 1 and accounted for 90% of the total variation (Table 7.2). T1 had high positive loadings on latency to touch the novel object and proportion of walk in SIT, and a high negative loading on percentage of time in contact with the novel object. T2 had high positive loadings on percentage of time displaying exploration during NET, SIT and SI/E, whereas T3 had high positive loadings on percentage of time walking during NET, SIT and SI/E. T4 had high positive loadings on percentage of time exploring and latency to walk away from the barrier during SI/E. Finally, T5 had a high negative loading on a single variable: percentage of time walking during NOT (Table 7.2).

Results of the PCA on learning variables showed that the first two PCs (referred to as L, for learning) had an eigenvalue above 1 and accounted for 82% of the total variation (Table 7.3). L1 had high positive loadings on presses per minute, number of rewards and session duration. L2 had a high positive loading on latency to finish first reward during forced choice and a high negative loading on presses per minute. L3, L4 and L5 each loaded high on a single variable: L3 had a high negative loading on deviation from the midpoint, L4 had a high negative loading on panel switch proportion, and L5 had a high positive loading on latency to do forced choice.

When temperament and learning variables or PCs were analysed for correlations, two negative correlations and one positive correlation emerged: 1) T3 tended to be

Table 7.3: Loadings for behavioural variables recorded during training, or hypothesised ‘learning variables’ on the first two principal components (L), and eigenvalues and percentages of explained variation for each L. High loadings (i.e.  $> 0.50$ ) are in bold.

Variables	L1	L2	L3	L4	L5
Deviation from midpoint	0.22	-0.12	<b>-0.94</b>	0.10	-0.19
Latency to eat first reward (s)	-0.03	<b>0.96</b>	0.06	-0.25	-0.05
Presses per minute	<b>0.56</b>	<b>-0.70</b>	-0.27	0.22	-0.26
Panel switch proportion	-0.31	0.41	0.13	<b>-0.85</b>	0.04
No. of rewards	<b>0.74</b>	-0.36	-0.38	0.30	-0.30
Session duration (s)	<b>0.88</b>	-0.03	-0.17	0.22	-0.38
Latency to do forced choice (s)	-0.40	0.02	0.23	-0.03	<b>0.89</b>
Eigenvalues	4.31	1.40	0.62	0.42	0.22
Variance explained (%)	62	20	9	6	3

negatively correlated with L1 ( $\beta = -0.60$ ,  $P = 0.088$ ); 2) T5 was negatively correlated with L3 ( $\beta = -0.68$ ,  $P = 0.042$ ); and 3) T5 was positively correlated with L5 ( $\beta = 0.67$ ,  $P = 0.050$ ).

## 4 Discussion

The present study confirms the multifactorial quality of calves’ behavioural response to challenging situations (Van Reenen et al., 2004). As mentioned in the introduction, temperament may be reflected in distinct ‘axes’. Our results showed five distinct axes regarding temperament. T1 is hypothesised to reflect underlying fearfulness for novel objects (Van Reenen et al., 2005, 2009), T2 is hypothesised to reflect exploratory motivation (Jensen et al., 1997; Graunke et al., 2013), T3 is hypothesised to reflect activity or coping style (Andersen et al., 2000; Van Reenen et al., 2005), while T4 may possibly reflect attention for environmental change. An increase in exploration following a change in the environment was previously reported in calves (De Passillé et al., 1995). This is separate from response to novelty, as calves in the present study were familiar with the barrier before it was introduced to the test pen. T5 only loaded high on walk during NOT, and is separate from hypothesised fear for novel objects, exploration and activity. Van Reenen et al. (2004) found this measure difficult to classify as it loaded high on different PCs in calves at different ages: i.e. ‘interaction with novel object’ and ‘vocalisations’ (3 wk), or ‘locomotion’ (16 and 29 wk). Fearfulness and coping style have been previously suggested as stable, consistent temperamental

traits in rodents, pigs and cattle (Courvoisier et al., 1996; Ramos et al., 1997; Andersen et al., 2000; Van Reenen et al., 2004; Koolhaas et al., 2007; Van Reenen et al., 2013). Coping styles have been linked to measures of activity in the past (Van Reenen et al., 2005), such as our T3, and have been suggested to be linked to exploration and attention towards the environment in challenge tests: 1) proactive pigs showed rapid superficial exploration compared to reactive pigs, which explore more gradually and intensively (Hessing et al., 1994); 2) rodents showing more routine-like behaviour, higher levels of activity and aggression were less influenced by environmental change (Benus et al., 1987, 1990; Teskey et al., 1998). However, in the present study, activity, exploration and attention for environmental change are suggested to be designated by three separate PCs, and are, therefore, suggested as independent mediators for behavioural response. We found fearfulness (T1, recorded in the presence of a novel object) and exploration (T2, recorded in a novel environment) to be accounted for by two different PCs suggesting that fear and exploration belong to two independent motivational systems, which is in concordance with Jensen et al. (1997).

Challenge tests are commonly used to investigate individual differences. In these tests, different potentially stressful phenomena may occur simultaneously, making it impossible to uncover actual causes of stress and subsequent behavioural responses (Boissy, 1995). A novel object test, for example, is often conducted in a novel arena during social isolation. The behavioural response of the animal could, consequently, be attributed to the novel object, novel environment, or social isolation, or to a combination of all three (Forkman et al., 2007). The present study used modified versions of commonly used challenge tests to avoid accumulation of stress factors, demonstrating that such modified tests can still uncover underlying temperamental traits in calves. Making challenge tests more specific to particular temperamental traits, in order to facilitate interpretation, is in line with recommendations proposed by Forkman et al. (2007).

Our results showed five distinct axes regarding hypothesised learning abilities, however, only two explained a substantial percentage of variance. L1 (62% of variance) and L2 (20% of variance) are hypothesised to reflect underlying feed motivation and working speed. L3 was hypothesised to reflect economic work, while L4 and L5 seem to both reflect hypothesised behavioural flexibility in that they represented switching between panels and latency to do the forced choice. Feed motivation (Marinier and Alexander, 1994; Visser et al., 2003b), working speed (Bokkers and Koene, 2002; Bokkers et al., 2004) and behavioural flexibility (Coppens et al., 2010; Melotti et al., 2013) have all been discussed in the context of learning ability in animals. However, feed motivation and behavioural flexibility tend to be seen as animal characteristics, whereas working speed is seen as a learning criteria. Our results suggest that feed mo-

tivation and behavioural flexibility do not affect working speed or economic work, as these were designated by separate PCs. A lack of a relationship between feed motivation and learning was previously suggested in horses (Visser et al., 2003b). Previous work on pigs by Melotti et al. (2013) found two strategies in a delay discounting task with two panels (small or large reward), carried out to measure impulsivity in the face of uncertainty (uncertain delay to delivery of large reward): i.e. Switching (switch between panels) and Omitting (focus on large reward). We seemed to have observed similar strategies in calves, which is why we included measures of ‘flexibility’ in the learning variables, i.e. switching between panels and latency to do forced choice. However, these two measures were designated by two separate PCs, hence not related to each other. Further studies might consider underlying learning ability rather than considering single learning variables (e.g. number of errors or trials until reach criteria), as is often the case (Fiske and Potter, 1979; Heird et al., 1981; Benus et al., 1990).

Three correlations between hypothesised temperament and learning traits were found. It appears that calves that walked more during NOT worked more economically and did the forced choice more rapidly than calves that walked less during NOT. It is unclear which underlying temperamental trait was mediating locomotion during NOT, consistent with previous work on calves (Van Reenen et al., 2004), and further research is encouraged to study this question including a larger number of animals. In addition, calves that had higher locomotion during the challenge tests in the test pen (or higher levels of activity, or possibly a proactive coping style) seemed to have a higher motivation for feed, or be more perseverant in the training phase. Measures of behavioural flexibility were not associated to hypothesised coping style (or activity) in this study, a finding both consistent (Melotti et al., 2013) and inconsistent (Bolhuis et al., 2004) with previous work on pigs. Moreover, the present study did not find that hypothesised fearfulness was related to aspects of learning ability in the calves. This finding is inconsistent with previous research suggesting that ‘emotionality’, handling experience (fear of humans), or presence of peers (sociality) affected learning ability, with more fearful animals exhibiting lower performances (Fiske and Potter, 1979; Heird et al., 1986; Boissy and Le Neindre, 1990; Marinier and Alexander, 1994; Le Scolan et al., 1997; Visser et al., 2003a).

The present study is the first to suggest that a relationship between temperament and learning exists in calves, and future studies will need to be carried out on greater numbers of calves to confirm this finding.

# Chapter 8

## Can tongue playing in veal calves be linked to cortisol or temperament?

Laura Webb, Kees van Reenen, Bas Engel, Harma Berends, Walter Gerrits, Eddie Bokkers

Submitted

*“Like a scar, a stereotype tells us something about past events.”* (Mason, 1991)

### Abstract

Stereotypies are often used as indicators of poor welfare in applied animal behaviour research, and it is, therefore, important to understand the underlying factors mediating the development of such behaviours. Stereotypies in captive animals have been previously related to chronic stress due to a sub-optimal environment and to temperamental traits. The aim of this study was to find whether individual levels of tongue playing in calves are related to cortisol responsiveness (a possible measure of chronic stress) and hypothesised measures of temperament. Eight-wk-old Holstein-Friesian bull calves ( $N = 48$ ) from a veal farm were group housed (3 calves per pen) and fed one of four different solid feed amounts (mixture of 25% maize silage, 25% wheat straw and 50% concentrate, on DM basis) alongside milk replacer (adjusted for similar growth). At 14-16 wk of age, calves were moved to and tethered inside metabolic cages (restraint) for a period of 10 days. Salivary cortisol was recorded before moving, and at +40, +80, +120 min and +48 h relative to tethering inside the cages. Behavioural response to restraint was recorded in the first 30 min using focal animal sampling and continuous recording, and these elements were entered into a Principal Component Analysis (PCA), to extract Principal Components (PC) (i.e. possible underlying factors). Tongue playing and abnormal oral behaviour (AOB) levels were recorded in the home pens in the 2 wk prior to and following restraint, using instantaneous scan sampling. Regression analyses were performed using a linear mixed model to investigate relationships between tongue playing and cortisol measures, tongue playing and PCs, and PCs and cortisol measures. PCA output supported the previously proposed multidimensionality of temperament in calves, with the first two PCs hypothesised to reflect underlying fearfulness (due to a relationship with cortisol response to stress) and activity. No relationship was found between tongue playing in the home pen and cortisol measures, suggesting this behaviour is not a good indicator of chronic stress at the individual level. Individual levels of tongue playing observed in the home pens were related to PCs hypothesised to reflect: 1) activity, and 2) propensity to perform tongue playing during challenge. This study supports the idea that stereotypies develop differently in individual animals, potentially as a result of differences in coping style.

## 1 Introduction

Abnormal stereotypies generally develop in sub-optimal environments in animals experiencing 'frustration' (Duncan and Wood-Gush, 1972), 'chronic stress' (Wiepkema, 1987) or 'boredom' (Wemelsfelder, 1993) over extended periods of time (Mason, 1991a). These behaviours are thought to be motivated (driven) by the 'need' to

perform specific natural behaviours, which are prevented in their full form in captive environments (Mason, 1991a; Rushen et al., 1993). For example, a limited opportunity to eat and ruminate in veal calves leads to the development of abnormal oral behaviours (**AOB**), including tongue playing/rolling and excessive, repetitive oral manipulation of the pen structure (Veissier et al., 1998). Tongue playing is often described as an oral stereotypy in cattle (Wiepkema, 1987; Redbo, 1998).

Establishing the relationship between stereotypies and chronic stress is difficult for two reasons: 1) measuring chronic stress is complex (Mormède et al., 2007), and 2) the relationship between stereotypic behaviour performance and physiological measures of chronic stress is not clear-cut (Ladewig et al., 1993).

The immediate physiological response to acute stress, most commonly investigated through changes in secretion of stress hormones (cortisol or adrenocorticotrophic hormone [**ACTH**]) mediated by the hypothalamic-pituitary-adrenal (**HPA**) axis, is relatively standardised across stressful contexts (Mormède et al., 2007). Inversely, physiological changes in animals exposed to chronic stressors are much more complex and difficult to measure and interpret (Mormède et al., 2007). In cattle, chronic stress can lead to both lower basal cortisol (Fisher et al., 1997; Van Reenen et al., 2000) and changes in the secretory pattern of cortisol (Ladewig and Smidt, 1989). Moreover, chronic stress was associated to both a blunting of the cortisol peak (tethered bulls: Ladewig and Smidt, 1989; reduced space allowance in beef heifers: Fisher et al., 1997; social isolation in calves: Van Reenen et al., 2000) and a higher cortisol peak in response to an ACTH challenge in cattle (tethered bulls: Friend et al., 1985, continuous regrouping of calves: Veissier et al., 2001). These differences most likely stem from differences in the timing of the measurement in relation to the stressor: it is thought that initially the HPA axis will become over sensitive, but then will adapt and become desensitised (Mormède et al., 2007). Because of the complexity in measuring chronic stress physiologically, it is more valuable to combine behavioural, health, performance and physiological indicators of chronic stress to get a better understanding of this process (Broom, 1986; Mormède et al., 2007).

The relationship between stereotypies and physiological measures of chronic stress is also unclear, as past studies have found both similar or lower basal cortisol (Van Reenen et al., 2001) or ACTH levels (Redbo, 1998) in stereotyping animals compared to non-stereotyping animals (Ladewig et al., 1993). In addition, a blunting of the cortisol peak in response to ACTH injection was found in stereotyping heifers (Redbo, 1998).

Stereotypies develop differently in individuals, in terms of form and frequency (Wiepkema, 1987). One hypothesis is that stereotypy development may depend on so-called 'temperamental traits' (Mason, 1991b). Temperament is now accepted as being multidimensional, and one temperamental trait in particular, namely coping

style, has been proposed as an important factor determining the propensity to develop stereotypic behaviour (Mason, 1991b). Koolhaas et al. (2007) describe coping styles as “alternative response patterns in reaction to a stressor”, and these are thought to be linked to behavioural flexibility, routine formation and attention towards environmental change (Benus et al., 1987, 1990; Bolhuis et al., 2004). The idea is that ‘proactive’ animals are more likely to develop high levels of stereotypies due to a higher inclination for routine formation, whereas ‘reactive’ animals in similarly poor conditions would perform lower levels of these behaviours (Mason, 1991b). In contrast, Redbo (1998) found that high stereotyping calves showed behavioural responses to challenge characteristic of the reactive coping style: namely low locomotion and high exploration. No further studies could be found in calves relating stereotypies to coping style. The success of these coping strategies in the wild depends on the environmental stability (Searle et al., 2010). However, there is some indication that developing stereotypies in sub-optimal captive settings may have a calming effect resulting in reduced levels of chronic stress (Mason and Latham, 2004).

The aim of this study was to establish whether tongue playing in veal calves is linked to physiological measures of chronic stress and temperamental traits. In order to attempt to achieve variation in individual calves’ level of tongue playing and, potentially, level of chronic stress, calves were fed different amounts of solid feed (including one group receiving no solid feed supplement). Chronic stress and temperament were assessed using the cortisol response and immediate behavioural response to a challenge. These measures were assumed to reflect chronic stress and temperament. This challenge was: moving to, and tethering inside a metabolic cage (henceforth referred to as restraint). Finally, restraint was extended (10 days) in order to model further chronic stress (in addition to that resulting from the feeding strategy) and look at effects of chronic stress on changes in tongue playing and AOB levels in the home pen.

## 2 Materials and methods

This study was conducted at the experimental facilities of Wageningen University, The Netherlands. All procedures met the terms of the Dutch law for animal experiments, which complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and was approved by the Wageningen University Committee on Animal Care and Use. The present study was part of a larger study (Berends et al., 2012a) which ran from March to August 2010.



## 2.1 Animals and husbandry

A detailed description of general husbandry procedures is provided in Berends et al. (2012a). In summary, 48 Holstein-Friesian bull calves ( $7.6 \pm 0.1$  wk of age;  $54.7 \pm 0.3$  kg body weight [BW]) were purchased from a Dutch veal farm. Calves were housed in groups of three in  $2.35 \text{ m} \times 2.45 \text{ m}$  pens with wooden-slatted floors. The barn was mechanically ventilated and lit by daylight and TL-lamps (06:00 to 23:00 h). Calves were assigned to one of four blocks (balanced based on BW) and within blocks randomly allocated to one of four solid feed treatments (0, 9, 18 or 27 g dry matter [DM]/kg of  $\text{BW}^{0.75}$  per day, Table 8.1). Each block, thus, comprised of four pens, one per solid feed treatment. Milk replacer (MR) allowance was adjusted per block in such a way that all pens within one block had similar average daily gains. This part of the methods was done based on the aims of the main study, which required each block to reach 108 kg BW in a staggered fashion. The solid feed was a mixture comprising of 25% chopped wheat straw, 25% maize silage and 50% concentrate, on DM basis. Solid feed provision was adjusted to BW on a weekly basis. All calves received MR. During the first study week, calves were fed according to the feeding regime of the commercial farm. From the second week onwards, calves were fed according to their given treatment. Calves were fed their allowance of MR and solid feed twice daily in open buckets (without teats) at 07:00 and 16:00 h, and were fixed during MR drinking. Water was offered ad libitum via nipple drinkers.

## 2.2 Restraint period

When calves were aged 14 to 18 wk they were subjected to a 10 d restraint period. MR schemes were adapted so that each block reached their respective restraint period at a similar BW, based on the aims of the main study (Berends et al., 2012a). During restraint periods, MR schemes were identical for all solid feed treatments (37.3 g DM/kg of  $\text{BW}^{0.75}$  per day). In order to reach these identical MR allowances (and intakes) for all treatments, the MR schedules were gradually modified in the 2 wk prior to restraint. This resulted in calves with a high MR allowance (i.e. low solid feed allowance) experiencing a decrease in their MR allowance, and calves with a low MR allowance experiencing an increase in their MR allowance in the week prior to restraint. Two adjacent pens from the same block (these groups of 6 calves are henceforth referred to as 'restraint group') were individually walked to a room adjacent to the home pen room. Calves were then individually placed inside metabolic cages ( $1.11 \text{ m} \times 0.79 \text{ m} \times 1.85 \text{ m}$ ) and tethered with a collar and chain. They were fitted with harnesses for the collection of faeces and urine (according to the aims of the main study). Calves remained in these cages for 5 d, during which they maintained visual

Table 8.1: Mean ( $\pm$  SEM) solid feed and milk replacer provision (g DM/d) for each solid feed treatment (given in g DM per kg of BW<sup>0.75</sup>) (Webb et al., 2012).

	Weeks of age		
	8-11	12-15	16-19
<i>Solid feed</i>			
0	0	0	0
9	242.6 $\pm$ 4.4	285.8 $\pm$ 5.6	347.2 $\pm$ 6.0
18	492.2 $\pm$ 7.3	578.0 $\pm$ 12.2	696.0 $\pm$ 11.0
27	742.9 $\pm$ 12.8	881.3 $\pm$ 18.8	1047.4 $\pm$ 16.7
<i>Milk replacer</i>			
0	1125.1 $\pm$ 18.9	1267.5 $\pm$ 13.9	1674.0 $\pm$ 21.0
9	1045.3 $\pm$ 16.5	1167.8 $\pm$ 10.9	1494.8 $\pm$ 20.5
18	961.6 $\pm$ 14.9	1080.0 $\pm$ 12.3	1310.9 $\pm$ 21.4
27	926.9 $\pm$ 12.5	1014.8 $\pm$ 13.9	1168.6 $\pm$ 24.7

and auditory contact with the other calves within the same restraint group. Average temperature and relative humidity in the metabolic cage room were: 18.5  $\pm$  1.2°C, 61.0  $\pm$  1.1%. After 5 d, calves were moved to other metabolic cages placed within respiratory chambers in groups of three (calves from the same home pen were housed together in the chambers and maintained visual and auditory contact with each other). Calves were weighed before and after their stay in the respiratory chambers. They remained in these chambers for an additional 5 d before returning to their home pens. TL-lamps in the respiratory chambers were switched on between 05:45 and 23:00 h. A small lamp was on at night. The temperature was maintained at 18°C, relative humidity at 65% and air velocity at <0.2 m/s. During the restraint period, the bags collecting faeces were changed and the trays collecting urine were emptied twice a day.

## 2.3 Behavioural observations

Direct and indirect (video) observations were carried out. These two observation methods were done by two separate observers, using The Observer XT (Noldus Information Technology, Wageningen, the Netherlands).

Direct behavioural observations were carried out in the home pens using instantaneous scan sampling at a 5 min interval for 30 min every 2 h from 06:00 to 19:00 h once a week, in the 2 wk prior to and following restraint. During these observations, AOB (i.e. tongue rolling/playing, oral manipulation of environment, and grazing of

Table 8.2: Ethogram for indirect (video) observations of immediate behavioural response of calves (N = 48) to restraint inside metabolic cages at 14-18 wk of age, using focal animal sampling and continuous recording.

Behaviour	Definition
Sniff	Muzzle close to any aspect of cage
Idle	No body movement for more than 3 seconds
Contact cage	Muzzle in contact with any aspect of cage
Look around	Head moving from side to side, with no other body movement
Idle with ear movement	No body movement except for ears for more than 3 seconds
Tongue play	Tongue extending and turned outside of mouth
Walk	Any movement of limbs
Hang on chain <sup>1</sup>	Chain fully extended for more than 3 seconds
Rapid locomotion	Rapid movement of all four limbs. With each change in direction (back or forth) a new event is recorded
Turn attempt <sup>1</sup>	Head of calf is turned towards back of cage

<sup>1</sup> These behaviours were recorded as behavioural events (i.e. counts). All other behaviours were recorded as behavioural states (i.e. durations).

the coat of other calves) were recorded (Webb et al., 2012). AOB and tongue playing are both considered in the present study.

Indirect behavioural observations were carried out in the metabolic cages in an attempt to record acute behavioural response to challenge, hence measures of temperament. When a calf was tethered inside the metabolic cage, focal animal sampling and continuous recording (Martin and Bateson, 1993) was carried out for 30 min to assess acute behavioural response to restraint. Behaviours recorded using indirect observations are described in Table 8.2.

## 2.4 Saliva sampling

Salivary cortisol was measured instead of plasma cortisol, because cortisol response to ACTH is similar in saliva and plasma (although less sensitive and with a smaller peak) in both pigs (Parrott et al., 1989) and cattle (Negrao et al., 2004), and because it requires a non-invasive sampling method that only measures ‘free’ cortisol (Mormède et al., 2007). Saliva sampling was done before and after the onset of restraint to measure physiological response to restraint at 14-16 wk of age. A baseline sample was collected at 09:00 h on the day when restraint was started, followed by samples at +40

min, +80 min, +120 min and +48 h, relative to the time calves were tethered inside the metabolic cages. These sampling time points were chosen based on previous research in calves comparing cortisol response in saliva and blood (Negrao et al., 2004). Two cotton swabs ( $150 \times 4$  mm, VWR®, Amsterdam, the Netherlands) were inserted into the mouth of a calf, who chewed on them voluntarily (calves were habituated to this procedure in their home pens), then placed into a salivette (Sarstedt B.V., Etten-Leur, the Netherlands) and immediately set on ice. Within 4 h the samples were centrifuged at 4650 rpm (4630 rcf) for 6 min, the cotton swabs removed, and the salivettes stored at  $-20^{\circ}\text{C}$  until analysis using enzyme immunoassay (Salimetrics Europe Ltd., Newmarket, UK). The final cortisol concentration was based on two independent 25  $\mu\text{L}$  samples for a given saliva sample, tested on the same microplate. The optical density, i.e. magnitude of colour (inversely proportionate to amount of cortisol in sample) change, was measured using a microplate spectrophotometer for all microplate wells containing control and test saliva. Two measures of optical density were taken: 450 nm (primary) and 650 nm (reference), and the difference calculated. Then the coefficient of variation percentage in optical density between both independent samples was calculated. If this coefficient was greater than 10, the sample was re-assayed.

## 2.5 Statistical analysis

All instantaneous scan sampling data were expressed as proportions of total scans. Continuously recorded behavioural data were expressed as proportion of total time if they were durations, or as counts. Models used for specific data are described in the sections below. In linear mixed models (**LMM**), the analysis was done with restricted maximum likelihood (McCulloch, 2006). In generalised linear mixed models (**GLMM**) (McCulloch, 2006) the distribution was specified as binomial with a logit link function, and an (over) dispersion parameter was introduced. For GLMM and GLM pseudo likelihoods were computed. For LMM and GLMM, approximate F-tests were constructed (Kenward and Roger, 1997). Statistical analyses were carried out in SAS (SAS Institute Inc., 2008). The effect of diet on behaviour is described extensively in Webb et al. (2012). The effect of diet on cortisol response was not considered here because each restraint group ( $N = 6$ ) comprised of one of two treatment pairs (0 and 9, or 18 and 27 g DM/kg of  $\text{BW}^{0.75}$  per day) for practical reasons. This results in an imbalance that prevents full comparison of diet treatments.

### **Cortisol response to restraint**

Cortisol data (log transformed because residuals were not normally distributed) were analysed for differences between sampling times using a LMM, with a first-order autoregressive structure on sampling time and the residuals. Treatment, sampling time point and the interaction between the two, as well as tethering groups nested in block and the interaction of tethering group nested in block and treatment, were included in the model as fixed main effects. Non-significant interactions were removed from the model. Pen, calf nested in pen and the interaction between pen and sampling time point were included as random effects.

### **Relation between tongue playing in the home pen, cortisol responsiveness and temperament**

Data for immediate behavioural response to restraint were first transformed: count (e.g. turn attempts) and continuous (i.e. cortisol) data were log transformed and proportions (e.g. % duration of sniffing) were logit transformed. These behavioural variables were included into a Principal Component Analysis (**PCA**) in order to extract underlying factors mediating these behavioural elements. PCA was carried out with varimax rotation. Principal components (**PC**) with eigenvalues above 1 were then included into regression analyses. PCs and tongue playing in the home pens were entered into regression analyses as explanatory variables (or covariables) for cortisol data. Finally, PCs were entered into a regression analysis with levels of tongue playing (proportion of total scans, recorded in the home pen) as explanatory variable (or covariable). Regression analysis was done with a LMM comprising fixed main effects of tethering group nested inside block, and diet treatment, and the interaction between the explanatory variable and diet treatment. Pen and calf nested inside pen were included as random components.

### **Chronic changes in tongue playing and AOB levels in response to restraint**

Tongue playing and AOB in the home pen were analysed to find whether restraint at 14-18 wk modified in some way these behaviours. These data were analysed using a GLMM. This model comprised fixed main effects for observation time (i.e. 2 wk prior or following restraint), treatment, and block, and interactions between treatment and observation time and treatment and block, and random effects for pen, and the interaction between pen and observation time.

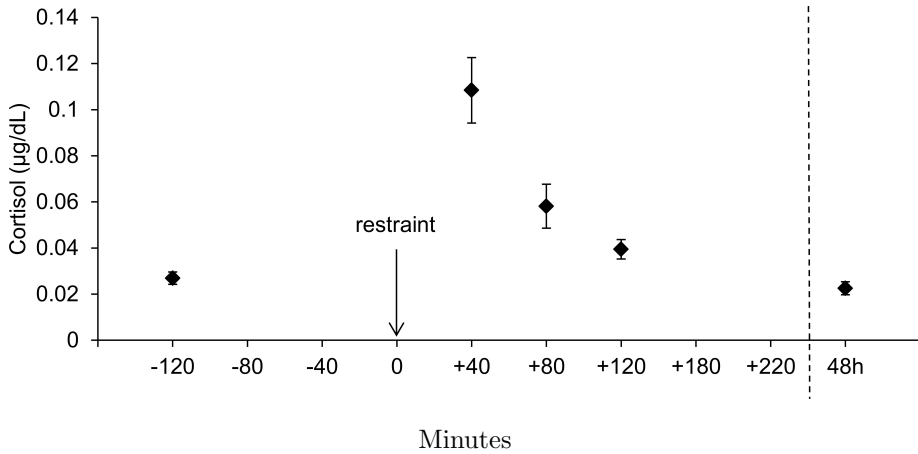


Figure 8.1: Salivary cortisol response ( $\mu\text{g}/\text{dL}$ ) of veal calves ( $N = 48$ ) to handling, walking to a metabolic cage, and tethering inside the cage (i.e. restraint). The baseline was taken 120 min before moving the first of the 6 calves.

## 3 Results

### 3.1 Cortisol response to restraint

Salivary cortisol response varied according to the sampling time point ( $P < 0.001$ ; Fig.8.1): all time points differed from each other except the baseline and the +48 h sampling, and the +80 and +120 min samplings.

### 3.2 Relation between tongue playing, cortisol responsiveness and temperament

Summary statistics for the behavioural variables recorded to monitor immediate behavioural response (first 30 min) to restraint are shown in Table 8.3. PCA on these behavioural elements revealed four PCs with eigenvalues above 1, which together accounted for 68% of total variation (Table 8.3). PC1 had a high positive loading on contact with cage as well as high negative loadings on idle and rapid locomotion. PC2 had high positive loadings on walk and turn attempts. PC3 had a high positive loading on tongue playing. Finally, PC4 had a high positive loading on look around and a high negative loading on idle with ear movements.

Regression analysis revealed four relationships between PCs and cortisol measures: PC1 was negatively associated with +40 ( $\beta = -0.42$ ;  $P = 0.014$ ), +80 ( $\beta = -0.38$ ;  $P = 0.006$ ), and +120 ( $\beta = -0.41$ ;  $P = 0.052$ ) min cortisol levels; PC4 was negatively

Table 8.3: Loadings on the first four principal components (PC) from the principal component analysis on variables collected during the first 30 min of restraint in a metabolic cage at 16 wk. Loadings higher than 0.5 are considered of interest and are in bold.

Variables	Mean $\pm$ SEM	PC1	PC2	PC3	PC4
Sniff (% time)	18.5 $\pm$ 1.1	0.39	0.23	0.17	0.11
Idle (% time)	58.7 $\pm$ 2.7	<b>-0.81</b>	-0.10	-0.17	-0.11
Contact cage (% time)	14.5 $\pm$ 2.2	<b>0.90</b>	0.01	0.14	-0.03
Look around (% time)	0.8 $\pm$ 0.2	-0.15	-0.11	0.25	<b>0.80</b>
Idle with ear movement (% time)	1.2 $\pm$ 0.4	-0.39	0.00	0.14	<b>-0.76</b>
Tongue play (% time)	0.2 $\pm$ 0.1	0.19	-0.19	<b>0.83</b>	0.10
Walk (% time)	3.0 $\pm$ 0.3	0.16	<b>0.78</b>	-0.07	-0.09
Hang on chain (count)	0.7 $\pm$ 0.2	-0.11	0.20	0.40	-0.06
Rapid locomotion (count)	1.0 $\pm$ 0.3	<b>-0.59</b>	-0.09	0.40	-0.29
Turn attempt (count)	10.1 $\pm$ 1.1	-0.05	<b>0.89</b>	-0.12	0.01
Eigenvalues		2.84	1.73	1.27	1.00
Variance explained		28%	17%	13%	10%

associated with +48 h cortisol level ( $\beta = -0.46$ ;  $P = 0.075$ ). Furthermore, a negative relationship was found between tongue playing level in the 2 wk prior to restraint and PC2 ( $\beta = -4.14$ ;  $P = 0.082$ ) and a positive relationship was found between tongue playing level and PC3 ( $\beta = 3.89$ ;  $P = 0.071$ ).

### 3.3 Chronic changes in tongue playing and AOB level in response to restraint

Individual differences in the level of tongue playing and AOB prior to restraint varied largely between calves, even within similar solid feed treatments (Fig.8.2). Tongue playing frequency observed in the home pens was not affected by a 10-d period of restraint (before:  $4.5 \pm 1.2$ ; after:  $4.9 \pm 1.2$ ;  $P = 0.513$ ). However, AOB frequency (which include tongue playing, oral manipulation of the environment and grazing of the coat of other calves) observed in the home pens was higher in the 2 wk following restraint compared to the 2 wk prior to restraint (before:  $20.8 \pm 2.5$ ; after:  $25.8 \pm 2.5$ ;  $P = 0.049$ ).

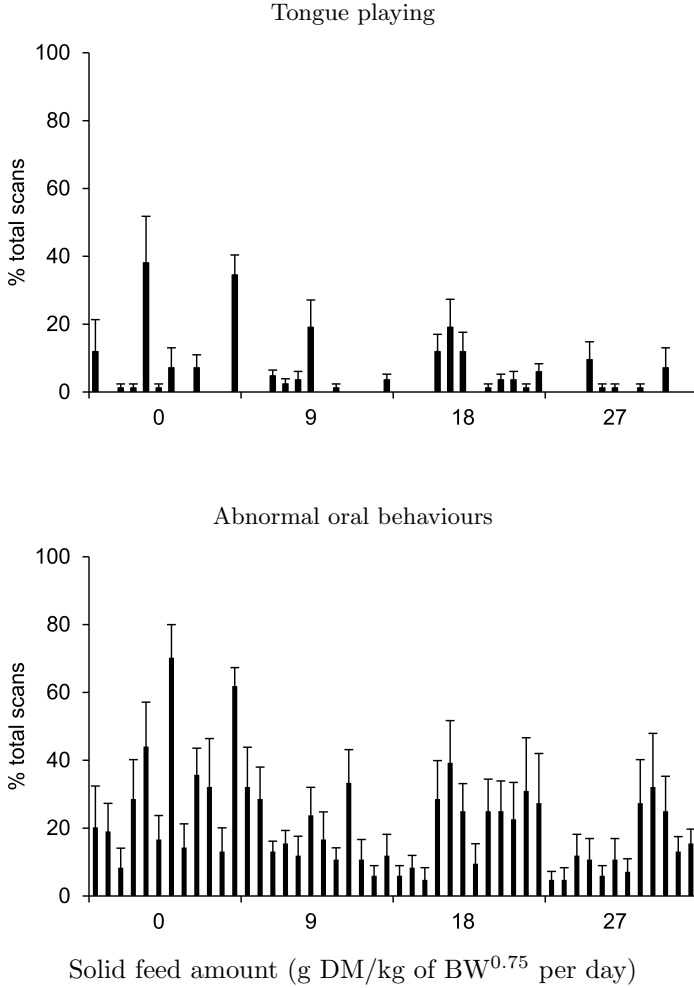


Figure 8.2: Tongue playing and abnormal oral behaviour levels (% totals scans) in individual calves from the four diet treatments. Levels of these behaviours were observed once a week in the 2 wk prior to restraint in metabolic cages, using instantaneous scan sampling. NB: each bar corresponds to an average of two observations on one calf (observations were first averaged across the 7 sessions in a day).



## 4 Discussion

The aim of this study was to find whether individual levels of tongue playing in calves are related to cortisol responsiveness (a possible measure of chronic stress) and hypothesised measures of temperament.

The average cortisol baseline and peak were lower in the present study than levels reported previously in cattle plasma (Mormède et al., 2007). However, cortisol levels are lower in calves compared to adult cattle (Redbo, 1998), as well as are lower in saliva compared to plasma (Negrao et al., 2004). Negrao et al. (2004) found salivary cortisol peaks of between 1 and 1.5 µg/dL in response to ACTH injection and milking in cows. A lower peak in the present study could result from younger animals being used. Alternatively, all calves in the present study may have been chronically stressed due to husbandry conditions (i.e. slatted floors, low roughage level, MR fed twice a day) resulting in an overall blunting of the cortisol peak.

The HPA axis of the calves in the present study seemed to rapidly (within 48 hours) adapt to the stressor of tethering inside metabolic cages, as seen in the return of salivary cortisol levels to baseline. This decline in cortisol levels despite continuation of the stressor is consistent with previous research in pigs (Jensen et al., 1996) and calves (Friend et al., 1985). Due to the continued application of the stressor (i.e. restraint), however, the calves may have still been stressed (Dellmeier et al., 1985; Jensen et al., 1996) and the HPA axis may have still been activated or at least modified (Mormède et al., 2007), potentially at the adrenal (Ladewig and Smidt, 1989) or hypothalamic level (Bhatnagar and Dallman, 1998). In this study, the continued behavioural response of calves after the initial 30 min was not studied, therefore, it is unknown whether the calves still showed behavioural signs of stress at this time. Salivary and plasma cortisol levels following the initial application of a stressor are not likely to be very informative (Mormède et al., 2007). Instead, cortisol response to a new stressor, or measuring different aspects of the continued activity of the HPA axis (e.g. adrenal gland size) may be more valuable (Mormède et al., 2007). In the present study, calves were believed to be differently chronically stressed due to feeding conditions prior to the restraint period (Webb et al., 2012), and this chronic stress was thought to be signalled by the development of tongue playing (and other AOB) (Veissier et al., 1998). However, the present study failed to find a relationship between tongue playing level and cortisol response to restraint. In chronically stressed calves and rats, increased cortisol responsiveness to challenge (stressor or ACTH injection) has been previously documented (Friend et al., 1985; Bhatnagar and Dallman, 1998; Veissier et al., 2001). Moreover, no relationship between tongue playing and cortisol baseline could be shown in the present study.

The lack of a relationship between tongue playing in the home pen and cortisol might indicate that tongue playing level is not a good measure of chronic stress, at least at the individual level. This idea is supported by the large individual variation in levels of oral stereotypies in the home pen regardless of cortisol baseline and cortisol response to a stressor. AOB frequency increased after a 10 d period of restraint. This could indicate that these behaviours are indeed good measures of chronic stress because a hypothesised chronic stressor (i.e. restraint) caused these behaviours to become more frequent. The fact that tongue playing frequency was unaffected by restraint, however, suggests instead that oral manipulation of the home pens may have increased due to increased exploration as a result of rediscovering the home pens after being restrained in cages. The idea that animals develop stereotypies differently due to individual characteristics, and therefore, that these behaviours are not good indicators of chronic stress at the individual level (in other words, stereotypies cannot be explained by differences in stress sensitivity), has previously been suggested (Mason, 1991b; Ijichi et al., 2013). Alternatively, animals that develop stereotypies may originally be more stressed than animals who do not develop these behaviours, but the performance of stereotypies may reduce stress levels (Van Reenen et al., 2001): i.e. ‘coping hypothesis’ (Rushen, 1993). This may at some point in time result in all calves showing equal levels of chronic stress despite very different levels of AOB.

PCA is used to summarise variables to find distinct (independent) underlying motivational systems, in the form of PCs. Applying PCA to the immediate behavioural response of calves to a stressor, here restraint, revealed four PCs. PC1 was hypothesised to represent underlying fearfulness, first and foremost because it correlated with salivary cortisol response to restraint, consistent with Van Reenen et al. (2005). In addition, low scores on PC1 were linked to longer durations of idling or displaying rapid locomotion, both of which could be taken as indicators of high levels of stress (or negative emotional state): Idle behaviour could be synonymous with freezing behaviour, and rapid locomotion could be synonymous with escape behaviour (Boissy, 1995; Reimert et al., 2013). High scores on PC1 indicated high levels of muzzle contact with the cage. The latter behaviour might be exploratory behaviour, which could have, in the present study at least, reflected a less fearful state. This is consistent with high PC1 scores being associated with a smaller salivary cortisol peak in response to restraint. PC2 was hypothesised to be linked to activity (Van Reenen et al., 2005), thus potentially coping style, with high scores indicating a more proactive coping and low scores a more reactive coping strategy (Van Reenen et al., 2005). PC3 was hypothesised to reflect propensity to tongue play during challenge. Given the time frame (30 min), it is very unlikely that tongue playing developed in response to restraint (Wiepkema, 1987). Mason and Latham (2004) offered four un-

derlying mechanisms/motivations for the onset of stereotypies once they are already established in an animal: 1) self-enrichment, 2) calming effect through repetition, 3) habit through automatic 'central control' processing, or 4) change in behavioural control. Tongue playing was observed very little in the first 30 min of restraint, and only in 10 of the 48 calves. However, the calves that did show this behaviour may have used it as a means of calming themselves or simply out of habit (Mason and Latham, 2004). PC4 could have reflected some aspect of vigilance, with lower scores indicating a more auditory form of vigilance, while higher scores indicated a more visual form of vigilance. A relationship between PC4 and cortisol levels 48 hours after the onset of restraint suggests that the manner by which calves observed their environment in the first 30 min of restraint may affect their physiological adaptation to a stressor. Calves that looked around, instead of only listened, may have been subsequently less stressed by the novel conditions, resulting in the more rapid decrease in their cortisol levels. A study on dogs found that long-ranged exploration, i.e. visual and auditory, increased with increasing fear and concluded that this behavioural response to a fearful situation was 'adaptively appropriate' (Goddard and Beilharz, 1984). The latter conclusion most likely refers to increased levels of vigilance in unknown situations increasing chances of survival in the wild.

If oral stereotypies, such as tongue playing, develop differently in animals due to differences in individual characteristics, or temperament, then they should be associated to behavioural response to challenge (Van Reenen et al., 2004). In the present study, two tendencies for associations between tongue playing frequency and PCs were found: i.e. with PC2 and PC3. The relationship between tongue playing and PC3 is straightforward, because calves with high levels of tongue playing in the home pen are more likely to use this behaviour to calm/sooth themselves during stressful events, or out of habit (Mason and Latham, 2004). Moreover, if PC2 indeed reflects coping style, then this study suggests that calves that display high levels of tongue playing in the home pen may respond less actively in challenge tests, and thus, show a more reactive coping profile. This finding is consistent with findings from Redbo (1998), who found that high levels of oral stereotypies in calves were associated with less running and walking and more exploration in an open field test. The latter behavioural response suggests a more reactive coping style. Interestingly, theoretical papers assessing the relationship between stereotypies and coping style consistently suggest that proactive animals are more likely to develop stereotypic behaviour compared with reactive animals (Mason, 1991b; Ijichi et al., 2013), because they share behavioural (e.g. high activity, low flexibility, routine formation) and physiological (e.g. higher dopamine) traits with individuals performing stereotypic behaviour (Mason, 1991b; Bolhuis et al., 2004; Ijichi et al., 2013). However, in experimental papers on horses, pigs and calves,

animals showing a reactive style were found to develop higher levels of stereotypies compared with proactive animals (Schouten and Wiepkema, 1991; Nagy et al., 2010; Redbo, 1998), consistent with the present study.

Results regarding the effect of diet on behaviour are presented in Webb et al. (2012) who showed that only the diet with the highest level of solid feed (i.e. 27 g DM per kg of  $BW^{0.75}$  per day) resulted in lower levels of tongue playing and AOB compared with feeding no solid feed supplement on top of MR. Due to the design of this experiment (restraint groups always comprising the same two solid feed treatment pairs), it was not possible to analyse the effect of solid feed treatment on cortisol responsiveness or behavioural response to restraint. It is, however, important to keep in mind that the diet treatments did not only comprise of differences in solid feed provision but also MR provision, as MR allowance was based on achieving equal average daily gains between treatments within a block. Moreover, calves fed little or no solid feed, and subsequently large MR quantities, experienced a decreased in their MR allowance prior to restraint. This could possibly have resulted in frustration or hunger, and might have increased the differences with respect to chronic stress between diet treatments in the week prior to restraint.

The present study reinforces the idea of a multidimensional attribute for underlying mechanisms mediating behavioural responses of calves to a stressor, with fearfulness, but not activity, being correlated with changes in cortisol responsiveness to restraint in calves. Furthermore, we support the idea that the propensity of calves to develop tongue playing in a sub-optimal environment is mediated by individual characteristics, possibly coping style. However, we suggest that a higher propensity to develop stereotypies as a response to prolonged stress is in fact a characteristic of reactive, instead of proactive, animals. These findings back up the proposition that stereotypies may not be good indicators for chronic stress at the individual level in sub-optimal environments (Mason and Latham, 2004).

# Chapter 9

## The role of solid feed amount and composition, and milk replacer supply in veal calf welfare

Laura Webb, Kees van Reenen, Harma Berends, Bas Engel, Imke de Boer, Walter Gerrits, Eddie Bokkers

Submitted

*“Let us not mince words: Animal welfare involves the subjective feelings of animals.”*  
(Dawkins, 1990)

### Abstract

Feeding strategies commonly used in veal production have been linked with a number of welfare impairments, including behavioural and gastrointestinal health problems. The aim of this study was to determine how indicators of calf welfare (here behaviour and characteristics of the faeces, reflecting gastrointestinal health status) may be affected by: 1) different amounts and compositions of solid feed (SF), including three positive (ad libitum) controls, and 2) milk replacer (MR) being fed via an open bucket or an automated milk dispenser (AMD). Two-wk-old Holstein-Friesian bull calves (N = 160) were fed different cumulative amounts of SF (25, 110, 200, 280 kg of DM: SF25, SF110, SF200, SF280) with two percentages of concentrate in the SF (50 or 80) from 2 to 28 wk of age. The roughage part of the SF consisted of 50% maize silage and 50% chopped straw (DM based). Three controls were used: 1) 20 calves fed SF110 with 80% concentrate received ad libitum long straw; 2) 25 calves received ad libitum SF with the possibility to freely choose between components (SEP); 3) 25 calves received an SF mixture ad libitum (MIX) with the composition of this mixture being equal to the choice of SEP calves in the preceding week. Calves received MR in open buckets. An additional 40 calves received one of two SF amounts (SF25 or SF200) with 50% concentrate, and received MR via an AMD. MR provision was adjusted for similar rates of carcass weight gain between treatments (excluding controls). Behaviour was recorded at 15 and 24 wk of age using instantaneous scan sampling. The prevalence of diarrhoea and clay-like faeces (which signal ruminal drinking) was monitored at 14 and 24 wk of age. Higher roughage provision, but not concentrate, increased rumination and decreased abnormal oral behaviours. STR calves had similar levels of rumination and abnormal oral behaviours as MIX and SEP calves. Offering MR via an AMD did not seem to affect behaviour much compared with calves fed MR in buckets, except that tongue playing was reduced at 15 wk. Tongue playing was related to both roughage amount and AMD feeding, suggesting two separate motivations (i.e. rumination and sucking) underlying the development of this behaviour. Only SF amount affected aspects of faeces: SF25 calves had the highest diarrhoea incidence. No effect of feeding strategy was found on clay-like faeces.

## 1 Introduction

Feeding strategies commonly used in veal production have been linked with a number of welfare impairments, including behavioural and gastrointestinal health problems (Bokkers and Koene, 2001; Brscic et al., 2011). The amount and composition of solid feed provided may be inadequate in terms of meeting calves' motivation to ruminate,

as signalled by abnormal behaviours such as excessive oral manipulation of the pen and abnormal rolling of the tongue, or tongue playing (Mattiello et al., 2002; Webb et al., 2013). These abnormal, repetitive behaviours with no apparent function should be taken as a warning sign for suffering and distress (Mason, 1991b). Consequently, new feeding strategies that improve veal calf welfare need to be addressed. Increased rumination can occur through increasing the provision of solid feed (Webb et al., 2012, 2013), increasing the proportion of roughage, or the presence of longer particles, in the solid feed (Balch, 1971; Mertens, 1997; Kahyani et al., 2013), or providing coarser roughage sources (Mattiello et al., 2002; Webb et al., 2013). However, most studies ignore potential differences in satiety, by providing equal amounts of milk replacer (**MR**) to calves, with the amounts of solid feed differing. It may be more valuable to maintain equal growth between different feeding treatments by adjusting MR provision, which most likely has no effect on rumination and subsequently no effect on abnormal oral behaviours (van der Borne, personal communication). In addition, increasing the solid feed provision of veal calves usually involves an increase in concentrate provision, rather than roughage provision, in order to maximise growth efficiency. It is, thus, important to understand how increased concentrate provision, as opposed to roughage, may affect behaviour and health.

Some authors have even suggested that allowing calves to select their own feeding strategy, from a sound choice of components, is the best, or only way, to meet individual nutritional, physiological, and behavioural requirements (Manteca et al., 2008). Therefore, the behaviour of calves offered novel feeding strategies, aimed at improving welfare, should be directly compared to the behaviour of calves with ad libitum access to solid feed components.

Other than rumination, natural sucking is another natural behaviour that may be thwarted in veal systems, due to the feeding of MR via open buckets or troughs without teats (Brscic et al., 2011). This restriction may result in non-nutritive sucking of pen mates, as found in dairy calves (De Passillé and Rushen, 1997). Limited MR feeding, albeit via teats, has also been associated to tongue playing and cross-sucking in dairy calves (Fröberg and Lidfors, 2009). A careful comparison between impacts of solid feed provision versus MR feeding method on the behaviour and health of veal calves is required.

The aim of this study was to determine how indicators of calf welfare (here behaviour and characteristics of the faeces, reflecting gastrointestinal health status) may be affected by: 1) different amounts and compositions of solid feed, including three (ad libitum) control treatments, and 2) MR being fed via an open bucket or an automated milk dispenser (**AMD**). The three aforementioned controls included one group provided ad libitum long wheat straw, and two groups fed ad libitum concentrate,

maize silage and chopped wheat straw in separate troughs or mixed together. General performance and post-mortem gastrointestinal health measurements are described in separate articles (Berends et al., 2014).

## 2 Materials and methods

All procedures complied with the Dutch law for animal experiments, which itself complies with the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive). Procedures were further sanctioned by Wageningen University's Committee on Animal Care and Use. The study ran from July to December 2012.

### 2.1 Animals and husbandry

Two-wk old bull calves ( $N = 270$ ;  $45.1 \pm 0.2$  kg) were purchased from a Dutch dairy calf trader and transported to the experimental facilities, Scherpenzeel, the Netherlands. The calves were selected for similar weights and good clinical health. All calves received MR and solid feed (see Table 9.1 for nutrient composition) throughout the study. From 2 to 6 wk of age, the calves were housed individually in  $0.9$  m<sup>2</sup> temporary pens inside the group pens (contact possible between calves from adjacent pens), to monitor MR drinking and health. From 6 wk onwards, calves were group-housed in pens (5 calves per pen) with wooden slatted floors and  $1.8$  m<sup>2</sup> per calf. Adjacent pens were separated by metal bars allowing contact between calves from adjacent pens. Average temperature and relative humidity were:  $20.0 \pm 0.3^\circ\text{C}$  and  $85.5 \pm 0.7\%$  from 2 to 14 wk, and  $12.6 \pm 0.6^\circ\text{C}$  and  $91.7 \pm 0.6\%$  from 15 to 25 wk. The barn was lit by natural light and by artificial lighting between 05:00 and 22:30 h. Health was controlled daily. Calves were treated with antibiotics at arrival at the experimental facilities, and thereafter, were only treated when needed according to a veterinary protocol. Sick calves were individually housed within the group pen for the monitoring of solid feed and MR intake. Sick calves that showed indications of ruminal drinking (pasty light-coloured faeces suggesting large amounts of MR were entering the rumen) were offered a floating teat during MR feeding. Haemoglobin levels were monitored 6 times during the experiment. Calves were injected with extra iron when needed to maintain haemoglobin levels above  $5.5$  mmol/L of blood. Calves were weighed every 2 wk. Water was offered for 20 min around noon in open buckets in the first 4 wk, and thereafter provided ad libitum via drinking nipples. The study started when calves were 2 wk old and ended when they were 28 wk old. Three calves died in the course of the study.



Table 9.1: Nutrient composition (g/kg DM) of solid feed ration components and milk replacer.

Nutrient	Concentrate <sup>1</sup>	Maize silage	Wheat straw	Milk replacer
Dry matter, g/kg product	898	297	931	970
Crude protein <sup>2</sup>	137	69	31	210
Crude fat	67	29	9	212
Starch	429	312	11	22
NDF	127	421	794	-

<sup>1</sup> Concentrate composition: 36.2% maize, 20.6% lupins, 20.3% barley, 12.5% carob meal, 4.4% maize gluten meal, 6% premix. Composition was designed to meet beef cattle requirements for minerals and vitamins (NRC, 2000).

<sup>2</sup> N × 6.25.

## 2.2 Feeding treatments

The experiment included two main factorial designs. First, a 4 × 2 factorial design (32 pens) was used to evaluate the effect of different amounts of solid feed (4 levels: 25 [**SF25**], 110 [**SF110**], 200 [**SF200**], or 280 [**SF280**] kg DM, provided from 2 to 28 wk of age) and percentages of pelleted concentrate in the solid feed (2 levels: 50 or 80% on DM basis). The roughage part of the solid feed mixture consisted of 50% maize silage and 50% chopped wheat straw (on DM basis). Calves involved in this 4 × 2 factorial set-up were fed MR in individual buckets. Three control treatments were further included in this experiment to assess the impact of different solid feed amounts and compositions. First, the effect of providing enrichment and increasing chewing opportunity was assessed by equipping 4 pens with a rack filled with long wheat straw (**STR**). STR calves received the feeding strategy that was considered closest to what was done on Dutch commercial veal farms at the time of the experiment, i.e. SF110 with 80% concentrate, and MR fed in buckets. These calves were compared to calves from the 4 × 2 factorial design that received the same feeding strategy but no straw rack. The number of straw bales provided to STR calves during the experiment was recorded. Second, two additional control treatments received concentrate, maize silage and chopped wheat straw ad libitum. The first control treatment (5 pens) consisted of separate feeding of the three solid feed components in three separate troughs, enabling calves to select their own composition of solid feed (**SEP**). The second control treatment (5 pens) offered a solid feed mixture (**MIX**). Intake of SEP pens was monitored weekly, and the percentage of concentrate, maize silage and straw was calculated across all pens. From these calculations, the mixture for MIX pens

Table 9.2: Average body weight (BW, kg), average daily gain (ADG, kg/d) and milk replacer (MR, g/d) provision ( $\pm$  SEM) for each solid feed treatment during the adaptation period (2 to 12 wk of age).

SF <sup>1</sup>	BW 2 wk	BW 12 wk	ADG	MR
25	45 $\pm$ 0.3	99 $\pm$ 0.6	0.7	525 $\pm$ 18.3
110	45 $\pm$ 0.4	100 $\pm$ 0.7	0.8	468 $\pm$ 16.8
200	45 $\pm$ 0.2	101 $\pm$ 0.7	0.8	428 $\pm$ 15.5
280	45 $\pm$ 0.5	102 $\pm$ 0.9	0.8	393 $\pm$ 14.0

<sup>1</sup> SF = total kg DM solid feed provision from 2 to 28 wk.

was prepared, and fed ad libitum the following week.

Second, a  $2 \times 2$  factorial design (16 pens, 8 of which were also included in the  $4 \times 2$  design) was used to evaluate the effect of different amounts of solid feed (2 levels: SF25 or SF200, with 50% concentrate) and two different ways of feeding MR (2 levels: open bucket or AMD). The two factorial designs were merged to minimise the number of animals needed for the experiment, i.e. the  $2 \times 2$  factorial design comprised of bucket-fed calves from the  $4 \times 2$  factorial design.

Each treatment combination was comprised of four pens, except ad libitum groups, which were comprised of 5 pens, to account for the large individual variation anticipated in these groups in terms of intake (based on Chapter 4).

### 2.3 General feeding procedures

From 2 to 12 wk (i.e. adaptation period), the calves within one level of solid feed (i.e. SF25, SF110, SF200 and SF280) were fed the same composition (50% concentrate), as well as the same MR allowance. MIX and SEP calves received the solid feed and MR allowance of SF200 calves with 50% concentrate (based on the expected intake of these calves). A commercial starter MR (32.2% whey powder, 30.0% skimmed milk powder; 223 g/kg crude protein, 180 g/kg DM crude fat) was fed to calves during the adaptation period. Body weights, ADG and MR allowance until 12 wk are shown in Table 9.2. Between 2 and 6 wk of age, calves received alfalfa hay instead of maize silage in an attempt to stimulate solid feed intake.

From 12 wk onwards (i.e. experimental period), feeding treatments were started and MR provision (see composition in Table 9.1) was fed in such a way that allowed for similar rates of carcass weight gain (taking presumed differences in dressing percentages into account) between treatments (Table 9.3). MR provision was adjusted every 2 wk based on the body weight (**BW**) gain achieved in the 2 wk prior to the

Table 9.3: Average body weight (BW, kg), average daily gain (ADG, kg/d) and milk replacer (MR, g/d) provision ( $\pm$  SEM) per treatment during the experimental period (12 to 28 wk of age). These averages are based on the entire period, but MR provision increased gradually from 12 to 28 wk of age.

MF <sup>1</sup>	%C <sup>2</sup>	SF <sup>3</sup>	AL <sup>4</sup>	BW 12 wk	BW 26 wk	ADG	MR
Bucket	50	25		99 $\pm$ 1.0	216 $\pm$ 4.1	1.2	1270 $\pm$ 12.6
		110		99 $\pm$ 1.6	230 $\pm$ 5.6	1.3	1118 $\pm$ 9.8
		200		102 $\pm$ 2.0	236 $\pm$ 5.1	1.4	973 $\pm$ 7.1
		-	SEP	101 $\pm$ 1.3	263 $\pm$ 4.3	1.6	973 $\pm$ 7.1
		-	MIX	104 $\pm$ 1.2	260 $\pm$ 6.0	1.6	973 $\pm$ 7.1
		280		102 $\pm$ 1.1	237 $\pm$ 4.1	1.4	854 $\pm$ 5.2
		80	25		103 $\pm$ 0.7	214 $\pm$ 6.3	1.1
	80	110		100 $\pm$ 1.3	221 $\pm$ 3.5	1.2	1054 $\pm$ 7.6
		110	STR	103 $\pm$ 1.0	233 $\pm$ 5.1	1.3	1054 $\pm$ 7.6
		200		100 $\pm$ 1.7	230 $\pm$ 4.7	1.3	867 $\pm$ 4.0
		280		101 $\pm$ 1.5	238 $\pm$ 4.7	1.4	697 $\pm$ 1.4
		AMD	50	25		95 $\pm$ 1.0	207 $\pm$ 5.4
		200		96 $\pm$ 1.9	228 $\pm$ 4.7	1.4	973 $\pm$ 7.1

<sup>1</sup> MF = milk replacer feeding method; AMD = automated milk dispenser.

<sup>2</sup> %C = percentage concentrate.

<sup>3</sup> SF = total kg DM solid feed provision from 2 to 28 wk.

<sup>4</sup> AL = ad libitum controls; SEP = ad libitum solid feed provision with components in separate troughs; MIX = ad libitum solid feed provision with components mixed together (ratio based on intake of SEP calves); STR = calves with ad libitum provision of long straw in a rack.

adjustment. Equal growth was not aimed for within STR, SEP, and MIX calves. STR calves received the same MR allowance as all calves fed 110 kg with 80% concentrate, and SEP and MIX treatments were coupled to the MR allowance of calves fed 200 kg with 50% concentrate. Bucket-fed calves were fed MR at 06:00 and 16:00 h. During MR feeding calves were locked in the feeding fence to prevent them from ingesting other calves' MR. AMD-fed calves obtained a new MR allowance in three 7-hour periods in the day (during the first 3 hours of the day AMD were automatically cleaned and MR was unavailable). MR concentration gradually increased from 125 to 188 g/L. MR refusals were recorded daily. Solid feed was fed immediately after MR feeding in the morning. Solid feed provision was increased throughout the study period with equal weekly increments. Intake at 15 and 24 wk for calves within the 4  $\times$  2 is shown in Table 9.4. Solid feed refusals were recorded daily. The treatments were randomly allocated to pens within the experimental facilities.

Table 9.4: Solid feed intake (g DM/d) of calves in the  $4 \times 2$  factorial design, i.e. fed one of four solid feed amounts comprised of one of two compositions, at 15 and 24 wk of age.

Total solid feed (kg DM)	% Concentrate	15 wk	24 wk
25	50	$132 \pm 0$	$181 \pm 0$
	80	$137 \pm 0$	$190 \pm 0$
110	50	$562 \pm 0$	$880 \pm 0$
	80	$577 \pm 0$	$910 \pm 0$
200	50	$916 \pm 67$	$1501 \pm 69$
	80	$1077 \pm 0$	$1674 \pm 0$
280	50	$1476 \pm 25$	$2321 \pm 0$
	80	$1569 \pm 0$	$2409 \pm 0$

## 2.4 Behavioural measurements

### Direct Observations

Behavioural observations were carried out for 2 d at 15 and 24 wk of age, using instantaneous scan sampling and the ethogram described in Table 9.5. The 54 pens were divided into three groups based on location in the barn, and each group was observed for 30 min every 2 h from 06:30 to 20:30 h (with 30 min lunch break at 12:30 h). Each calf within each pen, within each group, was scanned at a 6 min interval. Calves that were individually housed within group pens for medical reasons were left out of the behavioural observations. Groups were observed in a random order, and this order varied each observation day. Calves were observed with instantaneous scan sampling (“a whole group of subjects is rapidly scanned [...] at regular intervals and the behaviour of each individual at that instant is recorded”, Martin and Bateson, 1993), using a hand held computer (Psion Teklogix Workabout Pro G2, Teklogix Int. Inc, Mississauga, Canada) with The Observer®XT (version 10, Noldus Information Technology, Wageningen, The Netherlands). All observations were done by a single observer.

### Indirect observations

Pens equipped with a straw rack were further observed indirectly to assess the level of utilisation of the straw by the calves. These pens were video recorded for one day a week at 18-19 and 23-24 wk of age. The videos were observed using instantaneous scan sampling at a 5 min interval from 05:00 to 22:00 h. At each scan, calves were scored as: 1) not in contact, or 2) in contact with the straw rack (including pulling

Table 9.5: Ethogram.

Behaviour	Definition
Oral manipulation of pen	Any oral (nose, lips or tongue) contact with any aspect of the pen structure including fences, floor and trough.
Tongue playing	Rolling and unrolling of the tongue inside or outside of the mouth, without oral contact with the environment.
Feed	Lateral movements of the jaw above a trough with solid feed present or oral contact with solid feed.
Ruminate	Lateral movements of the jaw away from the trough or above an empty trough.
Lie	Body not supported by limbs.
Sniff	Nose less than 10 cm away from an aspect of the environment, with movement of nostrils sometimes, but not always, noticeable.
Play	Any one of the following behaviours: run, jump, buck, head butt calf, head butt object.
Lick calf	Oral contact with body of another calf, without any part of the body being inside the mouth.
Suck calf	Mouth is around a part of the body (mouth, ear, tail, joint, prepuce) of another calf with suction sounds.
Groom	Oral contact with any part of own body.

straw out of the rack, or masticating near and facing the rack or with straw strands sticking out of the mouth facing or not the rack).

## 2.5 Characteristics of faeces

Characteristics of the faeces, reflecting gastrointestinal health status, were recorded when calves were 14 and 24 wk old. Three measures were recorded at pen level: presence or absence of fresh diarrhoea and presence or absence of clay-like faeces. Diarrhoea was defined as: very thin, watery faeces that is often different from normal faeces in color. Clay-like faeces were defined as: thicker and firmer consistency than pudding with a white/grey colouring. Clay-like faeces signal ruminal drinking, i.e. “the milk is forced into the rumen where it undergoes abnormal decomposition causing physiological disturbances” (Van Weeren-Keverling Buisman et al., 1988). These measures were recorded by a veterinarian.

## 2.6 Statistical analysis

### General

All data were grouped per pen and age. Behavioural data collected using instantaneous scan sampling were expressed as proportions of total scans and analysed with a generalised linear mixed model (**GLMM**) with a logit link and specifying the variance as a multiple (dispersion parameter) of the binomial variance function. Separate analyses were carried out for the  $4 \times 2$  (with additional controls) and  $2 \times 2$  factorial designs, and for analysing effects of providing ad libitum straw in a rack. Models comprised of fixed main effects and interactions for feeding treatments (i.e. solid feed level, concentrate percentage, ad libitum straw provision, MR feeding method, depending on the analysis) and age (15 vs. 24 wk). A random pen effect was introduced to account for dependence between repeated measurements of the same pen. Inference was based on pseudo-likelihood (Wolfinger and O’connell, 1993), which is equivalent to penalised quasi-likelihood (Schall, 1991; Breslow and Clayton, 1993; Engel and Keen, 1994). It was checked whether dispersion parameters were equal for both ages. Approximate F-tests were employed for the fixed effects (Kenward and Roger, 1997). The GLIMMIX procedure in SAS (SAS Institute Inc., 2008) was used for all analyses.

### $4 \times 2$ with added controls SEP and MIX

The STR control treatment was not analysed as an added control to the  $4 \times 2$  factorial design, as was the case for the other two controls. The STR control was paired to a

specific amount and composition of solid feed (i.e. SF110 and 80% concentrate) and, therefore, only compared to SF110 calves with 80% concentrate in a separate analysis (described above). To incorporate the added controls in the  $4 \times 2$  factorial design a dummy experimental factor  $a$  with three levels was created. The experimental factors for solid feed amount and % concentrate were extended with two extra levels, corresponding to the added controls. Two levels of  $a$  corresponded to the two controls, and the third level corresponded to the combinations in the original  $4 \times 2$  factorial design for solid feed amount and % concentrate. Next, the main effects and interactions for the extended factors for solid feed and % concentrate were nested within factor  $a$ . Tests were performed for main effects of  $a$ , solid feed amount and % concentrate within  $a$ , and age, and all (up to 4-way) interactions. Significant main effects and interactions were identified, suggesting what relevant contrasts, such as pairwise comparisons, should be inspected in detail next. A second analysis was performed with an alternative parametrisation of the same GLMM, to extract test results for the relevant contrasts. All treatments, i.e. the combinations of solid feed amount and % concentrate, as well as the two added controls, were included as levels of a single factor  $\beta$  with 10 levels. The GLMM comprised main effects and interactions for  $\beta$  and age, and random effects for pen. Inference for relevant contrasts was obtained with the CONTRAST statement in the GLIMMIX procedure in SAS.

### Characteristics of faeces

The prevalence (at pen level) of each type of faeces (i.e. diarrhoea and clay-like) was analysed using Fisher's exact test (SAS routine PROC FREQ).

## 3 Results

### 3.1 Solid feed amount, percentage concentrate and added controls SEP and MIX

Mean % total scans for behaviours of calves fed different amounts of solid feed with different % concentrate are shown in Table 9.6. Only SF280 with 50% concentrate considerably reduced the frequency of abnormal oral behaviours, i.e. manipulation of the pen and tongue playing. The frequency of feeding behaviours increased with solid feed amount, but was unaffected by solid feed composition. The frequency of rumination increased with solid feed amount, although this observation was mostly restricted to a solid feed composition of 50% concentrate, as opposed to 80%. A main effect of age was found on the frequency of oral manipulation of the pen ( $P =$

Table 9.6: Frequency of behaviours (mean % total scans) and SEM, averaged over two ages (15 and 24 wk) and observed in veal calves fed different amounts of solid feed with different percentages of concentrate. Behaviour was recorded using instantaneous scan sampling. P-values for main effects and interactions that include solid feed amount and % concentrate are shown. Milk replacer was provided in such a way that aimed for equal carcass weight gain across treatments.

kg DM	50% concentrate				80% concentrate				P-values <sup>1</sup>			
	25	110	200	280	25	110	200	280	SEM	SF	CON	SF×CON
Manip <sup>2</sup>	7.6 <sup>a,x</sup>	7.3 <sup>a,x</sup>	7.5 <sup>a,x</sup>	4.6 <sup>b,x</sup>	11.1 <sup>a,y</sup>	7.8 <sup>b,y</sup>	6.4 <sup>b,x</sup>	7.2 <sup>b,y</sup>	0.40			0.049
Tongue	1.2 <sup>x</sup>	1.5 <sup>x</sup>	1.0 <sup>x</sup>	0.5 <sup>x</sup>	1.8 <sup>y</sup>	2.0 <sup>y</sup>	2.1 <sup>y</sup>	1.4 <sup>y</sup>	0.18	NS	0.045	NS
Feed	1.8 <sup>a</sup>	6.3 <sup>b</sup>	9.6 <sup>bc</sup>	9.7 <sup>c</sup>	1.1 <sup>a</sup>	6.1 <sup>b</sup>	6.5 <sup>bc</sup>	9.2 <sup>c</sup>	0.58	<0.001	NS	NS
Ruminate	8.1 <sup>a,x</sup>	11.2 <sup>b,x</sup>	13.0 <sup>b,x</sup>	19.7 <sup>c,x</sup>	6.6 <sup>a,x</sup>	7.3 <sup>ab,y</sup>	8.5 <sup>ab,y</sup>	9.3 <sup>b,y</sup>	0.59			0.041
Lie	55.2	51.2	51.4	57.9	47.4	51.9	51.2	53	0.85	NS	NS	NS
Sniff	4.0	3.8	4.6	3.3	3.8	3.4	3.3	2.8	0.18	NS	NS	NS
Play	1.6	1.2	1.5	1.5	2.3	1.3	1.9	1.3	0.13	NS	NS	NS
Lick calf	1.2	2.0	1.3	1.0	1.3	1.6	1.7	2.1	0.13	NS	NS	NS
Snuck calf	1.2	1.1	0.5	0.4	1.0	0.5	1.0	0.6	0.11	NS	NS	NS
Groom	2.6	2.4	3.2	1.7	2.4	1.9	2.5	2.4	0.16	NS	NS	NS

<sup>1</sup> SF = main effect of solid feed amount; CON = main effect of % concentrate; SF×CON = interaction between SF and CON.

<sup>2</sup> Manip = oral manipulation of pen.

<sup>a-c</sup> Means with no common superscripts differ ( $P < 0.05$ ): pairwise comparison of SF within CON.

<sup>x-z</sup> Means with no common superscripts differ ( $P < 0.05$ ): pairwise comparison of CON within SF.



Table 9.7: Frequency of behaviours (mean % total scans) and SEM, averaged over two ages (15 and 24 wk) and observed in veal calves fed different amounts of solid feed with different percentage of concentrate, or ad libitum (added controls). Behaviour was recorded using instantaneous scan sampling. Pairwise comparisons are shown between added controls and solid feed amount on the one hand, and % concentrate on the other hand. Milk replacer was provided in such a way that aimed for equal carcass weight gain across treatments (excluding added controls).

	Total solid feed (kg DM)					Concentrate		Added controls <sup>1</sup>		P-values <sup>2</sup>	
	25	110	200	280	50%	80%	MIX	SEP	SEM	Ctrl/SF	Ctrl/CON
Manip <sup>3</sup>	9.4 <sup>a</sup>	7.6 <sup>a</sup>	6.9 <sup>b</sup>	5.9 <sup>c</sup>	6.8 <sup>x</sup>	8.1 <sup>y</sup>	4.7 <sup>c,z</sup>	5.0 <sup>bc,xz</sup>	0.33	<0.001	<0.001
Tongue	1.5 <sup>a</sup>	1.8 <sup>a</sup>	1.6 <sup>b</sup>	1.0 <sup>c</sup>	1.1 <sup>x</sup>	1.8 <sup>y</sup>	0.3 <sup>c,x</sup>	0.5 <sup>bc,x</sup>	0.15	0.019	0.007
Feed	1.5 <sup>a</sup>	6.2 <sup>a</sup>	8.0 <sup>b</sup>	9.5 <sup>c</sup>	6.8 <sup>x</sup>	5.7 <sup>x</sup>	9.6 <sup>bc,y</sup>	11.0 <sup>c,y</sup>	0.49	<0.001	<0.001
Ruminate	7.3 <sup>a</sup>	9.2 <sup>a</sup>	10.7 <sup>a</sup>	14.5 <sup>b</sup>	13.0 <sup>x</sup>	7.9 <sup>x</sup>	15.8 <sup>b,y</sup>	18.3 <sup>c,y</sup>	0.57	<0.001	<0.001
Lie	51.3	51.5	51.3	55.4	53.9	50.8	56.6	53.9	0.70	NS	NS
Sniff	3.9	3.6	4.0	3.1	3.9	3.3	3.0	2.7	0.15	NS	NS
Play	1.9	1.2	1.7	1.4	1.5	1.7	1.5	2.1	0.12	NS	NS
Lick calf	1.3	1.8	1.5	1.6	1.4	1.7	1.4	1.4	0.10	NS	NS
Suck calf	1.1	0.8	0.7	0.5	0.8	0.8	0.8	1.1	0.09	NS	NS
Groom	2.5	2.2	2.8	2.1	2.5	2.3	2.3	2.5	0.13	NS	NS

<sup>1</sup> MIX = ad libitum solid feed mixture provision; SEP = ad libitum solid feed provision with components in separate troughs.

<sup>2</sup> Ctrl/SF = pairwise comparisons between added controls and levels of solid feed; Ctrl/CON = pairwise comparisons between added controls and concentrate percentage.

<sup>3</sup> Manip = oral manipulation of pen.

a-c Means with no common superscripts differ ( $P < 0.05$ ): Ctrl/SF.

x-z Means with no common superscripts differ ( $P < 0.05$ ): Ctrl/CON.

0.014), sucking pen mates ( $P < 0.001$ ) and grooming ( $P < 0.001$ ). At 24 wk of age, calves orally manipulated the pen less ( $6.8 \pm 0.6\%$ ) than at 15 wk ( $8.1 \pm 0.5\%$ ). The frequency of both sucking pen mates and grooming also decreased from 15 to 24 wk of age (Suck:  $1.2 \pm 0.1$  and  $0.4 \pm 0.1\%$ ; Groom:  $3.0 \pm 1.8$  and  $1.8 \pm 0.1\%$ , at 15 and 24 wk respectively). For play behaviours, an interaction between % concentrate and age was found ( $P = 0.023$ ), with calves fed 80% concentrate decreasing their play frequency from 15 ( $2.3 \pm 0.3\%$ ) to 24 ( $1.0 \pm 0.2\%$ ) wk of age. Pairwise comparisons of added control with solid feed amount on the one hand, and % concentrate on the other hand are described in Table 9.7. MIX and SEP calves did not differ for any of the behaviours recorded in this study. For abnormal oral behaviours, MIX and SEP calves were similar to SF200 and SF280, and to pens fed 50% concentrate. For feeding behaviours, MIX and SEP calves were similar to SF200 and SF280. MIX and SEP showed higher frequencies of feeding than pens fed both 50% and 80% concentrate. For rumination, MIX and SEP calves were only similar to SF280, and showed higher rumination frequencies than pens fed 50 and 80% concentrate. Daily variations in frequency of oral manipulation of the pen, tongue playing and lying are shown in Fig.9.1.

### 3.2 Intake of calves in SEP and MIX

Total kg fresh product intakes were  $425.3 \pm 9.0$  and  $465.6 \pm 19.0$  for MIX and SEP from 12 to 28 wk of age. For SEP calves this intake translates to  $298.3 \pm 9.7$  kg/d of DM. In comparison, intakes were  $267.8 \pm 0.8$  and  $288.2 \pm 0.5$  kg/d of DM for SF280 calves fed 80% and 50% concentrate, respectively from 12 to 28 wk of age. At 15 wk of age, SEP calves consumed  $1146 \pm 125.1$ ,  $368 \pm 37.4$ , and  $134 \pm 16.0$  g/d of DM concentrate, maize silage and straw. At 24 wk of age, SEP calves consumed  $2325 \pm 63.3$ ,  $644 \pm 66.3$ , and  $313 \pm 26.2$  g/d of DM concentrate, maize silage and straw. The average percentages (on DM basis) of concentrate, maize silage and straw selected by SEP calves were rather constant between ages and were  $70.8 \pm 0.4\%$ ,  $20.2 \pm 0.4\%$ , and  $9.1 \pm 0.3\%$ .

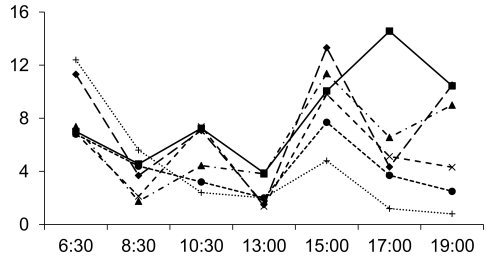
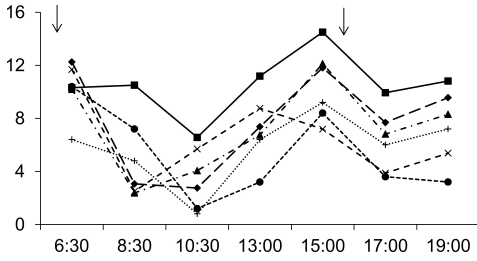
### 3.3 Ad libitum long straw (STR)

The provision of ad libitum long straw on top of a conventional veal feeding strategy resulted in lower levels of oral manipulation of the pen and tongue playing (Table 9.8). An interaction between the presence of a straw rack and age was found for feeding, rumination and licking other calves. STR calves were observed feeding less often at 15 than 24 wk and more so than calves without a straw rack at 24 wk of age (Table 9.8). STR calves showed more rumination at both ages compared to calves without ad

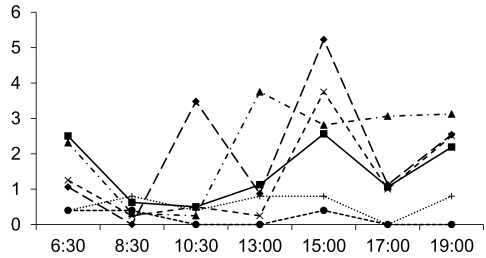
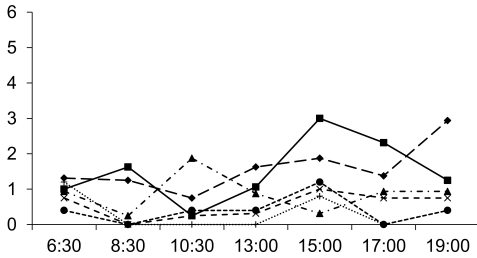
15 wk

24 wk

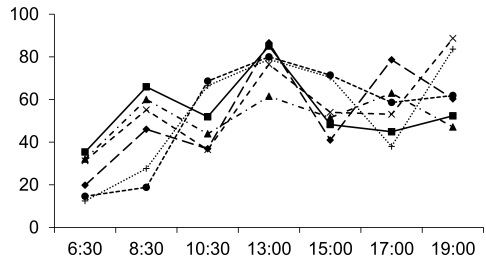
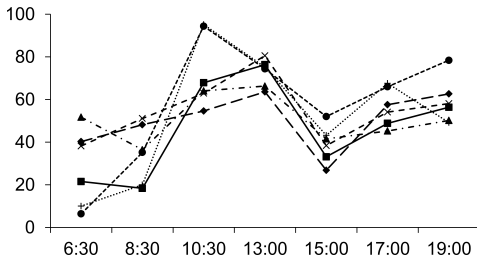
Oral manipulation of the pen



Tongue playing



Lie



Observation time

Figure 9.1: Frequency (mean % total scans) at which calves were observed manipulating the environment, performing tongue movements and lying throughout the day at 15 and 24 wk of age in calves fed 25, 110, 200, or 280 kg solid feed (square, diamond, triangle, and × respectively) and in calves fed ad libitum solid feed with ration components mixed (open circle), or in separate troughs (cross). Arrows in the first graph indicate the start of feeding (06:00 and 16:00 h).

Table 9.8: Frequency of behaviour (mean % total scans) and SEM, observed in veal calves with or without a straw rack. Behaviour was recorded using instantaneous scan sampling. P-values for the fixed main effect of the presence of a straw rack and the interaction between presence of straw rack and age are given.

	Age	Straw rack		SEM	P-value	
		Absent	Present		Straw rack	Straw rack × Age
Manip <sup>1</sup>		7.8	4.0	0.71	0.021	NS
Tongue		2.0	0.4	0.36	0.027	NS
Feed	15 wk	8.1 <sup>a,x</sup>	8.5 <sup>a,x</sup>	1.37		0.021
	24 wk	4.0 <sup>a,x</sup>	15.8 <sup>b,y</sup>			
Ruminate	15 wk	8.6 <sup>a,x</sup>	13.0 <sup>b,x</sup>	1.18		0.041
	24 wk	6.0 <sup>a,x</sup>	16.5 <sup>b,x</sup>			
Lie		51.9	57.1	1.79	NS	NS
Sniff		3.4	3.1	0.21	NS	NS
Play		1.3	1.1	0.31	NS	NS
Lick calf	15 wk	2.1 <sup>a,x</sup>	1.2 <sup>a,x</sup>	0.21		0.003
	24 wk	1.2 <sup>a,y</sup>	1.9 <sup>a,y</sup>			
Suck calf		0.5	0.5	0.11	NS	NS
Groom		1.9	3.1	0.27	NS	NS

<sup>1</sup> Manip = oral manipulation of pen.

<sup>a-b</sup> Where there is an interaction, means with different superscripts in a row differ ( $P < 0.05$ ).

<sup>x-y</sup> Where there is an interaction, means with different superscripts in a column for one behaviour differ ( $P < 0.05$ ).

libitum straw (Table 9.8). Finally, both these groups of calves changed the frequency at which they licked their pen mates, but in opposite directions, with STR calves licking less and calves without a straw rack licking more at 15 compared with 24 wk of age (Table 9.8). The usage of the straw in the racks (% observations), derived from video observations, is shown in Fig.9.2. This graph shows two rack usage peaks at 18-19 wk, one before the afternoon feed (16:00 h) and one just before the lights were switched off (22:00 h), and three rack usage peaks at 23-24 wk, the same two as for 18-19 wk plus one after the morning feed. The usage of straw was approximately 650 g/d per calf, including spilling the straw on the floor as well as consumption.

### 3.4 Solid feed amount and MR feeding method

MR feeding method affected sniffing frequency, with AMD calves showing less sniffing than bucket-fed calves (Table 9.9). An interaction between MR feeding method and

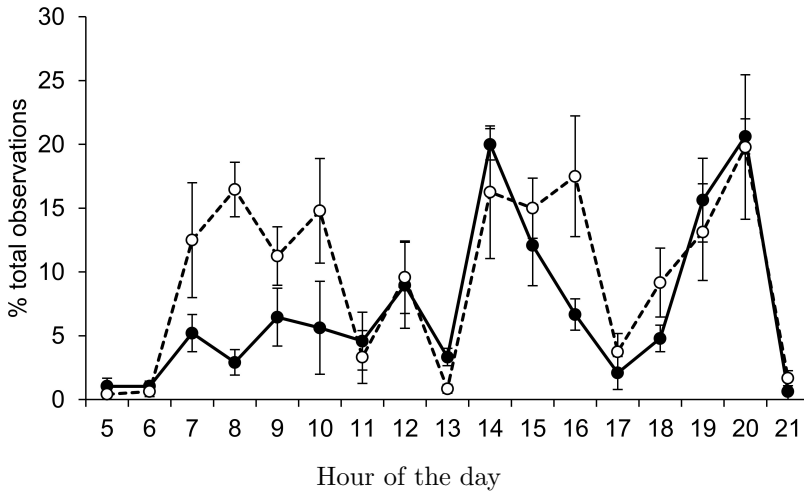


Figure 9.2: Average percentage of observations (out of 60 = 12 scans on 5 calves) within a pen using the straw rack between 05:00 and 22:00 h at 18-19 (continuous line) and 23-24 wk of age (dashed line). These data are based on four pens (5 calves per pen), with two repetitions at each age (1 wk apart). The error bars show the variation between pens.

age was found for tongue playing and lying. At 15 wk of age, AMD calves tongue played less than bucket-fed calves. However, AMD calves increased their tongue playing frequency from 15 to 24 wk of age, resulting in similar levels of tongue playing between these two groups at 24 wk (Table 9.9). AMD calves decreased their lying frequency from 15 to 24 wk, in such a way that they were lying less than bucket-fed calves at 24 wk but not at 15 wk (Table 9.9). MR feeding did not have an effect on any of the other behaviours monitored (Table 9.9). An age effect was found for sniffing and grooming (Table 9.9), with calves increasing sniffing (15 wk:  $3.4 \pm 0.3\%$ ; 24 wk:  $4.2 \pm 0.3\%$ ) and decreasing grooming (15 wk:  $3.3 \pm 0.4\%$ ; 24 wk:  $1.9 \pm 0.2\%$ ) with age.

### 3.5 Characteristics of faeces

The presence of diarrhoea within a pen seemed affected by solid feed level (plus added controls), but not by % concentrate, MR feeding method, or provision of ad libitum straw (Table 9.10). SF25 calves showed the highest levels of diarrhoea at both 14 and 24 wk of age. Clay-like faeces was not affected by any of the feeding treatments.

Table 9.9: Frequency of behaviours (mean % total scans) and SEM, observed in veal calves fed milk replacer with an automated milk dispenser or a bucket, and one of two solid feed amounts. Behaviour was recorded using instantaneous scan sampling. No interaction with solid feed level was found. Milk replacer was provided in such a way that aimed for equal carcass weight gain across treatments.

	Age	MF <sup>1</sup>		SF <sup>2</sup>			P-value <sup>3</sup>		
		AMD	bucket	25	200	SEM	MF	SF	Age
Mamip <sup>4</sup>	7.1	7.5	8.5	6.1	0.78	NS	NS	NS	NS
Tongue	15 wk	0.4 <sup>a,x</sup>	1.1 <sup>b,x</sup>	1.2	0.9	0.15	NS	NS	0.005
	24 wk	1.6 <sup>a,y</sup>	1.1 <sup>a,x</sup>						
Feed	7.4	5.7	2.1	10.9	1.18	NS	0.002	NS	NS
Ruminante	9.4	10.6	7.0	12.9	0.69	NS	<0.001	NS	NS
Lie	15 wk	54.8 <sup>a,x</sup>	50.3 <sup>a,x</sup>	53.2	50.0	1.49	NS	NS	0.007
	24 wk	45.1 <sup>a,y</sup>	56.3 <sup>b,x</sup>						
Sniff	3.3	4.3	3.9	3.7	0.24	NS	NS	0.041	NS
Play	1.3	1.6	1.4	1.5	0.16	NS	NS	NS	NS
Lick calf	1.3	1.3	1.4	1.2	0.13	NS	NS	NS	NS
Suck calf	0.5	0.8	0.9	0.5	0.14	NS	NS	NS	NS
Groom	2.4	2.9	2.1	3.2	0.25	NS	0.016	0.003	NS

<sup>1</sup> MF = milk feeding method; AMD = automated milk dispenser.

<sup>2</sup> SF = solid feed amount (in total kg DM from 2 to 28 wk of age).

<sup>3</sup> MF = main effect for milk feed method; MF×Age = interaction between milk feeding method and age.

<sup>4</sup> Mamip = oral manipulation of pen.

<sup>a-b</sup> Where there is an interaction, means with different superscripts in a row differ ( $P < 0.05$ ).

<sup>x-y</sup> Where there is an interaction, means with different superscripts in a column for one behaviour differ ( $P < 0.05$ ).

Table 9.10: Prevalence of diarrhoea, thick manure and clay-like manure (at pen level) within different feeding treatments at 14 and 24 wk of age.

	SF <sup>1</sup>				Controls <sup>2</sup>		%C <sup>3</sup>		MF <sup>4</sup>		STR <sup>5</sup>	
	25	110	200	280	MIX	SEP	50	80	AMD	bucket	No	Yes
<i>14 wk</i>												
Diarrhoea	75 <sup>a</sup>	13 <sup>b</sup>	0 <sup>b</sup>	13 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	13	38	50	25	25	0
Clay-like	13	13	0	0	0	0	6	6	25	13	25	0
<i>24 wk</i>												
Diarrhoea	88 <sup>a</sup>	13 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	40 <sup>a,b</sup>	20 <sup>b</sup>	13	38	50	38	25	0
Clay-like	25	13	0	0	0	0	6	6	50	13	0	0

<sup>1</sup> SF = solid feed level in total kg DM from 2 to 28 wk of age.

<sup>2</sup> MIX = ad libitum solid feed mixture; SEP = ad libitum solid feed with ration components in separate troughs.

<sup>3</sup> %C = percentage concentrate.

<sup>4</sup> MF = milk feeding method; AMD = automated milk dispenser.

<sup>5</sup> STR = Presence of a straw rack providing ad libitum long wheat straw.

<sup>a-c</sup> Means with different superscripts differ ( $P < 0.05$ ): pairwise comparisons between solid feed amount and added controls (Fisher's exact test).

## 4 Discussion

This study aimed to investigate the impact of various feeding strategies on veal calf behaviour and faeces characteristics reflecting gastrointestinal health status.

Abnormal oral behaviours, feeding and rumination were affected by changes in solid feed amount and percentage concentrate, including the three added ad libitum controls. Increasing solid feed provision when the composition included 80% concentrate had little impact on rumination and abnormal oral behaviours, relative to 50% concentrate. The only feeding strategy that seemed to substantially reduce the frequency of oral manipulation of the pen was SF280 with 50% concentrate. This is also the feeding strategy that resulted in a high level of rumination (i.e. approximately 20%). A rumination frequency of 20% was also reported in previous work with similar behavioural observation methods (Webb et al., 2013) and in a study where calves were given ad libitum access to MR and five solid feed components (Webb et al., 2014a). Providing solid feed with 50% instead of 80% concentrate increased the frequency of rumination and decreased the frequency of abnormal oral behaviours.

Consistent with previous findings, tongue playing seemed less associated with feeding and activity times, than oral manipulation of the pen (Webb et al., 2012). This may suggest that these two forms of abnormal oral behaviour have two distinct underlying motivations. We suggest that tongue playing is directly related to chewing activity (eating and rumination), and, therefore, is frequent around feeding times as well as during periods when calves normally ruminate (i.e. between feeding times: Veissier et al., 1998; Webb et al., 2012). In contrast, oral manipulation of the pen might be related to anticipation (arousal) of an up-coming meal (Mason, 1991b; Lawrence and Terlouw, 1993), and/or to positive feedback/reinforcement caused by the meal being too short and not satisfying veal calves' eating motivation (Lawrence et al., 1993). This would explain why oral manipulation of the pen is most frequent before and after feeding times.

Calves in pens fitted with a straw rack mostly used the straw after the morning meal and before and after the afternoon meal, which coincides with the times at which calves in pens not fitted with straw racks were most active. Ad libitum provision of long straw to calves otherwise fed a relatively low level of solid feed (i.e. 110 kg DM) with 80% concentrate, resulted in similar levels of abnormal oral behaviours and chewing activity as in calves fed ad libitum solid feed (SEP and MIX). This reinforces the idea that coarse roughages are beneficial in terms of increasing chewing activity and decreasing abnormal oral behaviours in veal calves (Webb et al., 2013). The negative impact of these roughage sources on gastrointestinal health, however, should not be taken lightly. Straw was associated with an exacerbation of abomasal damage



relative to calves fed only MR, or calves fed MR and a less abrasive roughage source (i.e. maize silage or hay) (Webb et al., 2013). However, Veissier et al. (1998) showed that if calves are initially fed concentrate (gradually increased from 100 to 500 g/d) without roughage at a young age, thereby promoting certain aspects of early rumen development (i.e. papillae development), then the provision of straw later on (i.e. 450 g/d from 7 wk of age onwards) may not exacerbate abomasal damage compared to calves fed only MR.

Calves provided with *ad libitum* solid feed, with components in separate troughs (SEP), consumed approximately 2.3 kg/d of DM concentrate and 0.9 kg/d of DM roughage at 24 wk of age. Previous work on veal calves revealed an average voluntary intake of hay of 1.2 kg of DM/d at 27 wk of age (Webb et al., 2013). Moreover, in a previous study, we found a total average voluntary intake of 3.2 kg of DM/d, with 2.1 kg of DM/d concentrate and 1.1 kg of DM/d roughage, in 27-wk-old calves with *ad libitum* access to MR, concentrate, hay, maize silage and straw (Chapter 4). Despite large differences in MR intake and experimental set-up, calves seem to consume similar amounts of concentrate (if present) and roughage in these studies. SF280 calves had the closest intake (although slightly lower) to that of MIX and SEP calves from 12 to 28 wk of age. Moreover, MIX and SEP calves consumed on average 70% concentrate, which makes these two control treatments similar to the SF280 with 80% concentrate in terms of solid feed intake. For most behaviours and for faeces measurements, MIX and SEP calves were similar to SF280 calves despite the MR and solid feed intakes being different (i.e. MR intake was higher for MIX and SEP calves, which had the same MR provision as SF200 calves with 50% concentrate). In terms of tongue playing and oral manipulation of the pen, MIX and SEP calves were more similar to calves fed 50% compared with 80% concentrate. This could be explained by 80% concentrate not stimulating optimum rumination in calves, and therefore, not being sufficient to prevent the development of abnormal oral behaviours, such as tongue playing and oral manipulation of the pen.

MR feeding method had an impact on the frequency of tongue playing. Along with oral manipulation of the pen, tongue playing is thought to stem from frustration resulting from limited chewing opportunity (whether feeding or rumination) (Mattiello et al., 2002; Webb et al., 2012, 2013). Other studies have also suggested a link between tongue playing and sucking on a teat (Seo et al., 1998b). The present study aimed to verify whether tongue playing was related to chewing or sucking, and a relationship to both these motivations was found. Rumination was especially affected by roughage amount, rather than total solid feed amount: higher amounts of solid feed in calves fed 80% concentrate had little impact on rumination frequency. Only in calves fed 50% concentrate, did solid feed amount have an impact on rumination

frequency, suggesting concentrate had little impact on rumination. This is logical since one of the main functions of rumination is to reduce the size of feed particles in the rumen before passage into the abomasum. Therefore, concentrate, with its small particle size, is unlikely to stimulate much rumination. A similar (although inverted) pattern of results was found for tongue playing, suggesting rumination and tongue playing are linked. Indeed, tongue playing was only affected by roughage provision and ad libitum provision of straw, and not by total solid feed provision. In addition, low levels of tongue playing were observed in AMD calves at 15 wk of age (but not at 24 wk), which may suggest that tongue playing is also related to sucking. It is unlikely that MR feeding frequency played a role here (van den Borne et al, unpublished data). Tongue playing can take two general forms: i.e. tongue rolling inside the mouth or tongue rolling outside of the mouth (personal observation). These two forms could potentially stem from different motivations: i.e. sucking and ruminating. Tongue rolling inside the mouth looks similar to a sucking movement (some calves tilt their head backwards whilst performing this behaviour), whereas tongue rolling outside of the mouth could mimic the tongue movement used to pull grass up from the ground. To confirm this hypothesis, studies would need to record these two types of abnormal tongue movements separately, and see whether they are differentially affected by roughage provision and the availability of a teat. Moreover, tongue rolling inside, as opposed to outside, the mouth should be more frequent in young calves (up to 8 wk of age), because sucking motivation is likely to be stronger at a young age (Wiepkema, 1987). Finally, observing both tongue movements in the same individual at different times of day may also point to separate motivations.

Playing frequency was affected by the composition of the solid feed, with calves fed a solid feed with 80% concentrate showing a decrease in playing frequency with age. Playing is considered an indicator of positive welfare, or ‘luxury’ behaviour in animals (Lawrence, 1987). This could be an indication that calves with 80% concentrate experienced a decrease in welfare. However, no age differences were observed for the frequencies of rumination or abnormal oral behaviours so this hypothesised decrease in welfare may have been related to (gastrointestinal) health, rather than behaviour.

The frequency of lying, sniffing, licking calf, sucking calf, and grooming were unaffected by aspects of the feeding strategy in the current study. Lying seems more linked to the housing system than to solid feed provision, with group-housed calves (40-80 calves per pen) fed MR via AMDs lying more than conventional group-housed (i.e. 5 to 7 calves per pen) or individually housed calves (Bokkers and Koene, 2001). In the present study we observed a decrease in lying (i.e. increase in activity) in AMD calves from 15 to 24 wk of age. Although no significant age effect was observed for lying behaviour for other treatments, SEP and MIX calves seem to have also decreased

their lying from 15 to 24 wk of age (Fig. 9.2). Calves at pasture naturally become more active (i.e. spend less time lying, more time standing and move across larger distances) as they grow older (0 to 8 wk of age) (Wood-Gush et al., 1984), and this could also be the case in calves from 15 to 24 wk of age. A failure to observe a decrease in lying in the other treatments could potentially indicate abnormal behaviour and might be a warning sign for poor welfare. Alternatively, AMD calves may have been more active due to competition around the AMD or due to an increased number of MR feeding times relative to bucket-fed calves. Therefore, differences in lying may have been unrelated to differences in welfare.

Sniffing could be a measure of exploration, which was previously related to welfare in beef cattle (Schulze Westerath et al., 2009). Sniffing could indicate feed searching and was, thus, expected to be performed more frequently in calves with lower solid feed provisions, but this was not found. This might be a consequence of this study adjusting MR provision for equal growth between treatments, which may have resulted in similar levels of satiety (e.g. similar nutrient uptake) despite differences in solid feed provision. It is unknown how rumen fill versus abomasum fill affects satiety. Licking pen mates may also signal feed searching and was linked to veal calf feeding strategy in past studies (Mattiello et al., 2002; Webb et al., 2013). Licking self (groom) or other calves was of particular interest in individually housed calves that seemed to excessively lick themselves (Bokkers and Koene, 2001), and in individual and group housed calves that developed hairballs in their rumen (Toofanian, 1976; Bokkers and Koene, 2001). The present study, however, failed to find an association between feeding strategy and licking of hair. Therefore, excessive licking of hair may be more related to housing than feeding conditions. Moreover, a causal link between the frequency of hair licking and hairball prevalence in veal calves is not corroborated (Morisse et al., 1999; Webb et al., 2013).

Preventing the sucking of body parts of other calves, including prepuce, nose, tail, ears and joints, (also referred to as cross-sucking, De Passillé, 2001), and urine drinking is, along with the monitoring of drinking behaviour and health, an important reason why veal calves typically spend the first few weeks of the fattening period in individual pens. Cross-sucking seems to be triggered by ingestion of milk (Lidfors, 1993). Minimising cross-sucking can be achieved by reducing MR flow out of a teat, providing dry teats or providing hay alongside MR in dairy calves (De Passillé, 2001). In the present study, calves fed via an AMD had MR available at least 3 times a day (as opposed to twice a day in bucket-fed calves), had the opportunity to suck, and had a reduced MR flow and abomasal fill compared with bucket-fed calves. Therefore, we hypothesised that cross-sucking would be reduced in calves fed MR via an AMD as opposed to open buckets. We also hypothesised that cross-sucking would be reduced

in calves fed more roughage. However, these hypotheses could not be supported by the current study. In fact, very low levels of cross-sucking were observed in all treatments, consistent with Smits and De Wilt (1991), and this possibly concealed treatment differences. Cross-sucking is more frequent in young calves and may not be an important behaviour past 8 wk of age, when calves start making the switch from pre-ruminants to full ruminants and when chewing motivation may override sucking motivation (Wiepkema, 1987; Margerison et al., 2003).

Diarrhoea was especially high in calves fed the lowest amount of solid feed (i.e. SF25), and, thus, the highest amounts of MR, at both 14 and 24 wk of age. However, no differences in diarrhoea incidence were found between the two concentrate percentages or the two MR feeding methods. It is unknown whether high diarrhoea incidence was caused by low solid feed provision, high MR provision or a combination of these two factors. Diarrhoea incidence was not increased in dairy calves given ad libitum access to MR, compared with calves fed restricted MR (Borderas et al., 2009), implying MR provision may not be the main cause for diarrhoea in the present study. However, in Borderas et al. (2009) calves were fed MR via AMDs not buckets, and this may reduce diarrhoea incidence by allowing more MR feedings. Clay-like faeces signal ruminal drinking, i.e. “the milk is forced into the rumen where it undergoes abnormal decomposition causing physiological disturbances” (Van Weeren-Keverling Buisman et al., 1988). This condition is thought to be related to feeding milk from open buckets, and, depending on solid feed sources fed and age of the animal, can cause severe bloating (Wise and Anderson, 1939; Van Weeren-Keverling Buisman et al., 1988). Slower ingestion of milk from a teat was hypothesised to reduce the incidence of ruminal drinking, but this was not observed in the present study as no effect of MR feeding method was observed on the incidence of clay-like faeces.

## 5 Conclusions

Increased roughage provision, but not concentrate, increased rumination and decreased tongue playing. This suggests that future feeding strategies should include increases in roughage amounts, regardless of concentrate amount, if rumination is to be stimulated and abnormal oral behaviour prevalence reduced. Moreover, the similarities in how feeding strategy characteristics affect rumination and tongue playing (although inversely) as well as the similarity in timing of these two behaviours throughout the day points to a causal link between the two. In other words, tongue playing may develop as a direct consequence of insufficient rumination, instead of feeding behaviour. STR calves had similar levels of rumination and abnormal oral behaviours as MIX and SEP calves. Therefore, offering ad libitum long straw may

offer a simple strategy to improve veal calf welfare. Offering MR via an AMD did not seem to affect behaviour much compared with calves fed MR in buckets, except that tongue playing was reduced at 15 wk. Tongue playing was related to both roughage amount and AMD feeding, suggesting two separate motivations underlying the development of this behaviour. Only solid feed amount affected aspects of faeces: SF25 calves had the highest diarrhoea incidence. The prevalence of clay-like faeces, which signal ruminal drinking, was not affected by feeding strategy.



# Chapter 10

## General discussion

*“An animal’s life would not be worth living when the balance of its negative experiences outweighs its positive experiences.” (Green and Mellor, 2011)*





# 1 Introduction

Current feeding strategies used to raise veal calves may be linked with welfare impairments. These impairments include the inability to perform sufficient levels of eating and rumination behaviours (henceforth referred to as chewing activity), which is signalled by the development of abnormal behaviours in the calves (Veissier et al., 1998). These abnormal behaviours include excessive oral manipulation of the environment, grazing of the coat of pen mates, as well as tongue playing (i.e. rolling and unrolling of the tongue inside and outside of the mouth) (De Wilt, 1985; Kooijman et al., 1991; Veissier et al., 1998; Mattiello et al., 2002) and are consequently termed abnormal oral behaviours (**AOB**).

The main aim of the work presented in this thesis was to develop novel feeding strategies to improve the welfare of veal calves. Improving the welfare of veal calves involved attempts to maximise chewing activity, subsequently minimising AOB, and optimising gastrointestinal health. AOB were used as an indicator of poor welfare when comparing veal calves fed different feeding strategies and it is therefore important to understand how these behaviours come about. Another approach used in this thesis was to assess the feeding preferences of calves, and it is therefore important to use the best available methods for the assessment of animal feed preferences.

This discussion is comprised of three main sections: 1) understanding underlying mechanisms of AOB in veal calves, 2) evaluating different methods for the assessment of animal feed preferences, and 3) practical implications of this work with the objective of developing novel feeding strategies for veal calves.

## 2 Underlying mechanisms for abnormal oral behaviours in veal calves

Identifying what causes AOB to develop in veal calves helps to understand why these behaviours are appropriate welfare indicators. The aim when devising novel feeding strategies to improve veal calf welfare is often to minimise these AOB.

Stereotypies are “behavioural elements that have a very constant form, that are repeated over and over again, (may) differ from individual to individual and that seem to have no function” (Wiepkema, 1987). For the sake of argument, I will consider all AOB in veal calves to be stereotypies, or at least abnormal behaviours that would, in time, become a form of ritualised, stereotypic behaviour. Certain of the AOB

recorded in the previous chapters may in fact reflect normal exploratory behaviour, as no distinction was made between ritualised and non-ritualised oral manipulation of the pen or bucket, and no attempt was made to find a threshold between normal and abnormal levels of a given oral behaviour in calves. However, in the previous chapters, oral manipulation of the pen/bucket was recorded, not in an attempt to find at which level or in which form oral behaviours in calves can be considered abnormal, but instead to compare the welfare of calves with different feeding regimes. In addition, distinguishing between normal and abnormal oral manipulation of the pen/bucket may bring a level of unwanted subjectivity to the behavioural observations. Tongue playing is accepted as an oral stereotypy, commonly studied in captive cattle (e.g. Wiepkema, 1987; Redbo et al., 1996; Redbo, 1998; Seo et al., 1998b; Morisse et al., 1999; Mattiello et al., 2002). Grazing the coat of other calves was observed very little in this thesis and is not thought to have affected the results much.

In this section, possible underlying mechanisms of AOB in veal calves were investigated in light of results from the chapters in this thesis. I will start with attempting to better understand chewing activity in calves, since AOB are thought to result from a frustrated drive to chew in veal calves.

## **2.1 Ruminants ruminate: understanding chewing activity in calves**

### **Is chewing activity a behavioural need in calves?**

One recurrent idea is that stereotypies occur in animals that are frustrated due to the inability to satisfactorily perform certain, internally driven, motivated behavioural patterns, such as for example feeding behaviours in feed restricted animals (Rushen et al., 1993). Since AOB are thought to be linked to a frustrated chewing activity (Veissier et al., 1998), I will first evaluate whether this latter behaviour could be such an internally driven, highly motivated behavioural pattern.

‘Behavioural needs’ have been put forward as behaviours, for which the motor act in itself, irrespective of the physiological need it is associated to or aims to fulfil, is internally motivated (Hughes and Duncan, 1988a; Jensen and Toates, 1993). The following criteria are associated with behavioural needs: 1) these behaviours are internally driven, 2) there is a progressive build-up in the tendency to perform them, 3) performing these behaviours, irrespective of environment or functional consequence, is rewarding (Hughes and Duncan, 1988b; Jensen and Toates, 1993). Therefore, identifying behavioural needs can be done by showing that a behaviour is internally driven and detached from its original function (Hughes and Duncan, 1988a), or by finding

behaviours for which animals are motivated to work hard (e.g. in an operant conditioning set up) (Jensen and Pedersen, 2008).

Showing that a behaviour is internally driven irrespective of functional consequence can be difficult. Given that rumination in the absence of a consequence on nutritional status or rumen function is difficult, even impossible, to achieve, it may be challenging to attribute the label of ‘behavioural need’ to the behaviour pattern for rumination with absolute certainty (Hughes and Duncan, 1988b). However, even in the absence of roughage, calves will show relatively high levels of chewing movements (i.e. 7% around feeding or 11% throughout the day (Fig.10.1), Chapter 3) without substrate in the mouth, also referred to as ‘sham chewing’ (Morisse et al., 1999). Sham chewing generally occurs in lying calves and involves regular pauses during which marked contraction of the abdomen occur, which resemble the rise of a bolus (personal observation). A rumination-like behaviour detached from its original function, i.e. the reduction of roughage particle size for passage from the rumen to the abomasum (Welch, 1982), suggests an internal drive for rumination, and, thus, suggests that rumination may indeed be a behavioural need.

Calves also seem highly motivated to ‘work’ for access to roughage. If we look at chewing activity as a whole, calves will pay a cost (press a panel with muzzle up to 35 times) to gain access to resources that promote chewing activity, i.e. 5 g of hay or straw, even when fed high levels of MR and concentrate in the home pen (Chapter 6). Calves even showed a preference for long particles (20-30 cm) over short particles (2-3 cm) of hay (Chapter 6). Longer particles of roughage are associated with increased chewing activity in cows (Couderc et al., 2006) and calves (Chapter 2). So calves may have been working for access to more chewing activity, whether immediate eating or rumination. Because it is difficult to separate chewing activity and improved rumen function, it is unknown, whether calves were working for chewing activity per se. But this finding does seem to be in line with chewing activity being a behavioural need in calves.

### **Chewing activity and solid feed intake**

Frustration is thought to occur in veal calves due to a limited opportunity for chewing activity, resulting from restricted solid feed provision (Veissier et al., 1998). As young as three weeks old, calves at pasture start nibbling and investigating grass and other plant material in their environment (De Vargas et al., 2011), slowly but surely developing the motor skills required for grazing and chewing, and ultimately, rumination. This early experience with roughage, which is self-driven (i.e. trial and error learning, imprinting) and dam-driven (i.e. social learning), is important in the learn-

ing of post-ingestive consequences of ingesting different types and amounts of plant material (Provenza and Balph, 1987). This learning process results in the development of dietary preferences, which in the long run are aimed at not only maximising nutrient uptake, but also minimising ingestion of aversive or toxic plant metabolites (Provenza and Balph, 1987). Cattle at pasture spend a large portion of their time budget grazing and ruminating (i.e. 95% of diurnal time budget, Kilgour et al., 2012), and animals unable to perform these behaviours at particular times of the day will adjust their time budget in order to maintain a relatively equal performance of these behaviours (Gregorini et al., 2009, 2012). Therefore, chewing activity is an important behavioural pattern in ruminants in that it represents a large proportion of an individual's diurnal time budget.

Chewing activity is important to calves, but how can we stimulate this behaviour? Understanding the relationship between chewing activity and solid feed provision is key to the development of animal-friendly diets for calves.

Interestingly, the same amount of solid feed at the beginning of the fattening period does not result in similar levels of chewing at the end of the fattening period in veal calves (Mattiello et al., 2002). Even when solid feed provision is based on metabolic weight, and, thus, increases with age, chewing activity decreases dramatically after the first couple of months (i.e. from 4 to 5 months of age) (Chapter 3). This could be due to more efficient chewing in older more experienced calves. Ageing is generally associated with body and muscle growth, which could lead to bigger mouthfuls and increased chewing speed and strength during eating and rumination. Changes in jaw motion during chewing across ages were observed in human infants (Wilson and Green, 2009). Alternatively, experience with roughage could be responsible for jaw muscle and motor skill development resulting in improved manipulation, ingestion and reduction of roughage particles (Provenza and Balph, 1987).

There might also exist an internally driven increase in motivational propensity to eat solid feed and ruminate, possibly mediated by physiological or psychological changes, and separate from the dam increasingly rejecting calves for suckling. The underlying function of this motivation may be to ensure that calves naturally increase their solid feed intake and make the switch from monogastric/pre-ruminant animals to full ruminants. A voluntary decrease in proportion of MR versus concentrate was observed in calves with ad libitum access to feed (Chapter 4). However, if there existed an internally driven increase in the motivation to express chewing activity, we might expect an increase in sham chewing over time in calves fed no solid feed, which was not observed (Fig.10.1) (Chapter 3). Another interesting finding is the absence of a relationship between chewing activity and intake of solid feed observed in Chapter 4, regardless of whether individual solid feed types (i.e. concentrate, barley straw,

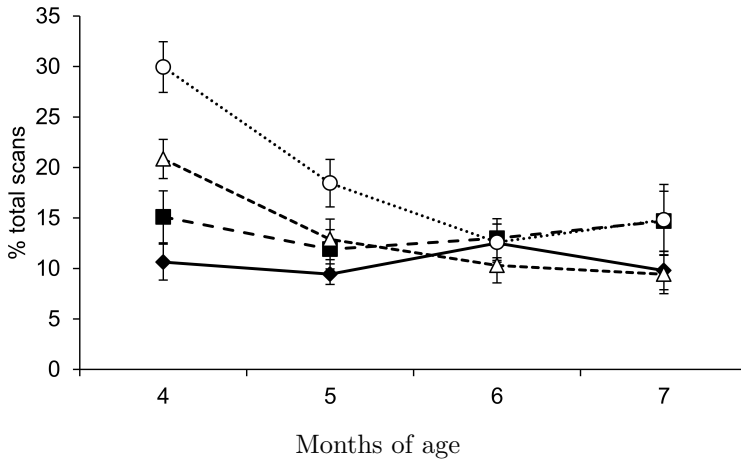


Figure 10.1: Chewing activity (% total scans) changes across age observed using instantaneous scan sampling in veal calves fed 0 (diamonds), 9 (squares), 18 (triangles), or 27 (circles) g DM/kg<sup>0.75</sup>/d of a solid feed mixture (50% concentrate, 25% maize silage, 25% chopped wheat straw, on DM basis) on top of milk replacer (adjusted for equal growth). Data from Chapter 3.

hay and maize silage) or all roughages or all solid feed components were considered. In addition, in Chapter 3 no difference in chewing activity was observed between treatments at the end of the fattening period despite large differences in solid feed provision (Fig.10.1).

Possibly as a result of ‘chewing efficiency’ increasing with age/experience, and varying between individuals, no simple linear relationship seems to exist between solid feed provision and chewing activity. These individual differences in chewing efficiency may in part be responsible for differences in solid feed intake between individuals of the same pen, where solid feed provision is restricted. This makes the conception of novel feeding strategies to maximise chewing activity in veal calves more complex.

### Relationship between chewing activity and abnormal oral behaviours

Past research has consistently implied an inverse relationship between chewing activity and level of AOB in veal calves (Kooijman et al., 1991; Mattiello et al., 2002). This thesis to a large extent confirms this relationship (Chapters 2, 3 and 9). Looking at individual calves from Chapters 3 and 4, there was a clear inverse relationship between chewing activity and AOB levels (Fig.10.2).

The previous section, however, suggests no simple relationship between chewing

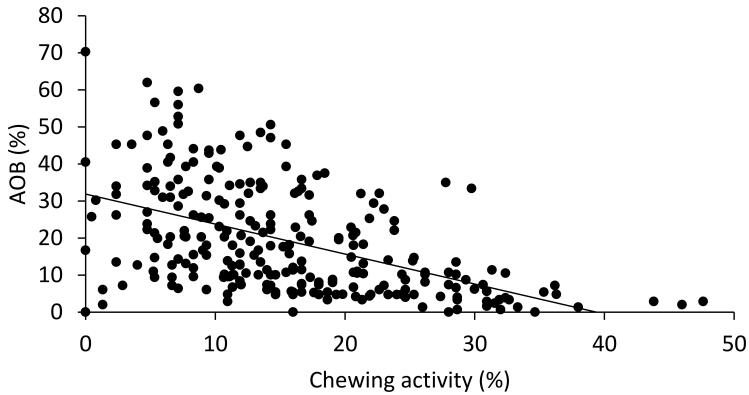


Figure 10.2: Relationship between chewing activity and abnormal oral behaviours. AOB = oral manipulation of pen structure, tongue playing, and grazing the coat of other calves. Data based on 48 calves, observed from 4 to 7 months of age (Regression analysis  $P < 0.001$ ) and 40 calves, observed at 3 and 6 months of age (Regression analysis  $P = 0.007$ ). Data from Chapters 3 and 4

activity and solid feed provision (or intake). Therefore, although larger amounts of solid feed may lead to higher levels of chewing and subsequently lower levels of AOB at group level, large individual variation in 1) chewing efficiency and 2) propensity to develop AOB may result in large individual differences in AOB levels (apparent in Fig.10.2 and Chapter 8). The inverse relationship between chewing activity and AOB may also be explained by differences in the type of AOB that are displayed. Sham chewing is difficult to separate from true chewing with absolute certainty, leading to these two behaviours being combined into a single behavioural element in Chapters 3 and 4. Calves will most likely show a ‘preferred’ stereotypic behaviour, meaning that calves that develop sham chewing may appear to have high levels of chewing activity and low levels of AOB, but may still be receiving little roughage. However, reductions in levels of chewing activity over time in calves with some amount of roughage (Chapter 3), signifies that most of the chewing observed in these calves was indeed true chewing and not sham chewing. Sham chewing would be expected to increase with time in a sub-optimal environment (Morisse et al., 1999), similarly to other AOB (Chapters 2, 3, 4, and 9).

### Conclusions on understanding chewing activity

This thesis provides evidence that supports the idea that chewing activity, including eating and rumination, is a behavioural need, or highly motivated behaviour that is

internally driven in calves. As such, one should aim to maximise opportunities to perform these behaviours in captive calves, such as veal calves, when devising novel feeding strategies. However, the complex relationship between solid feed provision and chewing activity, and the importance of individual differences in both chewing efficiency and propensity to develop AOB pose a challenge in the development of animal-friendly diets for veal calves.

## 2.2 Mediating mechanisms and possible functions of abnormal oral behaviours in calves

The following section attempts to tackle underlying factors mediating the development of AOB in veal calves. In the past, understanding stereotypies has been tackled from two angles: 1) looking at physiological correlates of stereotypies that point to a relationship with chronic stress (e.g. Van Reenen et al., 2001), 2) understanding the underlying motivation behind stereotypies (e.g. Lawrence et al., 1993).

### Chronic stress

**The HPA axis.** The hypothalamic-pituitary-adrenal (**HPA**) axis is responsible for a chain reaction in response to stress, that ultimately results, among other things, in adrenocorticotrophic hormone (**ACTH**) and cortisol (in most mammals) being released into the body (Tsigos and Chrousos, 2002) (see general introduction for more detail).

AOB in veal calves occur in sub-optimal environments suggesting an association between AOB and chronic stress. However, possibly due to large individual variations in chewing efficiency and propensity to develop AOB, studies in the past have not found relationships between treatments with high AOB levels (e.g. low solid feed provision) and physiological measures of chronic stress, e.g. cortisol response to ACTH challenge (Veissier et al., 1998; Mattiello et al., 2002). However, at the individual level, high AOB level was associated with lower chronic stress in veal calves (Redbo, 1998; Van Reenen et al., 2001). This is consistent with the idea that stereotyping animals are better off than non-stereotyping animals in sup-optimal environments, and that stereotypies are a sort of coping behaviour (Mason, 1991a; Mason and Latham, 2004).

When cortisol baseline and cortisol response to restraint were used as physiological measures of chronic stress, no relationship between the level of tongue playing and chronic stress was found in veal calves (Chapter 8). Previous studies that reported such a relationship used cortisol response to ACTH challenge as a measure of chronic stress. When stressors such as moving to and tethering inside a metabolic cage are used (Chapter 8), it may be more difficult to standardise the challenge, possibly making it more difficult to observe subtle differences in cortisol peaks between animals

performing different levels of AOB in the home pen. This could explain why no relationship was found between tongue playing level and cortisol response to a stressor in Chapter 8.

Moreover, if the stressor is very intense, the cortisol peak might reflect maximum adrenal output and variation between animals in terms of cortisol response to the stressor may reduce. In Chapter 8, the stressor used was individually moving 16-week-old calves to metabolic cages. This procedure involved individual calves being handled and walked from their home pen to a novel room, individually tethered inside cages (although they maintained audiovisual contact with pen mates), and getting harnesses fitted (these procedures were in line with the aims of the main study, Berends et al., 2012a). During the same experiment, calves were moved to the cages a second time at 24 weeks of age and received catheters in both jugular veins. Salivary cortisol response to catheterisation was also measured but unpublished (Fig.10.3). Figure 10.3 clearly shows a much higher cortisol response to catheterisation compared with moving to cages, and therefore, suggests that cortisol response to moving to cages was not so intense that it would have hidden individual differences. However, age of the calves confounded with stressor type in this experiment (the more intense stressor of catheterisation was imposed at 24 weeks, whereas the less intense stressor of moving to cages was observed at 16 weeks). Age differences in baseline ACTH and cortisol response to ACTH have been documented in cattle, with heifers having lower baseline plasma ACTH levels and higher cortisol peaks following synthetic ACTH injection compared with calves (Redbo, 1998).

**Gastrointestinal health.** Another way of measuring chronic stress, is looking at health, as long-term stress is known to suppress immune function in animals (Dantzer and Mormède, 1983) and cause gastric ulceration in certain mammalian species, e.g. humans, rats, pigs and cattle (e.g. Wiepkema et al., 1987). Wiepkema et al. (1987) found a negative relationship between the performance of tongue playing in veal calves and ulceration in the abomasum, but did not find a relationship between measures of anxiety and abomasal damage. They concluded that tongue playing may reduce stress and to some extent ‘protect’ calves against further abomasal ulceration. A similar relationship between abomasal damage and tongue playing on the one hand, and oral manipulation of the environment on the other hand was found in this thesis (Fig.10.4). If abomasal damage in calves can be related to stress, irrespective of AOB, then the performance of AOB in calves may reduce stress in sub-optimal environments and subsequently have beneficial effects on gastrointestinal health.



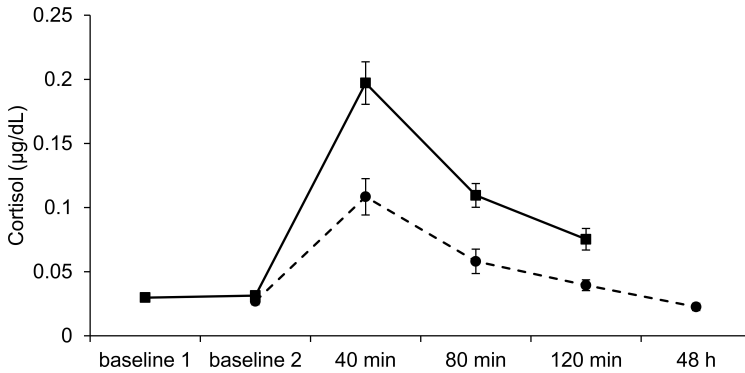


Figure 10.3: Cortisol response ( $\mu\text{g/dL}$ ) to moving to metabolic cages at 16 weeks (circles) or catheterisation at 24 weeks (squares). For cortisol response to moving to cages, only one baseline was taken in the home pen prior to moving (baseline 2). For cortisol response to catheterisation two baseline cortisol measures were taken: 1) in the home pen before moving to metabolic cages (baseline 1), and 2) after one day in the cages, just before catheterisation (baseline 2). Unpublished data.

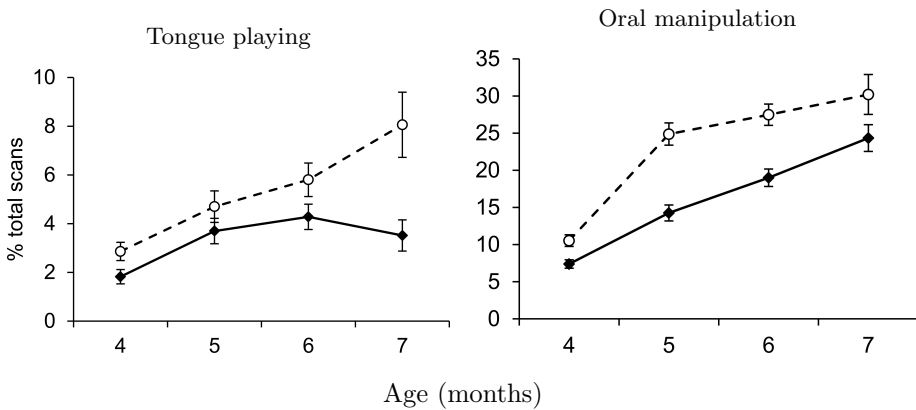


Figure 10.4: Relationship between tongue playing and oral manipulation of the environment (% total scans) and presence of abomasal lesions (present: continuous line; absent: dashed line) at slaughter in veal calves aged between 4 and 7 months. Behavioural data was collected using instantaneous scan sampling weekly. There was a significant difference between calves with and without abomasal damage in terms of oral manipulation of the pen ( $P = 0.052$ ). Unpublished data.

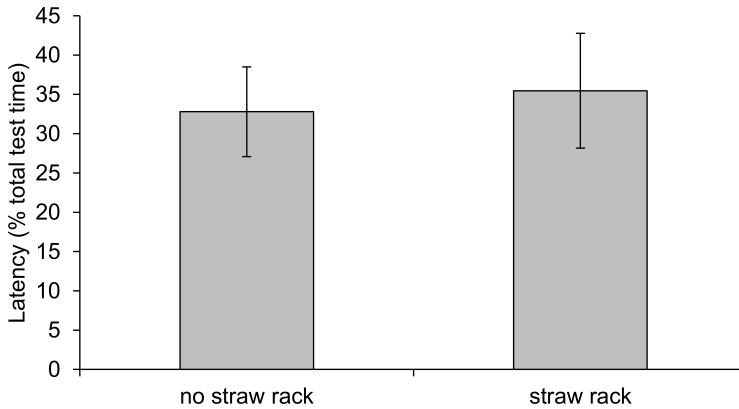


Figure 10.5: Latency (% of total test time) to touch a novel object (orange cone) in veal calves with or without a straw rack (i.e. ad libitum access to long wheat straw). Unpublished data.

### Lack of stimulation and boredom

If veal calves develop AOB as a result of a lack of stimulation, due to little roughage provision and little chewing activity, and subsequently experience a form of boredom, they should show a heightened interest to any novel stimuli, as compared to calves with more roughage or showing higher levels of chewing activity. In line with this idea, we observed that calves with low levels of, or no, solid feed, and consequently showing higher levels of AOB at group level, had shorter latencies to touch a novel ball, and orally manipulated a coverall more than calves with higher amounts of solid feed (Chapter 3).

However, calves with restricted access to solid feed did not show more interest in a coverall than calves with ad libitum access to five diet components (unpublished data). Moreover, no difference in latency to touch a novel object was found between calves with or without ad libitum access to long straw in a rack (Fig.10.5). If half the calves with no additional straw experienced apathy (i.e. lower interest in new stimuli) and the other half experienced boredom (i.e. increased interest in new stimuli) (Meagher and Mason, 2012) then differences could have been obscured in these results. Similar contradictions exist in previous literature, with studies finding increases or decreases in exploratory behaviour in animals housed in non-enriched environments (Meagher and Mason, 2012).

These results are inconclusive and preclude the drawing of conclusions regarding a possible relationship between AOB development and boredom in veal calves.

### **Arousal/anticipation and positive reinforcement**

It was previously suggested that veal calves display most of their AOB in the period immediately before and after feeding times (Veissier et al., 1998). A more detailed investigation of AOB in veal calves throughout the day revealed that only oral manipulation of the environment is more common around feeding, whilst tongue playing is performed throughout the day at a relatively even frequency (Chapters 3 and 9). This could indicate that these two forms of AOB may stem from different (frustrated) motivations, and that tongue playing is, for example, not a form of oral manipulation that became detached from objects (i.e. via a process called emancipation: Mason, 1991b). This suggests that arousal is not an important causal factor for tongue playing performance in calves. Tongue playing may be directly related to a motivation to chew, including eating and rumination. Eating would be located around meal times, whilst rumination is found mostly between the two meals fed to veal calves (Veissier et al., 1998), consistent with results from Chapter 3 and 9. As a consequence, chewing activity was observed throughout the day in veal calves with adequate levels of solid feed, similarly to the observation of tongue playing in veal calves with too little solid feed.

Oral manipulation of the environment may instead be related to behavioural arousal around feeding time. Arousal makes animals more likely to partake in active behaviours (Lawrence and Terlouw, 1993), which might lead calves to excessively orally manipulate the pen and trough. Anticipation of an upcoming meal (Mason, 1991b) might also stimulate oral behaviours in calves. In addition, there is a chance that the short meal actually initially applies positive feedback on feeding behaviour, because negative feedback from ingestion is insufficient (Lawrence et al., 1993). This would result in a heightened motivation to perform oral behaviours directly following a meal (Lawrence and Terlouw, 1993). General behavioural arousal, anticipation and positive feedback have all previously been suggested as underlying mediators of abnormal behaviour in feed restricted pigs (Lawrence and Terlouw, 1993; Lawrence et al., 1993). It is logical to expect different stereotypies to reflect anticipation before a meal on the one hand and positive feedback on feeding motivation after a meal on the other hand. In veal calves, AOB 1 h before and after a meal seem similar (Fig.10.6), but this does not exclude the possibility of the same stereotypies being elicited by a combination of different mechanisms or factors (Mason and Latham, 2004).

### **Iron deficiency**

Nutrient deficiency may explain the development of stereotypies in feed-restricted animals, or animals fed a total mixed ration (Lawrence and Terlouw, 1993). One com-

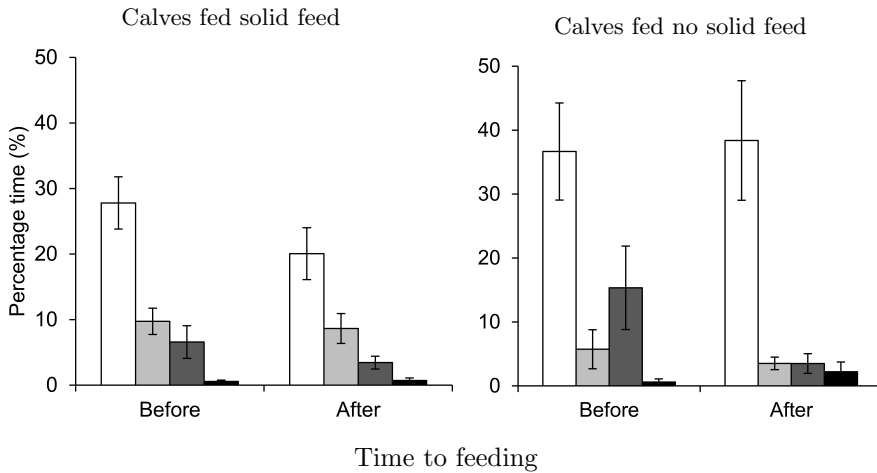


Figure 10.6: Percentage of time (mean  $\pm$  SEM) performing oral behaviours (licking pen [white], licking bucket [light grey], tongue playing [dark grey], or grazing the coat of other calves [black]) in 5 to 7-month-old veal calves with ( $N = 36, 9, 18$  or  $27$  g DM/kg<sup>0.75</sup>/d of a solid feed mixture) or without solid feed provision ( $N = 12$ ) in the hour before and after feeding. Observations were carried out using continuous recording and focal sampling for 10 min per calf. Unpublished data.

monly known nutrient deficiency in veal calves is iron deficiency (Lindt and Blum, 1994). In Chapter 2, two groups of calves with no solid feed supplement received MR with or without additional iron. The only marked difference between these two treatments was observed at 12 weeks of age, and was a higher tongue playing performance in calves with the iron supplement. This suggests that iron deficiency is not a possible causal factor in the development of AOB in calves. Other nutrient deficiencies (e.g. selenium, copper), or imbalances, were not investigated in this thesis.

### Individual calf characteristics

Another interesting finding is the substantial variation in AOB levels between individual calves fed the same diet (Chapter 8). Several explanations for this inter-individual variation are put forward: 1) calves differ in their chewing efficiency; 2) calves differ in their motivation to chew; 3) calves within the same pen have different access to solid feed (due to competition over resources); 4) underlying temperamental traits, such as differences in coping style, mediate the propensity of individual calves to develop AOB in a sub-optimal environment.

I have already discussed individual differences in chewing efficiency above. It is

very likely that these differences in chewing efficiency result in differences in AOB levels. For example, calves who eat and ruminate faster may need more solid feed to satisfy their ‘chewing need’ and, therefore, may be more likely to get more frustrated by a restricted feeding regime and thus be more likely to develop AOB. Another possibility, that is not mutually exclusive from the previous one, is that calves differ in their motivation to chew, or their ‘need’ for chewing activity. Calves with a higher motivation to chew would also be more likely to get frustrated and develop AOB. These two possibilities were not investigated in this thesis *per se*. However, calves differed in how much they were willing to work for roughage on the one hand, and longer particles of roughage on the other hand (Chapter 6). This suggests calves differ in their chewing efficiency and/or motivation to chew. Competition for feed was also not investigated in this thesis, but there is no doubt that calves within the same pen did not consume the exact same amount of solid feed (personal observation). Differences in intake can also be explained by chewing efficiency and motivation, as fast-eating, highly motivated calves will be more likely to displace their pen mates from solid feed buckets.

As mentioned in the introduction of this thesis, coping styles have previously been offered as an explanation for individual differences in propensity to develop stereotypic behaviour in captive animals (Mason, 1991b). The idea is that proactive animals, which are more likely to respond to challenge with activity and be more prone to form routines, are more likely to develop stereotypical behaviour (Mason, 1991b; Ijichi et al., 2013). However, in Chapter 8 we found the opposite result: calves showing a reactive coping style when faced with a stressor (i.e. restraint) were those which displayed higher levels of tongue playing in the home pens. This finding is inconsistent with theoretical articles, but consistent with other experimental articles that investigated horses (Nagy et al., 2010), pigs (Schouten and Wiepkema, 1991) or calves (Redbo, 1998).

## **Conclusions on mechanisms and functions of abnormal oral behaviours**

AOB in veal calves appear to develop due to a number of factors, starting with the thwarting of chewing activity, which is most likely a behavioural need in ruminants. AOB are also likely linked to frustration and subsequent chronic stress, which can be measured physiologically. This is complex and requires the combination of physiological and behavioural measurements. Of great importance is the understanding of individual variation in the propensity to develop AOB, as the performance of stereotypic behaviour in sub-optimal environments has been linked to improvements in welfare. This means that individuals in sub-optimal environments that do not develop

stereotypic behaviour may well be the individuals that are suffering the most.

### 3 How best to assess dietary preferences in ruminants?

#### 3.1 Choice test

One commonly used method to assess dietary preferences in ruminants is the choice test. These tests, however, have a number of limitations: 1) only one measure of preference (e.g. intake), 2) short testing periods, 3) social isolation, 4) limited choices, 5) no cost is imposed on choice (Kirkden and Pajor, 2006). Limitations 1 through 4 were addressed in Chapter 4, where calves' preferences were recorded using intake, duration and visit frequency, over a one week period at 3 and 6 months (with calves having the same dietary choice continuously from 1 to 6 months of age). Calves were tested for their feed preferences in groups of four familiar calves, and solid feed sources included variation in terms of digestible fibre and structure. Moreover, calves were offered free choice between MR, concentrate, maize silage, long barley straw and long hay. When intake, duration and visit frequency were used together to assess dietary preferences in these calves, these three variables showed slightly different preference rankings. In particular, the preference ranking for hay and concentrate varied depending on whether intake (concentrate preferred) or duration (hay preferred) was considered. Similar conclusions were observed for straw and maize silage. Considering only one variable could result in incomplete or wrong conclusions being drawn about feed preferences, and consequently inadequate feeding strategies being devised to improve welfare. Moreover, the feed choices of calves were observed to vary throughout the day (Fig.10.7) and with age (Chapter 4) demonstrating the importance of investigating preferences across an entire 24 h period, and across different ages. Moreover, inter-calf differences in terms of voluntary proportion of roughage, concentrate and MR in the diet seem to reduce with age (Chapter 4), possibly indicating a period of trial and error learning early on (Provenza and Balph, 1987). Intake is also known to vary across days for solid feed in beef calves (Atwood et al., 2001) and MR in dairy calves (Borderas et al., 2009).

Although we tried to address most limitations associated with choice test in Chapter 4, this study still included a number of drawbacks. First, the limited use of one test pen resulted in preferences of calves only being recorded at 3 and 6 months of age, rather than continuously from 1 to 6 months. Calf preferences may vary significantly before 3 months and between 3 and 6 months of age. Future studies should use

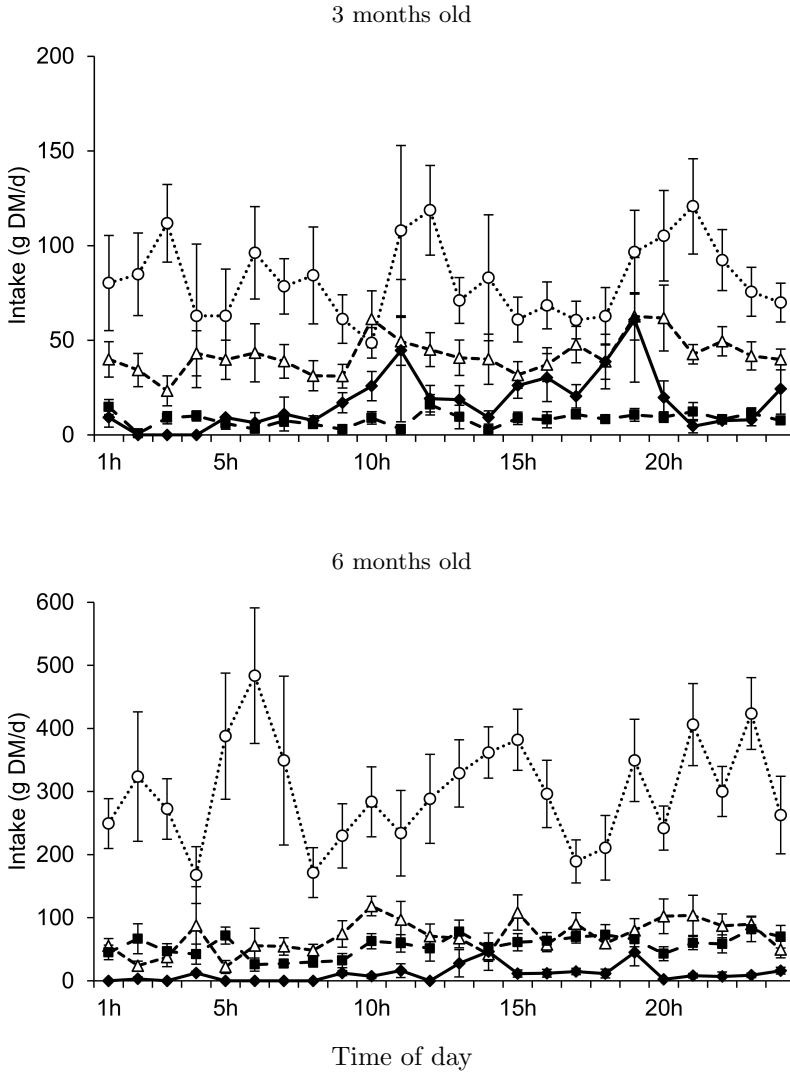


Figure 10.7: Average intake (g DM/d) at 3 and 6 months of age for concentrate (circles), hay (triangles), maize silage (squares) and straw (diamonds) at each hour of the day in Holstein-Friesian bull calves with ad libitum access to all diet components. Unpublished data.

automated pens in which calves could be housed continuously throughout the fattening period, during which calf preferences would be automatically recorded. Second, no cost was imposed on choices, therefore, the strength of feed preferences was not assessed.

### 3.2 Cross point analysis of double demand functions

Currently, the best method to assess preferences for two resources in animals is the cross point analysis of double demand functions, which was previously applied to cattle, pigs and rats (Matthews and Temple, 1979; Sørensen et al., 2001, 2004; Pedersen et al., 2005; Holm et al., 2007; Jensen and Pedersen, 2007). The main drawback of this method is the time-consuming training of individual animals. This generally means that these studies have a limited number of animals (Holm et al., 2002; Pedersen et al., 2005).

Moreover, differences in individual characteristics, or ‘temperament’, may mean that different animals require different training schedules and that certain animals require much longer training times. Previous studies found indications that fearfulness and coping style had an impact on how fast animals learnt tasks, e.g. running through a maze (Benus et al., 1987, 1990; Teskey et al., 1998; Bolhuis et al., 2004). In Chapter 7, we found some indication that temperament was related to learning in calves. To our knowledge, this is the first time that relationships between temperament and learning have been studied in calves. In particular, it seems that locomotion level during challenge tests was linked to learning ability measurements in a double demand operant conditioning set-up. High locomotion in novel environment and social isolation tests (which may reflect a proactive coping style, Van Reenen et al., 2005) tended to be associated with a lower motivation to work for roughage, as measured by less presses of the panel per minute, less rewards achieved per session, and shorter session durations. Since proactive animals are more likely to show routine-like behaviour and are less focused on the environment (Benus et al., 1987, 1990; Hessing et al., 1994; Teskey et al., 1998), we expected these calves to be more focused on the operant task and that this would result in them appearing to be more motivated to perform the operant task. This is not what was observed in Chapter 7. I speculate that the level of behavioural inflexibility associated with a proactive coping style may result in frustration in a double demand operant set-up due to the need to switch between panels (within and between sessions), which may result in reduced motivation to perform the task. Less flexible behaviour in the wild is thought to be advantageous in a continuously changing environment, because it reduces the chances of responding inadequately in a new situation (Searle et al., 2010). However, in the operant set-up,



behavioural flexibility was required at least at the beginning of training when needed to find out what workloads and roughage types were attributed to the left and right panel. Moreover, high locomotion in a novel object test was associated with good selection of the panel with the lowest workload (i.e. economic working) and shorter latencies to move away from a non-responding panel to check the other panel (referred to in Chapter 7 as ‘Latency to do forced choice’). Locomotion in novel object tests was previously shown to be difficult to link to a particular temperamental trait. Van Reenen et al. (2004) reported that this behavioural element could sometimes be related to hypothesised fearfulness and at other times to hypothesised activity. In addition, in Chapter 7 this behaviour did not seem to explain much of the variance observed between the calves (i.e. only 7%). It is, therefore, difficult to make strong conclusions about the two correlations involving locomotion in a novel object test. Chapter 7 included only nine calves, which means that correlations between temperament and learning measurements had to be strong for significant relationships to be found. This indicates the need for further research investigating the relationship between temperamental traits and learning ability in calves. This research can be applied to devising more effective and individually-based training schedules for double demand operant conditioning in calves.

Next to training, methods for analysing data statistically from double demand operant conditioning needed to be addressed, and this was done in Chapter 5. Methods used in previous studies included the number of rewards as the dependent variable and entered resource type as a fixed effect (e.g. Sørensen et al., 2004). This approach fails to address the dependence between both panels (or resources) which are available simultaneously: at any given point in time during a test session, a calf can only choose to work on one of the two panels present. We suggest using proportion of rewards achieved for one type of resource over the total number of rewards accessed during a session as a dependent variable. This dependent variable takes into account the level of competition present between both panels, and hence between both resources.

### 3.3 Conclusions on methods to assess feed preferences

Although choice tests and operant conditioning set-ups both present advantages and drawbacks, when assessing the preference of animals for two substitutable resources, cross point analysis of double demand function with a double alternating procedure is most sensitive and better mimics the complexity of natural environments. However, when using this method, training schedules need to be based on individual animals and appropriate statistical methods, which take into account the dependence structure between the two resources, should be applied.

## 4 Practical implications

The main aim of this thesis was to develop novel feeding strategies to improve the welfare of veal calves, and consequently, provide practical advice on this matter. As explained above, improving veal calf welfare in the context of feeding necessitates maximising chewing activity and minimising AOB frequency. Apart from behaviour, one should also consider impacts on gastrointestinal health when devising novel feeding strategies. The following two sections will consider feeding strategies that best enable the expression of natural behaviour and maximise gastrointestinal health, including rumen and abomasal health.

### 4.1 Which amount, type and particle length of roughage for the expression of natural behaviour?

#### Amount of roughage and concentrate

The 2008 EU Directive stipulates a minimum solid feed amount for calves ranging from 50 g at 2 weeks to 250 g/d fibrous feed at 20 weeks of age (EU Council, 2008). Previous research, however, has continuously demonstrated that 250 g (DM or fresh product) of different types or mixtures of solid feed was too small an amount for the ‘sufficient’ performance of natural chewing behaviours in milk-fed calves. Whether veal calves received 250 g DM/d of wheat straw, beet pulp, maize silage, maize cob silage, or 100 to 300 g/d of concentrate (pelleted straw and cereal), or even up to 400 g DM/d of a solid feed mixture, with maize silage as the main ingredient, they displayed increasing levels of AOB with increasing age (Chapters 2 and 3; Morisse et al., 1999; Mattiello et al., 2002).

Although we now know that calves appear to become more efficient in terms of chewing activity, and thus, that calves’ need for solid feed increases with age (whether because of body characteristics, motor skill development or a change in internal motivation), the question remains as to how much solid feed calves actually need. This is an extremely difficult question to answer given the large individual variation in intake and dietary preferences that exist in calves (Chapter 4). In theory, the best, maybe the only, way to meet the chewing needs of all individuals would be to enable them to select their own diet from a (sound) selection of diet components (Villalba et al., 2010). So far however, this is rarely done in practice. Calves with a dietary choice showed high levels of chewing activity and low levels of AOB in comparison with restrictedly-fed calves, suggesting they were able to maximise the expression of natural behaviour, satisfy their behavioural needs and subsequently show signs of good welfare (Chapters 2 and 4). This is in line with the suggestion of Forbes and

Kyriazakis (1995) that ruminants can select diets to maximise their comfort.

Six-month-old Holstein-Friesian calves fed 2750 g DM/d of MR, voluntarily consumed 1125 g DM/d of hay on average (Chapter 2). Six-month-old Holstein-Friesian calves with a free choice of MR, hay, barley straw, maize silage, and concentrate, consumed on average 1250, 1040 and 2170 g DM/d of MR, roughage and concentrate, respectively (Chapter 4). Finally, 6-month-old calves (most of which were Holstein-Friesian) fed 1050 g DM/d MR and with a free choice of straw, maize silage and concentrate consumed 2300 g DM/d concentrate and 950 g DM/d of roughage on average (Chapter 9). Despite very different set ups and large differences in MR and concentrate intake, Chapters 2, 4 and 9 found similar voluntary intakes for roughage, which could give an indication as to how much roughage calves should be fed, i.e. about 1000 g DM/d at 6 months. This amount is roughly four times the EU minimum provision of fibrous feeds for calves at 20 weeks of age. On top of this roughage amount, free choice calves consumed 2300 to 3200 g DM/d concentrate, which could also be encompassed under the term ‘fibrous feed’, bringing the voluntary intake of solid feed up to 3000 to 4000 g DM/d, i.e. over 12 times the EU minimum requirement. Although concentrate provision most likely has little impact on chewing activity (Morisse et al., 2000), it may be beneficial in terms of rumen development (see below).

### Type and particle size of roughage

Knowing how much roughage calves may need for the expression of natural feeding behaviours leads us to the following question: what roughage sources should we feed calves? Types of solid feed that contain long fibres and have a high neutral detergent fibre (**NDF**) content, such as straw, have a higher potential to reduce AOB for longer compared with less-fibrous roughages. For instance, chopped straw and hay were better types of solid feed, compared with the less-fibrous maize silage and maize cob silage, in terms of increasing chewing activity and decreasing AOB (Chapter 2). Due to its high iron content, hay is an uncommon diet component in veal systems, because meat colour is linked to iron uptake and consumers prefer pale veal. In addition, beneficial effects of hay in Chapter 2 are confounded with the amount provided, as hay was offered ad libitum and other roughage sources were offered in limited amounts (i.e. 250 or 500 g DM/d).

Doubling the amount of straw from 250 to 500 g DM/d does not result in an increase in chewing activity (Chapter 2), and calves do not show a clear preference for long straw over chopped straw (Chapter 6). This may suggest that a relatively small amount of chopped straw (e.g. 250 g DM/d) is sufficient to significantly increase

chewing activity in veal calves. However, given that this amount of straw given as a sole roughage source still resulted in AOB, especially at the end of the fattening period (Chapter 2; Mattiello et al., 2002), it is likely that other roughage sources need to be fed alongside this. This is consistent with the amount of straw that calves voluntarily select. If straw is the only coarse roughage present (i.e. no hay), 5-month-old calves select 200 and 300 g DM/d barley straw whether the straw was long or chopped (Chapter 6). Not surprisingly, if straw is fed alongside hay, which is preferred over straw (Chapter 6), then calves select little straw (i.e. 30 g DM/d) (Chapter 4). I suggest that 200 to 300 g DM/d (depending on what other roughages are provided) of straw should be fed to 6-month-old veal calves on top of MR and concentrate in order to maximise chewing activity and minimise AOB.

Maize silage includes more nutrients than straw and is preferred by calves at 6 months of age (Chapters 4 and 9). The quality of roughages may differ between batches, and this is especially true for maize silage, which is wet. Therefore, the preference of calves for maize over straw at 6 months may depend on maize quality. However, when the quality of the maize is good, providing some maize silage to calves might be of benefit despite it not being as good as straw in stimulating chewing activity. It is possible that calves need to habituate to this roughage, with intake being low at a young age, suggesting a small amount could be provided at the beginning of the fattening period (Chapter 4). Depending on whether hay is present and how much MR is fed, calves voluntarily selected between 400 and 800 g DM/d of maize silage at the end of the fattening period. Moreover, large amounts and longer particle sizes (i.e. chopped instead of ground) of maize silage increased chewing activity in calves fed only maize silage as a solid feed source (Chapter 2). This suggests that if calves must rely only on maize silage for their chewing activity, large amounts (i.e. > 500 g DM/d) of chopped maize silage should be provided at the end of the fattening period.

## 4.2 Gastrointestinal health and solid feed

Feeding strategies currently used on European veal farms are associated with a number of gastrointestinal health issues: including abomasal damage, poor rumen development, ruminal plaque (“rumen mucosa containing focal or multifocal patches with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris”, Suárez et al., 2007) and hyperkeratinisation, and ruminal hairballs. In a large cross-sectional study on veal farms in the Netherlands, France and Italy, Brscic et al. (2011) found on average 74% calves with abomasal lesions in the pyloric area, 60% calves with low rumen development, 31% calves with plaque, and 6% calves with

hyperkeratinisation, with some of their batches from one farm having up to 100% of calves with low rumen development, plaque or abomasal lesions.

### **Abomasum and lesions**

Abomasal lesions are thought to occur as a result of overfilling of the abomasum due to large quantities of milk replacer being fed in few meals, which leads to local ischemia and, consequently, lesioning of the abomasal wall (Breukink et al., 1991). Poor rumen development and the provision of roughage, and especially coarse feed stuffs, have been involved in the exacerbation of this existing damage (Katchuik, 1992; Mattiello et al., 2002; Berends et al., 2012b), consistent with findings from Chapter 2. A poorly developed rumen may let under-digested feed particles pass into the abomasum, and the abrasive action of these particles may cause further damage to the abomasal wall.

The provision of straw as sole solid feed source for veal calves is associated with particularly severe abomasal damage, most likely due to the combined effect of poor stimulation of rumen development and high abrasiveness (Chapter 2; Mattiello et al., 2002). Maize silage, maize cob silage, and dried beet pulp are feed types that led to less abomasal damage in comparison with straw (Chapter 2; Mattiello et al., 2002). Interestingly, Veissier et al. (1998) found no exacerbation of abomasal damage associated to the provision of solid feed compared with feeding only MR. In this particular study, calves started solid feed provision at an early age, i.e. 1 week old, and were initially only fed concentrate (100 to 500 g/d) until 7 weeks of age, at which point chopped straw (450 g/d) was mixed with the concentrate. Early rumen development with starchy, volatile fatty acid (VFA)-stimulating, and low-abrasiveness feed types may have improved feed digestion and, subsequently, protected these calves against exacerbation of abomasal damage when abrasive roughage particles were introduced (Veissier et al., 1998; Mattiello et al., 2002; Berends et al., 2012b).

Supporting this idea is the finding that different concentrate diets did not differentially affect abomasal damage (Morisse et al., 1999, 2000), most likely because each diet had an equally low abrasiveness value. Interestingly, a recent study found that increasing amounts of a solid feed mixture comprised of a 80:20 concentrate:roughage ratio exacerbated abomasal damage, whereas the same increase in amount of a solid feed mixture comprised of 50:50 concentrate:roughage ratio did not (Berends et al., 2014). It is surprising that concentrate, which included only fine particles, could have a more detrimental effect on abomasal health compared with roughage, which included chopped wheat straw and maize silage. Instead, large quantities of concentrate may have affected the rumen or abomasum in such a way that a small amount

of roughage became more detrimental to abomasal health. The aetiology of abomasal lesions observed in veal calves seems to involve a complex interaction between rumen development and abomasal health, and its understanding will require further research.

### **Rumen development, pH, motility, and a brief note on ruminal hairballs**

Looking at abomasal health only, a diet of concentrate with no roughage seems like the best option for veal calves with poorly developed rumens, as all roughage sources seem to exacerbate abomasal damage compared with a diet comprised only of MR (Chapter 2). Diets comprised of a high concentrate proportion relative to roughage result in an increased propionate to acetate ratio (Suárez et al., 2007), and propionate has been shown to better stimulate rumen papillary growth compared with acetate (Sander et al., 1959). In other words, feeding concentrate to pre-ruminant calves will stimulate rumen wall development with a low risk of exacerbating abomasal damage. In the long run, however, feeding only concentrate, or high starch feed types with fine particles, may: 1) increase rumen acidity because of an imbalance in ratios of VFA (Nocek, 1997; Beharka et al., 1998), 2) lead to the formation of plaque and hyperkeratinisation on the rumen wall (Suárez et al., 2007) most likely because of insufficient abrasion limiting removal of fine particles stuck between papillae (Haskins et al., 1969), and 3) decrease rumen muscularisation and motility (Harrison et al., 1960; Tamate et al., 1962). Increased rumen acidity for long periods of time (i.e. acidosis), as well as plaque, result in a non-optimal rumen environment (Suárez et al., 2007; González et al., 2012), which most likely negatively impacts on nutrient uptake efficiency in the rumen and impairs papillary growth (Suárez et al., 2007). In the case of plaque, this reduced efficiency is at least partly due to poor development of rumen papillae (Suárez et al., 2007). Acidosis in cattle can result in lower feed intake and disease, e.g. laminitis, although this is relatively uncommon (González et al., 2012).

Low abrasiveness of a diet associated to low rumen motility may further lead to hairball formation in the rumen (Chapter 2), which has been suggested to both impair digestion (Cozzi et al., 2002) and to cause no digestive disturbance in calves (Toofanian, 1976). One author even speculated that hairballs in the rumens of calves fed only MR might replace roughage in the promotion of rumen motility and muscularisation (Osborne, 1976). It was initially believed that ruminal hairballs found in veal calves were a result of excessive hair licking, which was mostly observed in individually housed calves (Bokkers and Koene, 2001). This idea was not supported by Morisse et al. (1999) or by findings in Chapter 2, where licking levels were similar between groups of calves with very different hairball prevalences. Providing even a small amount of straw (250 g DM/d) will prevent hairball formation altogether

(Chapter 2). Straw will moreover increase rumination, and thus stimulate salivation, and saliva is known to be a buffer-agent in the rumen, likely involved in increasing rumen pH (González et al., 2012). The abrasive nature of straw will also help reduce the prevalence of ruminal plaque and hyperkeratinisation in calves (Suárez et al., 2007). Hay, as a source that provides both abrasiveness and higher VFA-production than straw, seems to promote rumen development without exacerbating abomasal damage, at least when fed *ad libitum* (Chapter 2).

### 4.3 A brief note on MR provision in veal systems

Although MR constitutes an important part of veal diets, this thesis focused on the solid feed component of the veal diet. Past studies investigating MR feeding in dairy calves give some indication as to how to feed MR to calves to maximise welfare, at least in the first months of life (e.g. Loberg and Lidfors, 2001; Jasper and Weary, 2002; Jensen and Holm, 2003; Borderas et al., 2009; Bach et al., 2013). Until now, most veal calves were fed MR throughout the fattening period, i.e. until slaughter at 6 months of age, and this MR feeding strategy raises the question: in what quantities, at what flow rate and in how many feedings should one feed MR to veal calves in order to maximise animal welfare? This thesis did not address these points. However, in Chapter 4 calves were given a choice as to how to consume their MR. If we assume that calves are able to select a diet that maximises their comfort (Forbes and Kyriazakis, 1995), results from Chapter 4 in terms of MR drinking may shed light over how to feed MR to veal calves.

Calves able to select their own MR feeding strategy (via an automated milk dispenser: **AMD**, with a slow flow rate) appeared to drink in a similar fashion to calves suckled by their dam (Chapter 4). They selected on average approximately 900 g DM/d at 3 months and 1200 g DM/d at 6 months, and drank this MR in approximately 7 feedings of 8 min per day. Interestingly, calves with a choice seem to replace MR with concentrate as they grow older, and as a consequence ingest less MR than veal calves would typically be fed (Chapter 4). This points to the need to adjust proportions of MR and solid feed in the diet with age in veal calves. Calves in Chapter 4 shared access to the AMD with a maximum of 11 other calves. This is different from farms, where up to 40 animals may share one MR feeding station. This limits the time calves are able to drink and increases competition at the station (Borderas et al., 2009).

Because low levels of tongue playing were observed in Chapter 4, even in calves fed restrictedly, we hypothesised that tongue playing may stem from a frustrated motivation to suck as opposed to a frustrated motivation to chew, or a combination

of both motivations. This was also in part due to the differences observed in the form of tongue playing: some calves extend their tongues outside of their mouths and this might resemble the tongue movement made to pull grass up, whereas other calves were observed rolling their tongue inside of their mouth, with the head sometimes somewhat inclined upwards (personal observation). In Chapter 9, tongue playing seemed related to both roughage provision and MR feeding method, suggesting this behaviour may stem from two underlying motivations, i.e. rumination and sucking.

#### 4.4 Conclusions on practical implications

Combining findings from behavioural and gastrointestinal health indicators, it appears that a combination of coarse roughage sources and concentrate in adequate proportions, differing depending on age, could provide an animal-friendly diet for veal calves. More specifically, veal calves up to around 8 weeks of age may benefit from being fed solid feed sources that maximise rumen wall development, i.e. high VFA-stimulating solid feeds such as concentrate and maize silage (and possibly hay as iron uptake is not an issue at the beginning of the fattening period), and that do not exacerbate abomasal damage (e.g. straw). In young calves, coarse roughages may do more damage (to the abomasum) than good (by stimulating chewing activity) because chewing motivation may be low at this age. Thereafter, coarser roughages should be introduced to stimulate chewing activity, and minimise ruminal plaque and hairball formation and to prevent long periods of low ruminal pH. By the age of 6 months, veal calves might benefit from a minimum of 1000 g DM/d roughage (including at least 250 g of straw), and 2000 to 3000 g DM/d concentrate (depending on how much MR is provided). If meat colour is not an issue, then calves may benefit from receiving mostly long hay as a roughage source. Alternatively, roughage sources differing in abrasiveness and VFA-stimulating potential could be provided *ad libitum* and in separate troughs to permit individual diet selection. Finally, based on the behaviour of calves in a choice test and based on the idea that ruminants are able to select a diet that maximises comfort (Forbes and Kyriazakis, 1995), calves may benefit from being able to drink their MR provision in seven 8-min feedings per day. However, allowing individual calves to select their own MR feeding schedule may be even more advantageous in terms of welfare.







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# Miscellaneous

**Summary**

**Samenvatting (Dutch summary)**

**References**

**Acknowledgements**

**About the author**

**List of publications**

**Education certificate**



# Summary

Veal calves are typically fed high levels of milk replacer supplemented with solid feed, which tends to contain a relatively small roughage component. Feeding strategies used in veal production have been associated with welfare issues, including the development of abnormal oral behaviours (AOB) and poor gastrointestinal health. AOB include tongue playing, excessive oral manipulation of the environment, grazing of the coat of other calves, and sham chewing, and are thought to develop in calves when chewing activity (i.e. eating and rumination) is not adequately stimulated. Common gastrointestinal health issues include poor rumen development and lesions in the abomasum.

The aim of this thesis was to develop novel feeding strategies to improve the welfare of veal calves, i.e. to minimise the development of AOB and gastrointestinal health disorders as well as maximise chewing activity.

The EU legislation stipulates a minimum of 250 g of ‘fibrous feed’ for 20 week-old calves, but this amount does not seem supported by previous research in terms of it optimising calf welfare. In addition, it does not specify what fibrous feed refers to in terms of source and particle length of roughage. Developing novel feeding strategies for calves necessitates a better understanding of how different roughage characteristics might affect behaviour and gastrointestinal health, and this is what was investigated in **Chapter 2**. Because none of the single roughage sources investigated were able to improve both behaviour and health, it is likely that a combination of roughage sources would be optimal. For example, an appropriate diet choice may include a combination of roughage sources that facilitate good ruminal papillae development (e.g. maize silage), minimise plaque formation, and encourage both rumen muscularisation and rumination (e.g. straw). This chapter also suggested that hay, as a roughage source with both high levels of structure and high levels of fermentable fibre, could achieve

both objectives of encouraging rumination and rumen development. Hay, however, is not used in veal production due to its high iron content that would lead to darker meat colour, which is less preferred by consumers.

In **Chapter 3**, different amounts of a solid feed mixture were fed to calves and behaviour was monitored. The results showed that calves fed no solid feed on top of their milk replacer still displayed a rumination-like behaviour, which was in previous literature referred to as ‘sham chewing’. This result gives an indication as to the importance of rumination in calves. Moreover, this chapter failed to find a straight-forward linear relationship between amount of solid feed provided and level of AOB displayed. Certain amounts of solid feed were found to initially stimulate chewing activity to a high level, but later, as calves grew older and more experienced with roughage, failed to stimulate chewing above the level displayed by calves fed no solid feed. Providing such an amount of roughage seemed to be more detrimental in terms of behaviour than providing an amount that results in a constant level of chewing activity throughout the fattening period.

In order to develop animal-friendly feeding strategies, it is important to know what the animals would choose when given free choice. Therefore, in **Chapter 4**, the feed preferences of calves for milk replacer, concentrate, hay, straw and maize silage were investigated. This study showed that at 6 months, calves selected on average approximately 1250 g dry matter (DM) milk replacer, 1000 g DM roughage and 2000 g DM concentrate. Although all calves with free choice showed high levels of chewing activity and subsequently low levels of AOB, large individual differences existed in intake levels and feed preferences. Moreover, outcomes were dependent on the variable used to assess preferences: i.e. intake (in g DM relative to metabolic body weight), duration of feeding, or number of visits to each diet component. On average, however, calves showed a preference for milk replacer, concentrate and hay, over straw and maize silage.

In contrast to free choice testing, as was used in Chapter 4, double demand operant conditioning gives an indication as to the strength of a preference. In **Chapter 5**, different methods to analyse data collected from double demand operant conditioning studies were investigated. Due to the dependence level between the two resources presented simultaneously, i.e. at any given time the test animal can only work for one resource, it would seem that proportions of rewards achieved for one resource over the total number of rewards achieved for both resources would be an adequate dependent variable in this type of analysis.

In **Chapter 6** the statistical method developed in Chapter 5 was used to assess the preference of calves for long and chopped hay and straw, and their preference for hay versus straw. Two to five month-old calves learned the double demand operant

task and were motivated to work for roughage on top of a high energy diet of milk replacer and concentrate. They showed a preference for long over chopped hay, but not for long over chopped straw, and showed a strong preference for hay over straw.

In **Chapter 7** it was investigated whether temperament might affect learning of a double demand operant task in calves. Studies in horses and voles previously found that certain individuals seemed unable to learn certain tasks. If one could find out why, individual training programs could be designed and non-learners would not be removed from studies, potentially avoiding biases in data due to only certain temperament profiles making it through the learning criteria. Chapter 7 gave some indication that temperament may affect learning in calves, and it is the first study in calves to do so. However, due to the low number of animals used, further research is necessary to confirm which temperamental traits affect learning ability in calves.

Relationships between tongue playing and: 1) hypothesised measures of chronic stress, and 2) hypothesised temperamental traits were investigated in **Chapter 8**. Large individual differences in the performance of tongue playing in calves subjected to similar husbandry conditions were found. This suggests that although tongue playing might well be a warning sign for chronic stress, and hence poor welfare, individual variation in the propensity to tongue play in response to stressful conditions exists. This could be due to differences in temperament. In contrast to what theoretical papers suggest, calves that showed more tongue playing showed characteristics of a reactive coping style. This result is, however, consistent with previous experimental papers on calves and other species.

Results from Chapters 2 to 8 were combined into the design of the experiment described in **Chapter 9**. In this chapter, various feeding strategies (i.e. different amounts of solid feed combined with different concentrate to roughage ratios, different types of ad libitum choice diets, and feeding milk replacer via an open bucket or automated milk dispenser[AMD]) were applied and the effect on behaviour was recorded. Rumination was mainly affected by roughage provision, regardless of concentrate provision. Therefore, increasing solid feed provision without increasing the roughage content would most likely have little effect on rumination, although it would probably increase eating time to a certain extent. Because of the timing of tongue playing and oral manipulation of the environment (found in both Chapters 3 and 9), we suggest that the first of these two AOB is related to chewing activity in general, whereas the second may be more related to anticipation of an upcoming meal and positive reinforcement of feeding behaviours following an unsatisfactory meal. Calves provided ad libitum access to long straw in racks showed high levels of chewing activity and low levels of AOB relative to calves that did not have access to a straw rack but otherwise received the same diet. Six-month-old calves with ad libitum access to

straw, maize silage and concentrate (but a restricted milk replacer allowance of 1050 g DM/d) consumed on average approximately 900 g DM/d roughage and 2300 g DM concentrate at 6 months of age. Feeding milk replacer via an AMD seemed to have little impact on behaviour, although it led to lower levels of tongue playing at 15 wk relative to bucket-fed calves.

In **Chapter 10**, I first reflect on possible underlying mechanisms of AOB and on the best methods to assess animal preferences. AOB seem to develop in veal calves due to a number of factors, starting with the thwarting of chewing activity, of which rumination at least is most likely a behavioural need. Other factors involved in the development of AOB include chronic stress resulting from the thwarting of chewing activity, anticipation of an upcoming meal, and positive reinforcement of feeding behaviours following a meal that was unsatisfactory. Of great importance is the understanding of individual variation in the propensity to develop AOB, because stereotypic behaviours in sub-optimal environments have been linked to improvements in welfare (relative to non-stereotyping animals). Ruminants seem to be able to select a diet that maximises their comfort. Developing feeding strategies to improve veal calf welfare, therefore, requires the assessment of calf feed preferences. Choice tests and cross point analysis of double demand functions are two possible methods for the assessment of animal preferences, and both these methods include drawbacks and benefits. In contrast to choice tests, double demand offers a setting that closer mimics the complexity of natural environments by imposing a cost on access to resources and enables quantification of the strength of preferences. However, this procedure requires appropriate statistical methods, which take into account the dependence structure between the two simultaneously available resources. Finally, practical implications of the research presented in this thesis are described in Chapter 10. The development of novel feeding strategies to improve the welfare of veal calves is challenged by individual differences in feed preferences, chewing efficiency, and behavioural response to chronic stress caused by inadequate feeding. The latter is demonstrated by only certain calves developing AOB when chewing activity is not stimulated enough by the feeding strategy, whilst others do not develop such behaviours. This complicates the evaluation of the effects of feeding strategy on veal calf behaviour. However, based on the results of this thesis and previous research it seems that young calves should first receive a diet that optimises rumen development, before receiving coarser roughages that stimulate chewing activity, rumen muscularisation, and minimise plaque and hairball prevalence in the rumen. Adequate amounts of roughage and concentrate at 6 months of age seem to be 1000 and 2000-3000 g DM, based on voluntary intake.



# Samenvatting

Vleeskalveren worden voornamelijk gevoerd met kunstmelk aangevuld met krachtvoer en een relatief klein gedeelte ruwvoer. Deze voerstrategie is kenmerkend voor de vleeskalverhouderij en wordt geassocieerd met dierenwelzijnsproblemen, zoals abnormaal oraal gedrag en aandoeningen aan het maagdarmkanaal. Voorbeelden van abnormaal oraal gedrag bij vleeskalveren zijn tongspelen, overmatig bijten, belikken en bezuigen (oraal manipuleren) van hekwerk, vloer of voerbak, begrazen van de vacht van andere kalveren, en schijnkauwen. Abnormaal oraal gedrag wordt verondersteld te worden veroorzaakt doordat kalveren niet voldoende kunnen kauwen (voer eten en herkauwen). Voorbeelden van aandoeningen aan het maagdarmkanaal zijn een slechte ontwikkeling van de pens en beschadigingen in de lebmaag.

Het doel van dit proefschrift was het ontwikkelen van nieuwe voerstrategieën om het welzijn van vleeskalveren te verbeteren. Deze nieuwe voerstrategieën zijn er op gericht de kauwactiviteit van kalveren te stimuleren en daarmee het ontstaan van abnormaal oraal gedrag en maagdarmgezondheidsproblemen in de vleeskalverhouderij te minimaliseren. Volgens de regelgeving in de Europese Unie moeten kalveren van 20 weken oud ten minste 250 gram vezelrijk voer per dag krijgen. Uit eerder onderzoek is echter niet gebleken dat deze hoeveelheid bijdraagt aan een optimaal dierenwelzijn. In de regelgeving is tevens niet gespecificeerd wat wordt verstaan onder vezelrijk voer: noch het type voer als de minimale lengte van de voerdeeltjes staat beschreven. Voor de ontwikkeling van nieuwe voerstrategieën is het belangrijk inzicht te krijgen in het effect van verschillende voercomponenten op het gedrag en maagdarmgezondheid bij kalveren (zie **hoofdstuk 2**). Aangezien geen van de onderzochte voercomponenten (stro, snijmaïs en maïskolfsilage) zowel gedrag als gezondheid verbeterden, lijkt een combinatie van verschillende voercomponenten aanbevolen. Een dergelijke combinatie moet bestaan uit componenten die een goede ontwikkeling van de papillen in de pens

bewerkstelligen (bijvoorbeeld snijmaïs), het ontstaan van plak in de pens tegen gaat, en dat herkauwen en de ontwikkeling van pensspieren stimuleert (bijvoorbeeld stro). De resultaten in hoofdstuk 2 laten tevens zien dat hooi, een voercomponent met veel structuur en verteerbare vezels, zowel het herkauwen als de pensontwikkeling stimuleert. Het nadeel van hooi is echter dat het relatief veel ijzer bevat. Dit kan leiden tot een donkere vleeskleur die veelal door de consument, en dus de vleeskalverhouderij, als ongewenst wordt ervaren.

**Hoofdstuk 3** beschrijft een onderzoek waarin verschillende hoeveelheden en typen voer aan een rantsoen van kunstmelk worden toegevoegd, om vervolgens het effect op gedrag van kalveren te analyseren. Kalveren die alleen kunstmelk kregen vertoonden schijnherkauwen. Het feit dat kalveren herkauwen zonder dat ze vast voer krijgen, geeft aan dat dit gedrag belangrijk voor ze is. Er werd geen eenduidig lineair verband gevonden tussen de hoeveelheid verstrekt vast voer en de mate van abnormaal oraal gedrag. Een bepaalde hoeveelheid vast voer stimuleerde eerst de kauwactiviteit aanzienlijk, maar naarmate de kalveren ouder werden kauwden ze net zo veel als de kalveren die geen vast voer kregen. Het voeren van een beperkte hoeveelheid vast voer lijkt uiteindelijk tot meer abnormaal gedrag te leiden dan wanneer er een hoeveelheid voer wordt gegeven dat resulteert in een constante kauwactiviteit gedurende de gehele mestperiode.

Voor de ontwikkeling van diervriendelijke voerstrategieën is het belangrijk inzicht te krijgen in de voorkeuren van kalveren aangaande hun rantsoen. In **hoofdstuk 4** is deze voorkeur onderzocht door een onbeperkte vrije keuze te bieden aan kunstmelk, krachtvoer, hooi, stro en snijmaïs. Uit dit onderzoek bleek dat kalveren na 6 maanden gemiddeld ongeveer 1250 g droge stof (DS) kunstmelk, 1000 g DS ruwvoer en 2000 g DS krachtvoer per dag aten. Hoewel alle kalveren uit dit onderzoek veel kauwactiviteit en weinig abnormaal oraal gedrag vertoonden, waren er grote individuele verschillen met betrekking tot voeropname en voorkeuren. Bovendien werd duidelijk dat de resultaten afhankelijk waren van de gebruikte indicator: voeropname per component (in g DS per kg metabolisch gewicht), de tijdsbesteding aan eten per voercomponent, en het aantal bezoeken aan een voerbak met een bepaald voercomponent. Gemiddeld genomen gaven de kalveren de voorkeur aan kunstmelk, krachtvoer en hooi boven stro en snijmaïs.

Naast het onderzoek beschreven in hoofdstuk 4, is operante conditionering (werken voor een beloning) een methodiek om te bepalen welke type voer een kalf verkiest en hoe graag het kalf dit voer wil hebben. In **hoofdstuk 5** worden statistische methodes onderzocht om resultaten van dergelijke experimenten te analyseren. Omdat in deze test de dieren tussen twee voertypes of voersoorten moeten kiezen, is de keuze voor het een afhankelijk van het andere (als het kiest voor het een, kiest het automatisch

niet voor het andere). Hierdoor blijkt dat het aantal verkregen beloningen per type voer of voersoort gedeeld door het totaal aantal verkregen beloningen (proporties) de beste variabele oplevert.

In **hoofdstuk 6** wordt de statistische analysemethode uit hoofdstuk 5 gebruikt om te bepalen of kalveren een voorkeur hebben voor lang hooi of kort gesneden hooi, voor lang stro of kort gesneden stro, en voor kort gesneden hooi of kort gesneden stro. Kalveren werden getraind om een keuze te maken uit twee mogelijkheden. Resultaten toonden aan dat kalveren die een energierijk rantsoen van kunstmelk en krachtvoer kregen, gemotiveerd waren om te werken voor hooi en stro. Daarnaast hadden de kalveren een grotere voorkeur voor lang hooi dan voor kort hooi, maar werd er geen verschil gevonden in voorkeur voor lang of kort stro. Tot slot hadden de kalveren een sterke voorkeur voor hooi ten opzichte van stro.

In **hoofdstuk 7** is onderzocht of het karakter van de kalveren invloed heeft op het leren van een operante taak waarbij het dier twee keuzes voorgehouden krijgt. Uit eerdere onderzoeken met paarden en woelmuizen bleek dat sommige individuen bepaalde handelingen niet konden leren. Als duidelijk wordt waarom niet, kunnen individuele trainingsprogramma's ontwikkeld worden om deze individuen te behouden en daarmee te voorkomen dat in dergelijke experimenten alleen dieren met een bepaald karakter worden gebruikt, wat de resultaten sterk zou kunnen beïnvloeden. Hoofdstuk 7 laat zien dat er aanwijzingen zijn dat het karakter van kalveren een effect kan hebben op het leren van een operante taak. Echter, het geringe aantal dieren waarop deze conclusie is gebaseerd maakt verder onderzoek noodzakelijk om te bepalen welke individuele kenmerken van kalveren het leren beïnvloeden.

In **hoofdstuk 8** is onderzocht of er een relatie is tussen het tongspelen bij kalveren en 1) indicatoren van chronische stress, en 2) karaktereigenschappen. Kalveren in dezelfde soort houderijsystemen laten grote individuele verschillen zien in tongspelen. Hoewel dit tongspelen algemeen wordt erkend als een teken van chronische stress en daarmee als signaal van verminderd welzijn, geven deze individuele verschillen aan dat kalveren verschillend met chronische stress omgaan. Dit zou een gevolg kunnen zijn van verschil in karakter. In tegenstelling tot de theorie die in veel artikelen wordt beschreven, vertoonden de kalveren die veel tongspelen kenmerken van een reactieve coping stijl. Dit resultaat is in overeenstemming met eerder experimenteel onderzoek bij kalveren en andere diersoorten.

De resultaten uit het onderzoek beschreven in hoofdstuk 2 tot en met 8 zijn gecombineerd tot een experiment dat beschreven is in **hoofdstuk 9**. In dit experiment werden verschillende voerstrategieën (verschillende hoeveelheden vast voer (een gemengde hoeveelheid krachtvoer, stro en snijmaïs) gecombineerd met verschillende verhoudingen van ruwvoer en krachtvoer, verschillende manieren van onbeperkte vrije keuze

van drie verschillende voercomponenten (krachtvoer, stro en snijmaïs), en het verstrekken van kunstmelk via een emmer of via een drinkautomaat) toegepast en het effect ervan op het gedrag van kalveren onderzocht. Herkauwen werd voornamelijk beïnvloed door de hoeveelheid ruwvoer die werd verstrekt, onafhankelijk van de hoeveelheid krachtvoer. Het verhogen van de hoeveelheid vast voer zonder de hoeveelheid ruwvoer te verhogen zal daarom weinig effect hebben op herkauwen, hoewel de tijdsbesteding aan eten wel iets zal toenemen. Zowel in hoofdstuk 3 als in hoofdstuk 9 staat beschreven dat tongspelen en het oraal manipuleren van hekwerk, vloer en voerbak voornamelijk op bepaalde momenten van de dag wordt uitgevoerd. Tongspelen bleek vooral gerelateerd aan de totale kauwactiviteit van het kalf, en het oraal manipuleren van hekwerk, vloer en voerbak bleek vooral gerelateerd aan het moment van voeren. Het vertonen van oraal manipulatief gedrag vr het voeren lijkt een anticipatie op de aankomende maaltijd, en na het voeren op een versterkte motivatie tot eetgedrag als gevolg van een maaltijd die niet voldeed aan de behoeften. Kalveren die onbeperkt beschikking hadden over lang stro in een ruif lieten veel kauwactiviteit en weinig abnormaal oraal gedrag zien in vergelijking met kalveren die hetzelfde voer kregen maar niet de beschikking hadden tot stro in een ruif. Kalveren van 6 maanden oud die naast een beperkte hoeveelheid kunstmelk van 1050 g DS/d, onbeperkt beschikking kregen over stro, snijmaïs en krachtvoer, aten gemiddeld ongeveer 900 g DS/d ruwvoer en 2300 g DS/d krachtvoer. Kunstmelk voeren via een drinkautomaat bleek in dit experiment weinig invloed te hebben op het gedrag, hoewel op een leeftijd van 15 weken minder tongspelen werd waargenomen dan bij kalveren die via emmers werden gevoerd.

In **hoofdstuk 10** worden mogelijke onderliggende mechanismes voor abnormaal oraal gedrag, en methoden om de voorkeur van kalveren te bepalen bediscussieerd. Verschillende factoren hebben invloed op de ontwikkeling van abnormaal oraal gedrag. De belangrijkste lijkt het beperken van mogelijkheden om te kauwen, waarbij herkauwen een zeer sterke gedragsmatige behoefte lijkt te zijn bij kalveren. Andere factoren die van invloed zijn op de ontwikkeling van abnormaal oraal gedrag zijn chronische stress als gevolg van te weinig kauwen, anticipatie op een volgende maaltijd, en versterking van gedragingen gerelateerd aan het eten van een maaltijd die niet voldeed aan de behoeften. Het begrijpen en onderkennen van individuele verschillen bij de ontwikkeling van abnormaal oraal gedrag bij kalveren is erg belangrijk. Het lijkt erop dat herkauwers in staat zijn een dieet te selecteren dat hun comfort maximaliseert. Inzicht in de voorkeuren van kalveren is daarom nodig om goede voerstrategieën te ontwikkelen die het welzijn van vleeskalveren werkelijk verbeteren. Vrije keuze experimenten en operante experimenten met keuze uit twee soorten beloningen zijn goede methoden om hier inzicht in te krijgen, al hebben beide methoden zowel voor- als

nadelen. De set-up van een experiment met keuze uit twee soorten beloningen lijkt op de complexe situatie van een natuurlijk omgeving omdat er kosten staan tegenover de beloning. Een dergelijke set-up laat zien in welke mate het kalf gemotiveerd is een bepaalde beloning te krijgen. Deze methode is echter arbeidsintensief en behoeft een statistische methode die rekening houdt met het feit dat de motivatie voor de ene beloning afhankelijk is van de motivatie voor de andere. Tot slot worden in hoofdstuk 10 de praktische implicaties van het onderzoek beschreven. Het ontwikkelen van nieuwe voerstrategieën om het welzijn van vleeskalveren te verbeteren is een uitdaging omdat kalveren verschillende individuele voorkeuren hebben, verschillen in hun effectiviteit van kauwen, en verschillen in de manier waarop ze omgaan met chronische stress ten gevolge van een rantsoen dat niet voldoet aan hun behoefte. Dit laatste blijkt uit het feit dat niet alle kalveren abnormaal oraal gedrag ontwikkelen wanneer ze niet voldoende kunnen kauwen. Dit maakt het evalueren van het effect van een bepaalde voerstrategie op het gedrag van kalveren moeilijk.

Tot slot, op basis van dit proefschrift en eerder onderzoek kan worden gesteld dat jonge kalveren eerst voer moeten krijgen dat zorgt voor een goede pensontwikkeling. Vervolgens moeten de kalveren voldoende ruwvoer krijgen om de kauwactiviteit te stimuleren, de pens-spieren te ontwikkelen, en plak en haarballen in de pens te voorkomen. Bij onbeperkte toegang tot voer lijken kalveren van 6 maanden oud voldoende te hebben aan 1000 g DS/d ruwvoer en 2000-3000 g DS/d krachtvoer om aan hun behoeften te voldoen.



# REFERENCES

- Al-Saiady, M. Y., Abouheif, M. A., Aziz Makkawi, A., Ibrahim, H. A., and Al-Owaimer, A. N. (2010). Impact of particle length of alfalfa hay in the diet of growing lambs on performance, digestion and carcass characteristics. *Asian-Australasian Journal of Animal Sciences*, 23(4):475.
- Allen, M. S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*, 83(7):1598–1624.
- Andersen, I. L., Bøe, K. E., Førevik, G., Janczak, A. M., and Bakken, M. (2000). Behavioural evaluation of methods for assessing fear responses in weaned pigs. *Applied Animal Behaviour Science*, 69(3):227–240.
- Appleby, M. C., Weary, D. M., and Chua, B. (2001). Performance and feeding behaviour of calves on ad libitum milk from artificial teats. *Applied Animal Behaviour Science*, 74(3):191–201.
- Atwood, S. B., Provenza, F. D., Wiedmeier, R. D., and Banner, R. E. (2001). Influence of free-choice vs mixed-ration diets on food intake and performance of fattening calves. *Journal of Animal Science*, 79(12):3034–3040.
- Bach, A., Domingo, L., Montoro, C., and Terré, M. (2013). Short communication: Insulin responsiveness is affected by the level of milk replacer offered to young calves. *Journal of Dairy Science*, 96(7):4634–4637.
- Balch, C. C. (1971). Proposal to use time spent chewing as an index of the extent to which diets for ruminants possess the physical property of fibrousness characteristic of roughages. *British Journal of Nutrition*, 26(03):383–392.
- Baumont, R. (1996). Palatability and feeding behaviour in ruminants. a review. *Annales de Zootechnie*, 45:385–400.
- Beharka, A. A., Nagaraja, T. G., Morrill, J. L., Kennedy, G. A., and Klemm, R. D. (1998). Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. *Journal of Dairy Science*, 81(7):1946–1955.
- Bekoff, M. (1977). Mammalian dispersal and the ontogeny of individual behavioral phenotypes. *American Naturalist*, 111(980):715–732.

- Benus, R. F., Bohus, B., Koolhaas, J. M., and Van Oortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia*, 47(10):1008–1019.
- Benus, R. F., Den Daas, S., Koolhaas, J. M., and Van Oortmerssen, G. A. (1990). Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice. *Behaviour*, 112(3-4):176–193.
- Benus, R. F., Koolhaas, J. M., and Van Oortmerssen, G. A. (1987). Individual differences in behavioural reaction to a changing environment in mice and rats. *Behaviour*, 100(1/4):105–122.
- Berends, H., Van den Borne, J. J. G. C., Alferink, S. J. J., Van Reenen, C. G., Bokkers, E. A. M., and Gerrits, W. J. J. (2012a). Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. *Journal of Dairy Science*, 95(11):6654–6664.
- Berends, H., Van den Borne, J. J. G. C., Mollenhorst, H., Van Reenen, C. G., Bokkers, E. A. M., and Gerrits, W. J. J. (2014). Utilization of roughages and concentrates relative to that of milk replacer increases strongly with age in veal calves. *Journal of Dairy Science*, 97(10):6475–6484.
- Berends, H., Van Reenen, C. G., Stockhofe-Zurwieden, N., and Gerrits, W. J. J. (2012b). Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. *Journal of Dairy Science*, 95(6):3190–3199.
- Bhatnagar, S. and Dallman, M. (1998). Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience*, 84(4):1025–1039.
- Boga, M., Sahin, A., Kilic, U., Gorgulu, M., et al. (2009). Behavioural responses of dairy calves to cafeteria feeding vs. single feeding. *Journal of Animal and Veterinary Advances*, 8(8):1573–1578.
- Boissy, A. (1995). Fear and fearfulness in animals. *The Quarterly Review of Biology*, 70(2):165–191.
- Boissy, A. and Le Neindre, P. (1990). Social influences on the reactivity of heifers: Implications for learning abilities in operant conditioning. *Applied Animal Behaviour Science*, 25(1):149–165.
- Bokkers, E. A. M. and Koene, P. (2001). Activity, oral behaviour and slaughter data as welfare indicators in veal calves: A comparison of three housing systems. *Applied Animal Behaviour Science*, 75(1):1–15.
- Bokkers, E. A. M. and Koene, P. (2002). Sex and type of feed effects on motivation and ability to walk for a food reward in fast growing broilers. *Applied Animal Behaviour Science*, 79(3):247–261.
- Bokkers, E. A. M., Koene, P., Rodenburg, T. B., Zimmerman, P. H., and Spruijt, B. M. (2004). Working for food under conditions of varying motivation in broilers. *Animal Behaviour*, 68(1):105–113.
- Bokkers, E. A. M., Leruste, H., Heutinck, L. F. M., Wolthuis-Fillerup, M., Van Der Werf, J. T. N., Lensink, B. J., and Van Reenen, C. G. (2009). Inter-observer and test-retest reliability of on-farm behavioural observations in veal calves. *Animal Welfare*, 18(4):381–390.
- Bolhuis, J. E., Schouten, W. G. P., Leeuw, J. A. d., Schrama, J. W., and Wiegant, V. M. (2004). Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behavioural Brain Research*, 152(2):351–360.



- Bolhuis, J. E., Schouten, W. G. P., Schrama, J. W., and Wiegant, V. M. (2005). Individual coping characteristics, aggressiveness and fighting strategies in pigs. *Animal Behaviour*, 69(5):1085–1091.
- Borderas, T. F., De Passillé, A. M. B., and Rushen, J. (2009). Feeding behavior of calves fed small or large amounts of milk. *Journal of Dairy Science*, 92(6):2843–2852.
- Breslow, N. E. and Clayton, D. G. (1993). Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association*, 88(421):9–25.
- Breukink, H. J., Wensing, T., and Mouwen, J. M. V. M. (1991). Abomasal ulcers in veal calves: Pathogenesis and prevention. In *EAAP Publication no.52. New Trends in Veal Calf Production*. Pudoc Wageningen, Netherlands.
- Broom, D. M. (1986). Indicators of poor welfare. *British Veterinary Journal*, 142(6):524–526.
- Broom, D. M. and Fraser, A. F. (2007). *Domestic Animal Behaviour and Welfare*. CABI.
- Brcsic, M., Heutinck, L. F. M., Wolthuis-Fillerup, M., Stockhofe, N., Engel, B., Visser, E. K., Gottardo, F., Bokkers, E. A. M., Lensink, B. J., Cozzi, G., and Van Reenen, C. G. (2011). Prevalence of gastrointestinal disorders recorded at postmortem inspection in white veal calves and associated risk factors. *Journal of Dairy Science*, 94(2):853–863.
- Brcsic, M., Leruste, H., Heutinck, L. F. M., Bokkers, E. A. M., Wolthuis-Fillerup, M., Stockhofe, N., Gottardo, F., Lensink, B. J., Cozzi, G., and Van Reenen, C. G. (2012). Prevalence of respiratory disorders in veal calves and potential risk factors. *Journal of Dairy Science*, 95(5):2753–2764.
- Castells, L., Bach, A., Araujo, G., Montoro, C., and Terré, M. (2012). Effect of different forage sources on performance and feeding behavior of Holstein calves. *Journal of Dairy Science*, 95(1):286–293.
- Centres for Disease Control (CDC) (1998). Recommendations to prevent and control iron deficiency in the united states. *Morbidity and Mortality Weekly Report*, 47(RR-3):1–36.
- Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, 9(2):129–136.
- Commun, L., Mialon, M. M., Martin, C., Baumont, R., and Veissier, I. (2009). Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *Journal of Animal Science*, 87(10):3372–3379.
- Conover, W. J. (1980). *Practical Nonparametric Statistics 2nd Ed.* John Wiley and Sons Inc, New York.
- Cooper, S. D. B., Kyriazakis, I., and Oldham, J. D. (1996). The effects of physical form of feed, carbohydrate source, and inclusion of sodium bicarbonate on the diet selections of sheep. *Journal of Animal Science*, 74(6):1240–1251.
- Cooper, S. D. B., Kyriazakis, I., and Nolan, J. V. (1995). Diet selection in sheep: The role of the rumen environment in the selection of a diet from two feeds that differ in their energy density. *British Journal of Nutrition*, 74(01):39–54.
- Coppens, C. M., De Boer, S. F., and Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1560):4021–4028.

- Couderc, J. J., Rearte, D. H., Schroeder, G. F., Ronchi, J. I., and Santini, F. J. (2006). Silage chop length and hay supplementation on milk yield, chewing activity, and ruminal digestion by dairy cows. *Journal of Dairy Science*, 89(9):3599–3608.
- Courvoisier, H., Moisan, M.-P., Sarrieau, A., Hendley, E. D., and Mormède, P. (1996). Behavioral and neuroendocrine reactivity to stress in the wkha/wky inbred rat strains: A multifactorial and genetic analysis. *Brain Research*, 743(1):77–85.
- Cox, D. R. and Hinkley, D. V. (1979). *Theoretical Statistics*. CRC Press.
- Cozzi, G., Gottardo, F., Mattiello, S., Canali, E., Scanziani, E., Verga, M., and Andrighetto, I. (2002). The provision of solid feeds to veal calves: I. Growth performance, forestomach development, and carcass and meat quality. *Journal of Animal Science*, 80(2):357–366.
- Cozzi, G., Gottardo, F., Mutinelli, F., Contiero, B., Fregolent, G., Segato, S., and Andrighetto, I. (2010). Growth performance, behaviour, forestomach development and meat quality of veal calves provided with barley grain or ground wheat straw for welfare purpose. *Italian Journal of Animal Science*, 1(2):113–126.
- Dallman, M. F., Strack, A. M., Akana, S. F., Bradbury, M. J., Hanson, E. S., Scribner, K. A., and Smith, M. (1993). Feast and famine: Critical role of glucocorticoids with insulin in daily energy flow. *Frontiers in Neuroendocrinology*, 14(4):303–347.
- Dantzer, R. and Mormède, P. (1983). Stress in farm animals: A need for reevaluation. *Journal of Animal Science*, 57(1):6.
- Darwin, C. (1859). *On the Origin of the Species by Natural Selection*. Murray.
- De Boever, J. L., Andries, J. I., De Brabander, D. L., Cottyn, B. G., and Buysse, F. X. (1990). Chewing activity of ruminants as a measure of physical structure - A review of factors affecting it. *Animal Feed Science and Technology*, 27(4):281–291.
- De Passillé, A. M. (2001). Sucking motivation and related problems in calves. *Applied Animal Behaviour Science*, 72(3):175–187.
- De Passillé, A. M. and Rushen, J. (1997). Motivational and physiological analysis of the causes and consequences of non-nutritive sucking by calves. *Applied Animal Behaviour Science*, 53(1):15–31.
- De Passillé, A. M., Rushen, J., and Martin, F. (1995). Interpreting the behaviour of calves in an open-field test: A factor analysis. *Applied Animal Behaviour Science*, 45(3):201–213.
- De Vargas, F. M. d., Wechsler, F. S., Rossi, P., Oliveira, M. V. M. d., and Schmidt, P. (2011). Voluntary intake of dry matter and performance of Nellore cows and their Nellore and crossbred Simental × Nellore calves. *Revista Brasileira de Zootecnia*, 40(11):2574–2581.
- De Wilt, J. G. (1985). *Behaviour and Welfare of Veal Calves in Relation to Husbandry Systems*. PhD thesis, Landbouwhogeschool te Wageningen.
- Dellmeier, G. R., Friend, T. H., and Gbur, E. E. (1985). Comparison of four methods of calf confinement. ii. behavior. *Journal of Animal Science*, 60(5):1102–1109.
- Duncan, I. J. H. and Wood-Gush, D. G. M. (1972). Thwarting of feeding behaviour in the domestic fowl. *Animal Behaviour*, 20(3):444–451.

- Engel, B. (1998). A simple illustration of the failure of pql, irrem1 and aphi as approximate ml methods for mixed models for binary data. *Biometrical Journal*, 40(2):141–154.
- Engel, B. and Keen, A. (1994). A simple approach for the analysis of generalized linear mixed models. *Statistica Neerlandica*, 48(1):1–22.
- Estermann, B. L., Wettstein, H.-R., Sutter, F., Erdin, D., and Kreuzer, M. (2003). Effect of calving period on herbage intake and nutrient turnover of Simmental and Angus suckler cows with Angus sired calves grazing subalpine and alpine pastures. *Livestock Production Science*, 79(2):169–182.
- EU Council (1997). Council Directive 97/2/EC of 20 January 1997 amending Directive 91/629/EEC laying down minimum standards for the protection of calves. Bruxelles, Belgium. *Official Journal*, L 025(28/01/1997):24–25.
- EU Council (2008). Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves. Bruxelles, Belgium. *Official Journal*, L 010(15/01/2009):0007–0013.
- Faleiro, A. G., González, L. A., Blanch, M., Cavini, S., Castells, L., Ruíz de la Torre, J. L., Manteca, X., Calsamiglia, S., and Ferret, A. (2011). Performance, ruminal changes, behaviour and welfare of growing heifers fed a concentrate diet with or without barley straw. *Animal*, 5(02):294–303.
- Farm Animal Welfare Council (FAWC) (1992). FAWC updates the five freedoms. *The Veterinary*.
- Favreau, A., Baumont, R., Duncan, A. J., and Ginane, C. (2010). Sheep use preingestive cues as indicators of postingestive consequences to improve food learning. *Journal of Animal Science*, 88(4):1535–1544.
- Felt, B. T. and Lozoff, B. (1996). Brain iron and behavior of rats are not normalized by treatment of iron deficiency anemia during early development. *Journal of Nutrition*, 126(3):693–701.
- Fisher, A. D., Crowe, M. A., Prendiville, D. J., and Enright, W. J. (1997). Indoor space allowance: Effects on growth, behaviour, adrenal and immune responses of finishing beef heifers. *Animal Science*, 64(01):53–62.
- Fiske, J. C. and Potter, G. D. (1979). Discrimination reversal learning in yearling horses. *Journal of Animal Science*, 49(2):583–588.
- Flatt, W. P., Warner, R. G., and Loosli, J. K. (1958). Influence of purified materials on the development of the ruminant stomach. *Journal of Dairy Science*, 41(11):1593–1600.
- Forbes, J. M. and Kyriazakis, I. (1995). Food preferences in farm animals: Why don't they always choose wisely? *Proceedings of the Nutrition Society*, 54(02):429–440.
- Forkman, B. (1991). Some problems with current patch-choice theory: A study on the mongolian gerbil. *Behaviour*, 117(3-4):3–4.
- Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E., and Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, 92(3):340–374.
- Friend, T. H., Dellmeier, G. R., and Gbur, E. E. (1985). Comparison of four methods of calf confinement. i. physiology. *Journal of Animal Science*, 60(5):1095–1101.

- Fröberg, S. and Lidfors, L. (2009). Behaviour of dairy calves suckling the dam in a barn with automatic milking or being fed milk substitute from an automatic feeder in a group pen. *Applied Animal Behaviour Science*, 117(3):150–158.
- Fujita, O., Annen, Y., and Kitaoka, A. (1994). Tsukuba high-and low-emotional strains of rats (*Rattus norvegicus*): An overview. *Behavior Genetics*, 24(4):389–415.
- Goddard, M. E. and Beilharz, R. G. (1984). The relationship of fearfulness to, and the effects of sex, age and experience on exploration and activity in dogs. *Applied Animal Behaviour Science*, 12(3):267–278.
- González, L. A., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K. S., and Ferret, A. (2012). Ruminant acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Animal Feed Science and Technology*, 172(1):66–79.
- Graunke, K. L., Nürnberg, G., Repsilber, D., Puppe, B., and Langbein, J. (2013). Describing temperament in an ungulate: A multidimensional approach. *PLoS ONE*, 8(9):e74579.
- Green, T. C. and Mellor, D. J. (2011). Extending ideas about animal welfare assessment to include ‘quality of life’ and related concepts. *New Zealand Veterinary Journal*, 59(6):263–271.
- Greenwood, R. H., Morrill, J. L., Titgemeyer, E. C., and Kennedy, G. A. (1997). A new method of measuring diet abrasion and its effect on the development of the forestomach. *Journal of Dairy Science*, 80(10):2534–2541.
- Gregorini, P., Clark, C. E. F., Jago, J. G., Glassey, C. B., McLeod, K. L. M., and Romera, A. J. (2009). Restricting time at pasture: Effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. *Journal of Dairy Science*, 92(9):4572–4580.
- Gregorini, P., DelaRue, B., McLeod, K., Clark, C. E. F., Glassey, C. B., and Jago, J. (2012). Rumination behavior of grazing dairy cows in response to restricted time at pasture. *Livestock Science*, 146(1):95–98.
- Haag, E. L., Rudman, R., and Houpt, K. A. (1980). Avoidance, maze learning and social dominance in ponies. *Journal of Animal Science*, 50(2):329–335.
- Haley, D. B., Rushen, J., Duncan, I. J. H., Widowski, T. M., and De Passillé, A. M. (1998). Effects of resistance to milk flow and the provision of hay on nonnutritive sucking by dairy calves. *Journal of Dairy Science*, 81(8):2165–2172.
- Harfeld, J. L. (2013). Telos and the ethics of animal farming. *Journal of Agricultural and Environmental Ethics*, 26(3):691–709.
- Harrison, H. N., Warner, R. G., Sander, E. G., and Loosli, J. K. (1960). Changes in the tissue and volume of the stomachs of calves following the removal of dry feed or consumption of inert bulk. *Journal of Dairy Science*, 43(9):1301–1312.
- Haskins, B. R., Wise, M. B., Craig, H. B., Blumer, T. N., and Barrick, E. R. (1969). Effects of adding low levels of roughages or roughage substitutes to high energy rations for fattening steers. *Journal of Animal Science*, 29(2):345–353.

- Heinrichs, A. J. (2005). Rumen development in the dairy calf. In *Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer from Calf to Calving. 60th University of Nottingham Easter School in Agricultural Science, Nottingham, UK. 23rd-24th March, 2004.*, pages 53–65. Nottingham University Press.
- Heird, J. C., Lennon, A. M., and Bell, R. W. (1981). Effects of early experience on the learning ability of yearling horses. *Journal of Animal Science*, 53(5):1204–1209.
- Heird, J. C., Whitaker, D. D., Bell, R. W., Ramsey, C. B., and Lokey, C. E. (1986). The effects of handling at different ages on the subsequent learning ability of 2-year-old horses. *Applied Animal Behaviour Science*, 15(1):15–25.
- Hessing, M. J. C., Hagelsø, A. M., Schouten, W. G. P., Wiepkema, P. R., and Van Beek, J. A. M. (1994). Individual behavioral and physiological strategies in pigs. *Physiology & Behavior*, 55(1):39–46.
- Hinde, R. A. (1953). Appetitive behaviour, consummatory act, and the hierarchical organisation of behaviour: With special reference to the great tit (*Parus major*). *Behaviour*, 5(3):189–224.
- Holm, L., Jensen, M. B., and Jeppesen, L. L. (2002). Calves' motivation for access to two different types of social contact measured by operant conditioning. *Applied Animal Behaviour Science*, 79(3):175–194.
- Holm, L. and Ladewig, J. (2007). The effect of housing rats in a stimulus rich versus stimulus poor environment on preference measured by sigmoid double demand curves. *Applied Animal Behaviour Science*, 107(3):342–354.
- Holm, L., Ritz, C., and Ladewig, J. (2007). Measuring animal preferences: Shape of double demand curves and the effect of procedure used for varying workloads on their cross-point. *Applied Animal Behaviour Science*, 107(1):133–146.
- Hughes, B. O. and Duncan, I. J. H. (1988a). Behavioural needs: Can they be explained in terms of motivational models? *Applied Animal Behaviour Science*, 19(3):352–355.
- Hughes, B. O. and Duncan, I. J. H. (1988b). The notion of ethological 'need', models of motivation and animal welfare. *Animal Behaviour*, 36(6):1696–1707.
- Hughes, B. O. and Wood-Gush, D. G. M. (1973). An increase in activity of domestic fowls produced by nutritional deficiency. *Animal Behaviour*, 21(1):10–17.
- Hursh, S. R. (1984). Behavioral economics. *Journal of the Experimental Analysis of Behavior*, 42(3):435–452.
- Hursh, S. R. (1993). Behavioral economics of drug self-administration: An introduction. *Drug and Alcohol Dependence*, 33(2):165–172.
- Hutson, G. D. (1984). Animal welfare and consumer demand theory: Are preference tests a luxury we can't afford? *Animal Behaviour*, 32(4):1260–1261.
- Ijichi, C. L., Collins, L. M., and Elwood, R. W. (2013). Evidence for the role of personality in stereotypy predisposition. *Animal Behaviour*, 85(6):1145–1151.

- Inglis, I. R. (2000). The central role of uncertainty reduction in determining behaviour. *Behaviour*, 137(12):1567–1600.
- Inglis, I. R., Forkman, B., and Lazarus, J. (1997). Free food or earned food? A review and fuzzy model of contrafreeloading. *Animal Behaviour*, 53(6):1171–1191.
- Inglis, I. R., Langton, S., Forkman, B., and Lazarus, J. (2001). An information primacy model of exploratory and foraging behaviour. *Animal Behaviour*, 62(3):543–557.
- Jasper, J. and Weary, D. M. (2002). Effects of ad libitum milk intake on dairy calves. *Journal of Dairy Science*, 85(11):3054–3058.
- Jelinski, M. D., Ribble, C. S., Campbell, J. R., and Janzen, E. D. (1996). Descriptive epidemiology of fatal abomasal ulcers in canadian beef calves. *Preventive Veterinary Medicine*, 26(1):9–15.
- Jensen, K. H., Pedersen, L. J., Nielsen, E. K., Heller, K. E., Ladewig, J., and Jørgensen, E. (1996). Intermittent stress in pigs: Effects on behavior, pituitary-adrenocortical axis, growth, and gastric ulceration. *Physiology & Behavior*, 59(4):741–748.
- Jensen, M. B. and Holm, L. (2003). The effect of milk flow rate and milk allowance on feeding related behaviour in dairy calves fed by computer controlled milk feeders. *Applied Animal Behaviour Science*, 82(2):87–100.
- Jensen, M. B. and Pedersen, L. J. (2007). The value assigned to six different rooting materials by growing pigs. *Applied Animal Behaviour Science*, 108(1):31–44.
- Jensen, M. B. and Pedersen, L. J. (2008). Using motivation tests to assess ethological needs and preferences. *Applied Animal Behaviour Science*, 113(4):340–356.
- Jensen, M. B., Vestergaard, K. S., Krohn, C. C., and Munksgaard, L. (1997). Effect of single versus group housing and space allowance on responses of calves during open-field tests. *Applied Animal Behaviour Science*, 54(2):109–121.
- Jensen, P. (1995). Individual variation in the behaviour of pigs—noise or functional coping strategies? *Applied Animal Behaviour Science*, 44(2):245–255.
- Jensen, P. and Toates, F. M. (1993). Who needs ‘behavioural needs’? Motivational aspects of the needs of animals. *Applied Animal Behaviour Science*, 37(2):161–181.
- Kagan, J., Reznick, J. S., and Snidman, N. (1988). Biological bases of childhood shyness. *Science*, 240(4849):167–171.
- Kahyani, A., Ghorbani, G. R., Khorvash, M., Nasrollahi, S. M., and Beauchemin, K. A. (2013). Effects of alfalfa hay particle size in high-concentrate diets supplemented with unsaturated fat: Chewing behavior, total-tract digestibility, and milk production of dairy cows. *Journal of Dairy Science*, 96(11):7110–7119.
- Kammes, K. L. and Allen, M. S. (2012). Nutrient demand interacts with grass particle length to affect digestion responses and chewing activity in dairy cows. *Journal of Dairy Science*, 95(2):807–823.
- Katchuik, R. (1992). Abomasal disease in young beef calves: Surgical findings and management factors. *The Canadian Veterinary Journal*, 33(7):459.

- Kenney, P. A. and Black, J. L. (1984). Factors affecting diet selection by sheep. 1. Potential intake rate and acceptability of feed. *Crop and Pasture Science*, 35(4):551–563.
- Kenney, P. A., Black, J. L., and Colebrook, W. F. (1984). Factors affecting diet selection by sheep. 3. Dry matter content and particle length of forage. *Crop and Pasture Science*, 35(6):831–838.
- Kenward, M. G. and Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, 53(3):983–997.
- Kilgour, R. J., Uetake, K., Ishiwata, T., and Melville, G. J. (2012). The behaviour of beef cattle at pasture. *Applied Animal Behaviour Science*, 138(1):12–17.
- Kirkden, R. D. and Pajor, E. A. (2006). Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. *Applied Animal Behaviour Science*, 100(1):29–47.
- Kooijman, J., Wierenga, H. K., and Wiepkema, P. R. (1991). Development of abnormal oral behaviour in group-housed veal calves: Effects of roughage supply. In *EAAP Publication no.52. New Trends in Veal Calf Production*. Pudoc Wageningen, Netherlands.
- Koolhaas, J. M., De Boer, S. F., Buwalda, B., and Van Reenen, C. (2007). Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and Evolution*, 70(4):218–226.
- Krause, K. M., Combs, D. K., and Beauchemin, K. A. (2002). Effects of forage particle size and grain fermentability in midlactation cows. ii. Ruminant pH and chewing activity. *Journal of Dairy Science*, 85(8):1947–1957.
- Kyriazakis, I., Anderson, D. H., and Duncan, A. J. (1998). Conditioned flavour aversions in sheep: The relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. *British Journal of Nutrition*, 79(01):55–62.
- Ladewig, J., De Passillé, A. M., Rushen, J., Schouten, W., Terlouw, E. M. C., and Von Borell, E. (1993). Stress and the physiological correlates of stereotypic behaviour. In Lawrence, A. B., editor, *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*. CAB International, Oxon UK.
- Ladewig, J. and Smidt, D. (1989). Behavior, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethering. *Hormones and Behavior*, 23(3):344–360.
- Latham, N. and Mason, G. (2010). Frustration and perseveration in stereotypic captive animals: Is a taste of enrichment worse than none at all? *Behavioural Brain Research*, 211(1):96–104.
- Lawrence, A. B. (1987). Consumer demand theory and the assessment of animal welfare. *Animal Behaviour*, 35(1):293–295.
- Lawrence, A. B. and Terlouw, E. (1993). A review of behavioral factors involved in the development and continued performance of stereotypic behaviors in pigs. *Journal of Animal Science*, 71(10):2815–2825.
- Lawrence, A. B., Terlouw, E. M., and Kyriazakis, I. (1993). The behavioural effects of undernutrition in confined farm animals. *Proceedings of the Nutrition Society*, 52(01):219–229.

- Le Neindre, P. and Petit, M. (1975). Nombre de tétées et temps de pâturage des veaux dans les troupeaux de vaches allaitantes. *Annales de Zootechniques*, 24(3):553–558.
- Le Scolan, N., Hausberger, M., and Wolff, A. (1997). Stability over situations in temperamental traits of horses as revealed by experimental and scoring approaches. *Behavioural Processes*, 41(3):257–266.
- Leruste, H. (2014). *Assessing Welfare of Veal Calves on Farms: Measures of Behaviour and Respiratory Disorders and Potential Ways for Welfare Improvement*. PhD thesis, Wageningen: Wageningen University.
- Lidfors, L. M. (1993). Cross-sucking in group-housed dairy calves before and after weaning off milk. *Applied Animal Behaviour Science*, 38(1):15–24.
- Lindt, F. and Blum, J. W. (1994). Occurrence of iron deficiency in growing cattle. *Journal of Veterinary Medicine Series A*, 41(1-10):237–246.
- Loberg, J. and Lidfors, L. (2001). Effect of milkflow rate and presence of a floating nipple on abnormal sucking between dairy calves. *Applied Animal Behaviour Science*, 72(3):189–199.
- Lozoff, B. (1987). Behavioral alterations in iron deficiency. *Advances in Pediatrics*, 35:331–359.
- Manninen, E., De Passillé, A. M., Rushen, J., Norring, M., and Saloniemi, H. (2002). Preferences of dairy cows kept in unheated buildings for different kind of cubicle flooring. *Applied Animal Behaviour Science*, 75(4):281–292.
- Manteca, X., Villalba, J. J., Atwood, S. B., Dziba, L., and Provenza, F. D. (2008). Is dietary choice important to animal welfare? *Journal of Veterinary Behavior: Clinical Applications and Research*, 3(5):229–239.
- Margerison, J. K., Preston, T. R., Berry, N., and Phillips, C. J. C. (2003). Cross-sucking and other oral behaviours in calves, and their relation to cow suckling and food provision. *Applied Animal Behaviour Science*, 80(4):277–286.
- Marinier, S. L. and Alexander, A. J. (1994). The use of a maze in testing learning and memory in horses. *Applied Animal Behaviour Science*, 39(2):177–182.
- Martin, P. and Bateson, P. P. G. (1993). *Measuring Behaviour: An Introductory Guide*. Cambridge University Press.
- Mason, G. J. (1991a). Stereotypies: A critical review. *Animal Behaviour*, 41(6):1015–1037.
- Mason, G. J. (1991b). Stereotypies and suffering. *Behavioural Processes*, 25(2):103–115.
- Mason, G. J. and Latham, N. R. (2004). Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? *Animal Welfare*, 13(Suppl):S57–S69.
- Matthews, L. R. and Temple, W. (1979). Concurrent schedule assessment of food preference in cows. *Journal of the Experimental Analysis of Behavior*, 32(2):245–254.
- Mattiello, S., Canali, E., Ferrante, V., Caniatti, M., Gottardo, F., Cozzi, G., Andrighetto, I., and Verga, M. (2002). The provision of solid feeds to veal calves: Ii. Behavior, physiology, and abomasal damage. *Journal of Animal Science*, 80(2):367–375.



- McAdie, T. M., Foster, T. M., Temple, W., and Matthews, L. R. (1993). A method for measuring the aversiveness of sounds to domestic hens. *Applied Animal Behaviour Science*, 37(3):223–238.
- McCullagh, P. N. J. A. and Nelder, J. A. (1989). An outline of generalized linear models. In *Generalized Linear Models*, pages 21–47. Springer.
- McCulloch, C. E. (2006). *Generalized Linear Mixed Models*. Wiley Online Library.
- Meagher, R. K. and Mason, G. J. (2012). Environmental enrichment reduces signs of boredom in caged mink. *PLoS ONE*, 7(11):e49180.
- Melotti, L., Thomsen, L. R., Toscano, M. J., Mendl, M., and Held, S. (2013). Delay discounting task in pigs reveals response strategies related to dopamine metabolite. *Physiology & Behavior*, 120:182–192.
- Mertens, D. R. (1997). Creating a system for meeting the fiber requirements of dairy cows. *Journal of Dairy Science*, 80(7):1463–1481.
- Miller-Cushon, E. K., Bergeron, R., Leslie, K. E., Mason, G. J., and DeVries, T. J. (2013). Effect of early exposure to different feed presentations on feed sorting of dairy calves. *Journal of Dairy Science*, 96(7):4624–4633.
- Morandfehr, P., Hervieu, J., Legendre, D., Gutter, A., and Deltedesco, L. (1987). Rapid tests to assess concentrate feed acceptability by goats. *Annales De Zootechnie*, 36(3):324–324.
- Morisse, J. P., Cotte, J. P., Huonnic, D., and Martrenchar, A. (1999). Influence of dry feed supplements on different parameters of welfare in veal calves. *Animal Welfare*, 8(1):43–52.
- Morisse, J. P., Huonnic, D., Cotte, J. P., and Martrenchar, A. (2000). The effect of four fibrous feed supplementations on different welfare traits in veal calves. *Animal Feed Science and Technology*, 84(1):129–136.
- Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., Van Reenen, C. G., Richard, S., and Veissier, I. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology & Behavior*, 92(3):317–339.
- Mormède, P., Foury, A., Terenina, E., and Knap, P. W. (2011). Breeding for robustness: The role of cortisol. *Animal*, 5(05):651–657.
- Nagy, K., Bodó, G., Bárdos, G., Bánszky, N., and Kabai, P. (2010). Differences in temperament traits between crib-biting and control horses. *Applied Animal Behaviour Science*, 122(1):41–47.
- Negrao, J. A., Porcionato, M. A., De Passille, A. M., and Rushen, J. (2004). Cortisol in saliva and plasma of cattle after ACTH administration and milking. *Journal of Dairy Science*, 87(6):1713–1718.
- Ngwa, A. T., Pone, D. K., and Mafeni, J. M. (2000). Feed selection and dietary preferences of forage by small ruminants grazing natural pastures in the Sahelian zone of Cameroon. *Animal Feed Science and Technology*, 88(3):253–266.
- Nocek, J. E. (1997). Bovine acidosis: Implications on laminitis. *Journal of Dairy Science*, 80(5):1005–1028.

## 234 | REFERENCES

- NRC (2000). *Nutrient Requirements of Beef Cattle*. National Academy Press, Washington, DC.
- Osborne, A. D. (1976). Hairballs in veal calves. *Veterinary Record*, 99(12):239–239.
- Osborne, S. R. (1977). The free food (contrafreeloading) phenomenon: A review and analysis. *Animal Learning & Behavior*, 5(3):221–235.
- Parrott, R. F., Misson, B. H., and Baldwin, B. A. (1989). Salivary cortisol in pigs following adrenocorticotrophic hormone stimulation: Comparison with plasma levels. *British Veterinary Journal*, 145(4):362–366.
- Pedersen, L. J., Holm, L., Jensen, M. B., and Jørgensen, E. (2005). The strength of pigs' preferences for different rooting materials measured using concurrent schedules of reinforcement. *Applied Animal Behaviour Science*, 94(1):31–48.
- Pedersen, L. J., Jensen, M. B., Hansen, S. W., Munksgaard, L., Ladewig, J., and Matthews, L. (2002). Social isolation affects the motivation to work for food and straw in pigs as measured by operant conditioning techniques. *Applied Animal Behaviour Science*, 77(4):295–309.
- Petherick, J. C., Seawright, E., and Waddington, D. (1993). Influence of motivational state on choice of food or a dustbathing/foraging substrate by domestic hens. *Behavioural Processes*, 28(3):209–220.
- Phillips, C. J. C. (2004). The effects of forage provision and group size on the behavior of calves. *Journal of Dairy Science*, 87(5):1380–1388.
- Prevedello, P., Andrighetto, I., Schiavon, E., and Gottardo, F. (2010). Administration of high amounts of two solid feeds to veal calves: Effects on growth performance and slaughter traits. *Italian Journal of Animal Science*, 8(2s):534–536.
- Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management Archives*, 48(1):2–17.
- Provenza, F. D. and Balph, D. F. (1987). Diet learning by domestic ruminants: Theory, evidence and practical implications. *Applied Animal Behaviour Science*, 18(3):211–232.
- Provenza, F. D., Scott, C. B., Phy, T. S., and Lynch, J. J. (1996). Preference of sheep for foods varying in flavors and nutrients. *Journal of Animal Science*, 74(10):2355–2361.
- Ramos, A., Berton, O., Mormède, P., and Chaouloff, F. (1997). A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behavioural Brain Research*, 85(1):57–69.
- Redbo, I. (1990). Changes in duration and frequency of stereotypies and their adjoining behaviours in heifers, before, during and after the grazing period. *Applied Animal Behaviour Science*, 26(1):57–67.
- Redbo, I. (1992). The influence of restraint on the occurrence of oral stereotypies in dairy cows. *Applied Animal Behaviour Science*, 35(2):115–123.
- Redbo, I. (1993). Stereotypies and cortisol secretion in heifers subjected to tethering. *Applied Animal Behaviour Science*, 38(3):213–225.

- Redbo, I. (1998). Relations between oral stereotypies, open-field behavior, and pituitary–adrenal system in growing dairy cattle. *Physiology & Behavior*, 64(3):273–278.
- Redbo, I., Emanuelson, M., Lundberg, K., and Oredsson, N. (1996). Feeding level and oral stereotypies in dairy cows. *Animal Science*, 62(2):199–206.
- Redbo, I. and Nordblad, A. I. I. (1997). Stereotypies in heifers are affected by feeding regime. *Applied Animal Behaviour Science*, 53(3):193–202.
- Reimert, I., Bolhuis, J. E., Kemp, B., and Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109:42–50.
- Reinhardt, V. and Reinhardt, A. (1981). Natural sucking performance and age of weaning in zebu cattle (*Bos indicus*). *The Journal of Agricultural Science*, 96(02):309–312.
- Rushen, J. (1993). The ‘coping hypothesis’ of stereotypic behaviour. *Animal Behaviour*, 45(3):613–615.
- Rushen, J., De Passillé, A. M., von Keyserlingk, M. A. G., and Weary, D. M. (2008). *The Welfare of Cattle*. Springer.
- Rushen, J., Lawrence, A. B., and Terlouw, E. M. C. (1993). The motivational basis of stereotypies. In Lawrence, A. B., editor, *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*. CAB International, Oxon UK.
- Sander, E. G., Warner, R. G., Harrison, H. N., and Loosli, J. K. (1959). The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. *Journal of Dairy Science*, 42(9):1600–1605.
- SAS Institute Inc. (2008). Statistical analysis system version 9.1.
- Schall, R. (1991). Estimation in generalized linear models with random effects. *Biometrika*, 78(4):719–727.
- Schouten, W. G. P. and Wiepkema, P. R. (1991). Coping styles of tethered sows. *Behavioural Processes*, 25(2):125–132.
- Schulze Westerath, H., Laister, S., Winckler, C., and Knierim, U. (2009). Exploration as an indicator of good welfare in beef bulls: An attempt to develop a test for on-farm assessment. *Applied Animal Behaviour Science*, 116(2):126–133.
- Scott, L. L. and Provenza, F. D. (1999). Variation in food selection among lambs: Effects of basal diet and foods offered in a meal. *Journal of Animal Science*, 77(9):2391–2397.
- Searle, K. R., Hunt, L. P., and Gordon, I. J. (2010). Individualistic herds: Individual variation in herbivore foraging behavior and application to rangeland management. *Applied Animal Behaviour Science*, 122(1):1–12.
- Seo, T., Sato, S., Kosaka, K., Sakamoto, N., and Tokumoto, K. (1998a). Tongue-playing and heart rate in calves. *Applied Animal Behaviour Science*, 58(1):179–182.

- Seo, T., Sato, S., Kosaka, K., Sakamoto, N., Tokumoto, K., and Katoh, K. (1998b). Development of tongue-playing in artificially reared calves: Effects of offering a dummy-teat, feeding of short cut hay and housing system. *Applied Animal Behaviour Science*, 56(1):1–12.
- Smits, A. and De Wilt, J. (1991). Group housing of veal calves. In *EAAP Publication no.52. New Trends in Veal Calf Production*. Pudoc Wageningen, Netherlands.
- Sørensen, D. B., Ladewig, J., Ersbøll, A. K., and Matthews, L. (2004). Using the cross point of demand functions to assess animal priorities. *Animal Behaviour*, 68(4):949–955.
- Sørensen, D. B., Ladewig, J., Matthews, L., Ersbøll, A. K., and Lawson, L. (2001). Measuring motivation: Using the cross point of two demand functions as an assessment of the substitutability of two reinforcers. *Applied Animal Behaviour Science*, 74(4):281–291.
- Suárez, B. J., Van Reenen, C. G., Gerrits, W. J. J., Stockhofe, N., Van Vuuren, A. M., and Dijkstra, J. (2006). Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. *Journal of Dairy Science*, 89(11):4376–4386.
- Suárez, B. J., Van Reenen, C. G., Stockhofe, N., Dijkstra, J., and Gerrits, W. J. J. (2007). Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. *Journal of Dairy Science*, 90(5):2390–2403.
- Tamate, H., McGilliard, A. D., Jacobson, N. L., and Getty, R. (1962). Effect of various dietaries on the anatomical development of the stomach in the calf. *Journal of Dairy Science*, 45(3):408–420.
- Tamminga, S., Van Straalen, W. M., Subnel, A. P. J., Meijer, R. G. M., Steg, A., Wever, C. J. G., and Blok, M. C. (1994). The dutch protein evaluation system: The DVE/OEB-system. *Livestock Production Science*, 40(2):139–155.
- Teskey, G. C., Ossenkopp, K.-P., Kavaliers, M., Innis, N. K., and Boon, F. H. (1998). Individual differences in radial maze performance and locomotor activity in the meadow vole, *Microtus pennsylvanicus*. *Physiology & Behavior*, 65(3):555–561.
- Toates, F. M. (1986). *Motivational Systems*. CUP Archive.
- Toates, F. M. (2004). Cognition, motivation, emotion and action: A dynamic and vulnerable interdependence. *Applied Animal Behaviour Science*, 86(3):173–204.
- Todd, C. G., DeVries, T. J., Leslie, K. E., Sargeant, J. M., Shore, K., Anderson, N. G., and Millman, S. T. (2010). Effects of free-access feeding and milk replacer acidification on calf performance and development of digestive anatomy. In *First North America Conference on Precision Dairy Management*. <http://www.precisiondairy2010.com/conferenceproceedings.htm>, Toronto, Canada.
- Toofanian, F. (1976). Hairballs in veal calves. *Veterinary Record*, 99(22):444.
- Tsigos, C. and Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, 53(4):865–871.
- Tulleners, E. P. and Hamilton, G. F. (1980). Surgical resection of perforated abomasal ulcers in calves. *The Canadian Veterinary Journal*, 21(9):262.
- Van Es, A. J. H. (1978). Feed evaluation for ruminants. i. The systems in use from May 1977-onwards in the Netherlands. *Livestock Production Science*, 5(4):331–345.

- Van Reenen, C. G., Engel, B., Ruis-Heutinck, L. F. M., Van der Werf, J. T. N., Buist, W. G., Jones, R. B., and Blokhuis, H. J. (2004). Behavioural reactivity of heifer calves in potentially alarming test situations: A multivariate and correlational analysis. *Applied Animal Behaviour Science*, 85(1):11–30.
- Van Reenen, C. G., Hopster, H., Van der Werf, J. T. N., Engel, B., Buist, W. G., Jones, R. B., Blokhuis, H. J., and Korte, S. M. (2009). The benzodiazepine brotizolam reduces fear in calves exposed to a novel object test. *Physiology & Behavior*, 96(2):307–314.
- Van Reenen, C. G., Mars, M. H., Leushuis, I. E., Rijsewijk, F. A. M., Van Oirschot, J. T., and Blokhuis, H. J. (2000). Social isolation may influence responsiveness to infection with bovine herpesvirus 1 in veal calves. *Veterinary microbiology*, 75(2):135–143.
- Van Reenen, C. G., O’Connell, N. E., Van der Werf, J. T. N., Korte, S. M., Hopster, H., Jones, R. B., and Blokhuis, H. J. (2005). Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. *Physiology & Behavior*, 85(5):557–570.
- Van Reenen, C. G., Strappini, J. T. N., Van der Werf, J. T. N., Schouten, W. G. P., and Veissier, I. (2001). Physiological correlates of abnormal oral behaviour in veal calves. In *35th International Society for Applied Ethology*, page 101, Davis, USA.
- Van Reenen, C. G., Van der Werf, J. T., O’Connell, N. E., Heutinck, L. F. M., Spoolder, H. A. M., Jones, R. B., Koolhaas, J. M., and Blokhuis, H. J. (2013). Behavioural and physiological responses of heifer calves to acute stressors: Long-term consistency and relationship with adult reactivity to milking. *Applied Animal Behaviour Science*, 147(1):55–68.
- Van Weeren-Keverling Buisman, A., Noordhuizen-Stassen, E. N. and Breukink, H. J., Wensing, T., and Mouwen, J. M. V. M. (1988). Villus atrophy in ruminal drinking calves and mucosal restoration after reconditioning. *Veterinary Quarterly*, 10(3):164–171.
- Van Weeren-Keverling Buisman, A., Wensing, T., Breukink, H. J., and Mouwen, J. M. V. M. (1991). Ruminal drinking in veal calves. In *EAAP Publication no.52. New Trends in Veal Calf Production*. Pudoc Wageningen, Netherlands.
- Veillette, M. and Reeb, S. G. (2011). Shelter choice by syrian hamsters (*Mesocricetus auratus*) in the laboratory. *Animal Welfare*, 20(4):603–611.
- Veissier, I., Boissy, A., Rushen, J., Van Reenen, C. G., Roussel, S., Andanson, S., and Pradel, P. (2001). Calves’ responses to repeated social regrouping and relocation. *Journal of Animal Science*, 79(10):2580–2593.
- Veissier, I., Ramirez De La Fe, A., and Pradel, P. (1998). Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. *Applied Animal Behaviour Science*, 57(1):35–49.
- Vicari, T., Van den Borne, J. J. G. C., Gerrits, W. J. J., Zbinden, Y., and Blum, J. W. (2008). Postprandial blood hormone and metabolite concentrations influenced by feeding frequency and feeding level in veal calves. *Domestic Animal Endocrinology*, 34(1):74–88.
- Villalba, J. J., Provenza, F. D., and Manteca, X. (2010). Links between ruminants’ food preference and their welfare. *Animal*, 4(07):1240–1247.

- Visser, E. K., Van Reenen, C. G., Engel, B., Schilder, M. B. H., Barneveld, A., and Blokhuis, H. J. (2003a). The association between performance in show-jumping and personality traits earlier in life. *Applied Animal Behaviour Science*, 82(4):279–295.
- Visser, E. K., Van Reenen, C. G., Schilder, M. B. H., Barneveld, A., and Blokhuis, H. J. (2003b). Learning performances in young horses using two different learning tests. *Applied Animal Behaviour Science*, 80(4):311–326.
- VSN-International (2012). Genstat for windows 15th edition.
- Webb, L. E., Bokkers, E. A. M., Engel, B., Gerrits, W. J. J., Berends, H., and Van Reenen, C. G. (2012). Behaviour and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. *Applied Animal Behaviour Science*, 136(2):108–116.
- Webb, L. E., Bokkers, E. A. M., Heutinck, L. F. M., Engel, B., Buist, W. G., Rodenburg, T. B., Stockhofe-Zurwieden, N., and Van Reenen, C. G. (2013). Effects of roughage source, amount, and particle size on behavior and gastrointestinal health of veal calves. *Journal of Dairy Science*, 96(12):7765–7776.
- Webb, L. E., Engel, B., Berends, H., Van Reenen, C. G., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2014a). What do calves choose to eat and how do preferences affect behaviour? *Applied Animal Behaviour Science*, DOI: 10.1016/j.applanim.2014.09.016.
- Webb, L. E., Jensen, M. B., Engel, B., Van Reenen, C. G., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2014b). Chopped or long roughage: What do calves prefer? Using cross point analysis of double demand functions. *PLoS ONE*, 9(2):e88778.
- Welch, J. G. (1982). Rumination, particle size and passage from the rumen. *Journal of Animal Science*, 54(4):885–894.
- Welchman, D. D. and De Baust, G. N. (1987). A survey of abomasal ulceration in veal calves. *Veterinary Record*, 121(25-26):586–590.
- Welfare Quality (2009). Welfare quality assessment protocol for cattle. Welfare quality consortium, Lelystad/Netherlands; ISBN. Technical report, EAN 978-90-78240-04-4.
- Wemelsfelder, F. (1993). The concept of animal boredom and its relationship to stereotyped behaviour. In Lawrence, A. B., editor, *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*. CAB International, Oxon UK.
- Wickens, C. L. and Heleski, C. R. (2010). Crib-biting behavior in horses: A review. *Applied Animal Behaviour Science*, 128(1):1–9.
- Wiepkema, P. R. (1987). Developmental aspects of motivated behavior in domestic animals. *Journal of Animal Science*, 65(5):1220–1227.
- Wiepkema, P. R., Van Hellemond, K. K., Roessingh, P., and Romberg, H. (1987). Behaviour and abomasal damage in individual veal calves. *Applied Animal Behaviour Science*, 18(3):257–268.
- Willner, P., Moreau, J.-L., Kurre Nielsen, C., Papp, M., and Sluzewska, A. (1996). Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. *Physiology & Behavior*, 60(1):129–134.

- Wilson, E. M. and Green, J. R. (2009). The development of jaw motion for mastication. *Early Human Development*, 85(5):303–311.
- Wilson, J. R. and Kennedy, P. M. (1996). Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. *Crop and Pasture Science*, 47(2):199–225.
- Wise, G. H. and Anderson, G. W. (1939). Factors affecting the passage of liquids into the rumen of the dairy calf. I. Method of administering liquids: drinking from open pail versus sucking through a rubber nipple. *Journal of Dairy Science*, 22(9):697–705.
- Wolfinger, R. and O'connell, M. (1993). Generalized linear mixed models a pseudo-likelihood approach. *Journal of Statistical Computation and Simulation*, 48(3-4):233–243.
- Wood-Gush, D. G. M., Hunt, K., Carson, K., and Dennison, S. G. C. (1984). The early behaviour of suckler calves in the field. *Biology of Behaviour*, 9(4):295–306.





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# About the author

Laura Webb was born in Rennes, France, in 1986. She obtained a joint honours BSc degree in Psychology and Zoology from the University of Bristol, England (2007) and a MSc degree in Applied Animal Behaviour and Welfare from Edinburgh University, Scotland (2008). During her BSc, Laura investigated the presence of wing-based acoustic crypsis in moths. During her MSc, Laura studied the social interactions surrounding the infant in a family group of captive Diana monkeys (Edinburgh Zoo, Scotland) and assessed the impact of a period of undernutrition during the first 90 days of pregnancy on ewe-lamb bond formation (Scottish Agricultural College, Scotland). Following her MSc, Laura conducted a project to provide an initial time period for the habituation of marmosets to the presence of a human observer (MRC laboratory, Edinburgh, Scotland), and worked as a field research assistant on a project monitoring the mating behaviour and courtship of male Satin bowerbirds (New South Wales, Australia). In 2009, she worked as a research assistant, conducting research on the alleviation of pain and distress in laboratory rodents (Institute of Neuroscience, Newcastle University, England).

In 2010, Laura started her PhD at the Animal Production Systems chair group of Wageningen University, where she investigated the feeding preferences and behavioural needs of veal calves with regards to solid feed. Her PhD research was part of an interdisciplinary project entitled ‘Novel roughage-based feeding strategies to improve the welfare of veal calves’, which integrated animal behaviour and health, animal nutrition, and modelling of environmental and economic impacts. This project was part of the program ‘The value of Animal Welfare’, instigated by the Netherlands Organisation for Scientific Research (NWO) and the Dutch Ministry of Economic Affairs.

Laura was awarded the Nederlandse Zoötechnische Vereniging (NZV) travel grant

in 2011 for her poster entitled ‘How much solid feed do veal calves really need?’. She received the Best Paper Award by the Nutrition Commission of the 62<sup>nd</sup> EAAP congress in Stavanger, Norway, for her presentation entitled: ‘How do different amounts of solid feed affect the behaviour and welfare of veal calves?’. Furthermore, she obtained the best poster award at the 2011 NWO Symposium ‘Waardering voor Dierenwelzijn’ for her poster entitled: ‘Towards a sustainable diet for veal calves: II. Can calves choose a diet that optimises their welfare?’. Laura was granted a UFAW Small projects and travel award in 2013 to go to Denmark for collaborative work with Margit Bak Jensen, Aarhus University.

Currently, Laura is writing a post-doctoral grant proposal designed to detect patterns in complex datasets, and this is applied to the detection of early signals for health and behaviour problems in calves. To undertake this work within the Animal Production Systems chair group, Laura was awarded a WIAS fellowship.



# List of publications

## Refereed scientific journals

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- Webb, L. E., Bokkers, E. A. M., Heutinck, L. F. M., Engel, B., Buist, W. G., Rodenburg, T. B., Stockhofe-Zurwieden, N., and Van Reenen, C. G. (2013). Effects of roughage source, amount, and particle size on behavior and gastrointestinal health of veal calves. *Journal of Dairy Science*, 96(12):7765-7776.
- Webb, L. E., Jensen, M. B., Engel, B., Van Reenen, C. G., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2014). Chopped or long roughage: what do calves prefer? Using cross point analysis of double demand functions. *PLoS ONE*, 9(2):e88778.
- Webb, L. E., Engel, B., Berends, H., Van Reenen, C.G., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2014). What do calves choose to eat and how do preferences affect behaviour? *Applied Animal Behaviour Science*, DOI: 10.1016/j.applanim.2014.09.016.
- Webb, L. E., Van Reenen, C.G., Jensen, M. B., Schmitt, O., and Bokkers, E. A. M. Does temperament affect learning in calves? *Submitted*.
- Webb, L. E., Van Reenen, C.G., Engel, B., Berends, H., Gerrits, W. J. J., and Bokkers, E. A. M. Can tongue playing in veal calves be linked to cortisol or temperament? *Submitted*.

- Webb, L. E., Van Reenen, C.G., Berends, H., Engel, B., De Boer, I. J. M., Gerrits, W. J. J., and Bokkers, E. A. M. The role of solid feed amount and composition, and milk replacer supply in veal calf welfare. *Submitted*.
- Berends, H., Gerrits, W. J. J., Webb, L. E., Bokkers, E. A. M., Gilbert, M. S., Engel, B., and Van Reenen, C.G. Effects of free diet selection on dietary preferences, health, and performance of Holstein-Friesian calves. *Submitted*.
- Engel, B., Webb, L. E., Jensen, M. B., Van Reenen, C. G., and Bokkers, E. A. M. (2014). Methods for cross point analysis of double-demand functions in assessing animal preferences. *Applied Animal Behaviour Science*, DOI: 10.1016/j.applanim.2014.09.004.

### Conference proceedings and abstracts

- Webb, L. E., Bokkers, E. A. M., Heutinck, L. F. M., and Van Reenen, C. G. (2010). Comparing the effects of different roughage diets on grooming behaviour and hairball prevalence in veal calves. In: *Proceedings of the 44<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 3-7 August, Uppsala, Sweden.
- Webb, L. E., Bokkers, E. A. M., and Van Reenen, C. G. (2011). How much solid feed do veal calves really need? (poster). *Wageningen Institute of Animal Sciences (WIAS) Science Day*, 3 February, Wageningen, the Netherlands.
- Webb, L. E., Bokkers, E. A. M., Engel, B., Gerrits, W. J. J., and Van Reenen, C. G. (2011). Behaviour of veal calves. *5<sup>th</sup> International Veal Congress*, 20 May, Noordwijk, the Netherlands.
- Webb, L. E., Bokkers, E. A. M., and Van Reenen, C. G. (2011). How do different amounts of solid feed in the diet affect time spent performing abnormal oral behaviours in veal calves? In: *Proceedings of the 45<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 31 July-4 August, Indianapolis, USA.
- Webb, L. E., Bokkers, E. A. M., Engel, B., and Van Reenen, C. G. (2011). How do different amounts of solid feed in the diet affect the behaviour and welfare of veal calves? In: *Proceedings of the 62<sup>nd</sup> Annual Meeting of the European Federation of Animal Science (EAAP)*, 2 September, Stavanger, Norway.
- Webb, L. E., Berends, H., and Mollenhorst, H. (2011). Towards a sustainable diet for veal calves: II. Can calves choose a diet that optimises their welfare?



- (poster). *Netherlands Organisation for Scientific Research (NWO) symposium*, 30 November, Utrecht, the Netherlands.
- Webb, L. E., Bokkers, E. A. M., and Van Reenen, C. G. (2011). What type, amount, and structure of solid feed do veal calves need for improved behaviour and health? *Farm Animal Health Group (GLL) congress*, 1 December, Doorn, the Netherlands.
  - Webb, L. E., Van Reenen, C. G., Engel, B., Berends, H., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2012). What do calves choose to eat and how does free choice affect behaviour? In: *Proceedings of the 46<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 31 July-4 August, Vienna, Austria.
  - Bokkers, E. A. M., Webb, L. E., Engel, B., Berends, H., Gerrits, W. J. J., and Van Reenen, C. G. (2012). How does solid feed provision and display of abnormal oral behaviours affect salivary cortisol in veal calves? (poster). In: *Proceedings of the 46<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 31 July-4 August, Vienna, Austria.
  - Webb, L. E., Jensen, M. B., Van Reenen, C. G., Engel, B., Gerrits, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2013). Chopped or long roughage: what do calves prefer? *Wageningen Institute of Animal Sciences (WIAS) Science Day*, 28 February, Wageningen, the Netherlands.
  - Webb, L. E., Jensen, M. B., Engel, B., Van Reenen, C. G., De Boer, I. J. M., Gerrits, W. J. J., and Bokkers, E. A. M. (2013). Chopped or long roughage: what do calves prefer? In: *Proceedings of the 47<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 2-6 June, Florianópolis, Brazil.
  - Webb, L. E., Van Reenen, C. G., Jensen, M. B., Schmitt, O., and Bokkers, E. A. M. (2014). Does personality affect learning in calves? (poster). *Wageningen Institute of Animal Sciences (WIAS) Science Day*, 30 April, Wageningen, the Netherlands.
  - Webb, L. E., Van Reenen, C.G., Berends, H., Engel, B., Gerrist, W. J. J., De Boer, I. J. M., and Bokkers, E. A. M. (2014). More solid or ad libitum long straw? Best diet to improve veal calf welfare (poster). In: *Proceedings of the 48<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE)*, 29 July-2 August, Vittoria, Spain.

### Other publications related to this thesis

- Wageningers zoeken uit wat kalf vreten wil (2011). *Agrarisch Dagblad dinsdag*, 5 July, p.3.
- Sikkema, A. (2012). Calves are happier on solid feed (Vleeskalveren gebaat bij meer vast voer). *Resource - voor studenten en medewerkers van Wageningen UR*, 6(13-23 February):10.
- Kalveren moeten meer vast voer krijgen (2012). *Nieuwe Oogst.nu*, 16 February.
- Veldman, J. W. (2012). Blankvleeskalveren zijn gebaat bij meer vast voer. *Boerderij.nl*, 16 February.

# Education certificate

## Completed training and supervision plan<sup>1</sup>

### The basic package (3 ECTS)

- WIAS Introduction course (2010)
- EL&I-NWO course 'Ethics & animal welfare', Lunteren, the Netherlands (2011)

### International conferences (9.4 ECTS)

- 44<sup>th</sup> ISAE congress, Uppsala, Sweden (2011)
- 5<sup>th</sup> International Veal Congress, Noordwijk, the Netherlands (2011)
- 45<sup>th</sup> ISAE congress, Indianapolis, USA (2011)
- 62<sup>nd</sup> EAAP Annual Meeting, Stavanger, Norway (2011)
- Farm Animal Health Group (GLL) congress, Doorn, the Netherlands (2011)
- 46<sup>th</sup> ISAE congress, Vienna, Austria (2012)
- 47<sup>th</sup> ISAE congress, Florianópolis, Brazil (2013)
- 48<sup>th</sup> ISAE congress, Vittoria, Spain (2014)

### Seminars and workshops (2.3 ECTS)

- NVG PhD workshop, Soesterberg (2010)
- WIAS Science Day (2011, 2013-14)

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<sup>1</sup>With the activities listed, the PhD candidate has complied with the educational requirements set by the Graduate School of Wageningen Institute of Animal Science (WIAS). One ECTS equals a study load of 28 hours.

- Scientific Research in Animal Welfare: Do we Make a Difference? Wageningen (2011)
- NWO Symposium 'Waardering voor Dierenwelzijn', Utrecht (2011)
- NWO Symposium 'Waardering voor Dierenwelzijn', Den Haag (2013)
- NWO Symposium 'Waardering voor Dierenwelzijn', Wageningen (2014)

### **Theatre and poster presentations (13 ECTS)**

- Poster, WIAS Science Day, Wageningen, the Netherlands (2011)
- Theatre, 44<sup>th</sup> ISAE congress, Uppsala, Sweden (2011)
- Theatre, 5<sup>th</sup> International Veal Congress, Noordwijk, the Netherlands (2011)
- Theatre, 45<sup>th</sup> ISAE congress, Indianapolis, USA (2011)
- Theatre, 62<sup>nd</sup> EAAP Annual Meeting, Stavanger, Norway (2011)
- Poster, NWO Symposium, Utrecht, the Netherlands (2011)
- Theatre, GGL congress KNMvD, Doorn, the Netherlands (2011)
- Theatre, 46<sup>th</sup> ISAE congress, Vienna, Austria (2012)
- Theatre, WIAS Science Day, Wageningen, the Netherlands (2013)
- Theatre, 47<sup>th</sup> ISAE congress, Florianópolis, Brazil (2013)
- Poster, WIAS Science Day, Wageningen, the Netherlands (2014)
- Theatre, NWO Symposium, Wageningen, the Netherlands (2014)
- Poster, 48<sup>th</sup> ISAE congress, Vitoria, Spain (2014)

### **In-depth studies (11 ECTS)**

- EL&I/NWO course 'Sustainable animal production', Lunteren (2010)
- EL&I/NWO course 'Animal behaviour & society behaviour', Warder-Zeevang (2011)
- EL&I/NWO course 'Market & animal welfare', Spaarnwoude (2012)
- EL&I/NWO course 'Governance & policy advice', Den Haag (2013)
- WIAS course 'Tropical farming systems with livestock', Wageningen (2013)
- WIAS course 'Advanced statistics course - Design of animal Experiments', Wageningen (2010)
- WIAS course 'Statistics for the life sciences', Wageningen (2013)
- WIAS Animal welfare discussion group, Wageningen (2010-2012)

### **Statutory courses (1 ECTS)**

- Use of laboratory animals, Wageningen (2010)

**Professional skills support courses (4 ECTS)**

- WGS course ‘Career assessment’, Wageningen (2010)
- ESD course ‘Teaching and supervising thesis students’, Wageningen (2011)
- WGS course ‘Technique for writing and presenting a scientific paper’, Wageningen (2011)
- Valley Consult course ‘Project and time management’, Wageningen (2013)

**Didactic skills training (14 ECTS)**

- Lecture MSc course ‘Health, welfare and management’ (2012-2013)
- Lecture BSc course ‘Sustainable development of animal systems: Issues & options’ (2012)
- Lecture BSc course ‘Global & sustainable animal production in the 21<sup>st</sup> century’ (2013)
- Coaching BSc project ‘Small ruminants’ (2012)
- Thesis supervision for 2 BSc and 6 MSc students

**Management skills training (3 ECTS)**

- Organisation WIAS seminar ‘Scientific research in animal welfare: Do we make a difference?’ (2011)
- Organisation WIAS Science Day (2012)
- Design of PhD course (2012)





## Colophon

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