Influence of weaning age on rumen development in calves

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Abstract

A neonatal calf is a pre-ruminant animal. The milk will bypass the rumen, reticulum and omasum by way of the reticular and omasal grooves and enters the abomasum. The development of the rumen is related to the diet of the calves. The intake of hay and concentrate is essential for the calf to become a functional ruminant. Early consumption of hay and concentrate is essential for successful early weaning. There are different weaning protocols that can be used, and every protocol has its own weaning age. The aim of this study was 1) to determine the effect of weaning age on rumen development and 2) try to relate it with the growth of the calves by using the chest girth.

18 Holstein calves were used in this experiment. All calves were weaned at 64 days of age. Twice a week the chest girth was measured and reported in the personal records of the calves. Three to five weeks after weaning the calves were euthanized and rumen development was assessed in 9 rumen areas by measuring the length and density per square centimeter of the rumen papillae.

Weaning at 9 weeks of age has a negative impact on rumen development compared with weaning at 12 weeks of age. But there was no correlation found between the length and density of the papillae and the weight of the calves. So weaning at 9 weeks instead of 12 weeks has no influence on the growth of the calves.

Introduction

A new-born calf is a pre-ruminant animal; the rumen of the neonatal calf occupies about 20% of the volume of the whole stomach (Sato et al., 2010). In dairy farming calves are separated from the cow within 24 hours of birth and fed restricted quantities of milk until weaning (Jasper and Weary, 2002). In these first weeks of life, the milk bypasses the rumen, reticulum and omasum. In this period the rumen, reticulum and omasum have no role in digestion. The milk will enter the abomasum by way of the reticular and omasal grooves (McGeady et al., 2006).

The development of the rumen is related to the diet of the calves. The intake of hay and concentrate is essential for the calf to become a functional ruminant. Because rumen development occurs primarily in response to the presence and subsequent fermentation of feed in the rumen, and because most milk bypasses the rumen, early consumption of hay and concentrate is essential for successful early weaning (Klein et al., 1987). Therefore a good weaning strategy is necessary, so when to start with hay and concentrate, and when to stop with the milk. The development of the rumen is completed by 14 to 16 weeks of age. The size is not fixated because an increase intake of hay and concentrate will result in a bigger rumen.

There are several weaning methods, for example concentrate-dependent weaning or conventional weaning (Roth et al., 2009). The goal of all weaning methods is to transform the calf from a pre-ruminant animal to a functional ruminant as quickly as possible but without health problems.

The period of weaning is a critical period regarding to disease problems in calves, like diarrhea and respiratory diseases, due to the abruptness of dietary change especially if dry matter intake is inadequate prior to weaning (Roth et al., 2009; Van Ackeren et al., 2010). And it is known that diseases have a negative impact on the welfare and development of young animals, but it also has a direct economic impact on the farming enterprise as they are likely to result in higher calf morbidity and lower growth rates (Lundborg et al., 2005). So it is important to have a good weaning strategy to reduce the disease problems during weaning.

When the calf consumes concentrate the rumen papillae will grow in size by the influence of starch that is converted by microorganisms to the volatile fatty acid (VFA) butyrate, which shifts the rumen into a light acid condition (Sato et al., 2010). The microorganisms in the rumen also start to develop and multiply so they can produce energy in form of the VFA acetate, propionate and butyrate. Volatile fatty acids, and especially butyrate are known to stimulate the papillae development, accelerates rumen motility and muscle development (Anderson et al., 1987; Kristensen et al., 2007). So the length of the papillae and the density of papillae per square centimeter are good parameters to evaluate the development of the rumen.

The rumen and reticulum are one big fermentation compartment together. An important part of nutrition of the cow is being severed by microbial fermentation in the rumen and reticulum. This is especially important for cellulose, the chief substance in the cell walls of plants, which cannot be digested in the intestine of mammals (Dyce and Wensing, 1983).

VFA are a good source of energy for the cow. If the rumen developed well and will be able to produce VFA from roughage and concentrate, the energy can be used for the development of other organs and systems in the calf, for example the immune system. So it is important that the rumen is well developed at the moment of weaning. A rumen is well developed if the amount of papillae and the size of the papillae are greatly increased so the total absorption surface is extended and more nutrients can be absorbed.

The objectives of this study were to determine the effect of weaning age on rumen development and try to relate it with the growth of the calves by using the chest girth. So if chest girth is a predictor for rumen development and also if chest girth is a predictor of weight in calves.

Materials and methods

Animals and housing:

The experiment was performed between January and May 2011 at an experimental barn used by University of Calgary. 18 Holstein calves were collected from farms around Calgary from January till the 10th of February when the last calf was collected. Among the 18 calves there was one heifer and 17 bull calves. The calves were used for Mycobacterium avium spp paratuberculosis research and as a consequence all calves were housed individually in pens with the entrances facing away from each other. Thus there was no visual contact in the beginning. The pens were supplied with saw-dust bedding. At day 0 (day of arrival at the barn) the calves were fed 2L of colostrum at arrival at the barn (or on the road if the trip back is more than 2hours). In total the calf received 6L of colostrum in 6 hours. The calves were fed milk replacer according to a calf feeding schedule (see table 1).Hay and water were available ad libitum. In the morning they also get a 150gram of concentrate. When the calves get weaned at day 64 they also get a 150gram of concentrate in the evening. Every morning the health status of every calf was evaluated and reported at their personal record and if necessary a treatment was started and also reported. This animal experiment was approved by the Canadian animal care committee.

Table 1. Calf feeding schedule

Day	7am	12pm	5pm
0	6L of colostrum in 6 hours		
1-3	225g powder + 1,5 L water	225g powder + 1,5 L water	225g powder + 1,5 L water
4-14	300g powder + 2L water	300g powder + 2L water	300g powder + 2L water
15-55	450g powder + 3L water		450g powder + 3L water
56-63	200g powder + 3L water		200g powder + 3L water
64	Weaned		

Calculation of weight gain:

We started to measure the circumference of chest twice a week. It was done with a simple measuring tape. The size was reported in centimeters and was also linked to the age of the calf (in days). A graphic drawing could be made for every calf with the size of the chest girth in cm at the Y-axis and the age in days at the X-axis. So a growth curve was made in the graphic. At the first week of weaning an extra measurement was done because that is a sensitive period (growth retarding) for the calves and they can lose some weight. Afterwards the centimeters were converted to kilograms of body weight using a Dutch validated body weight tape measure, to be able to report the weight gain.

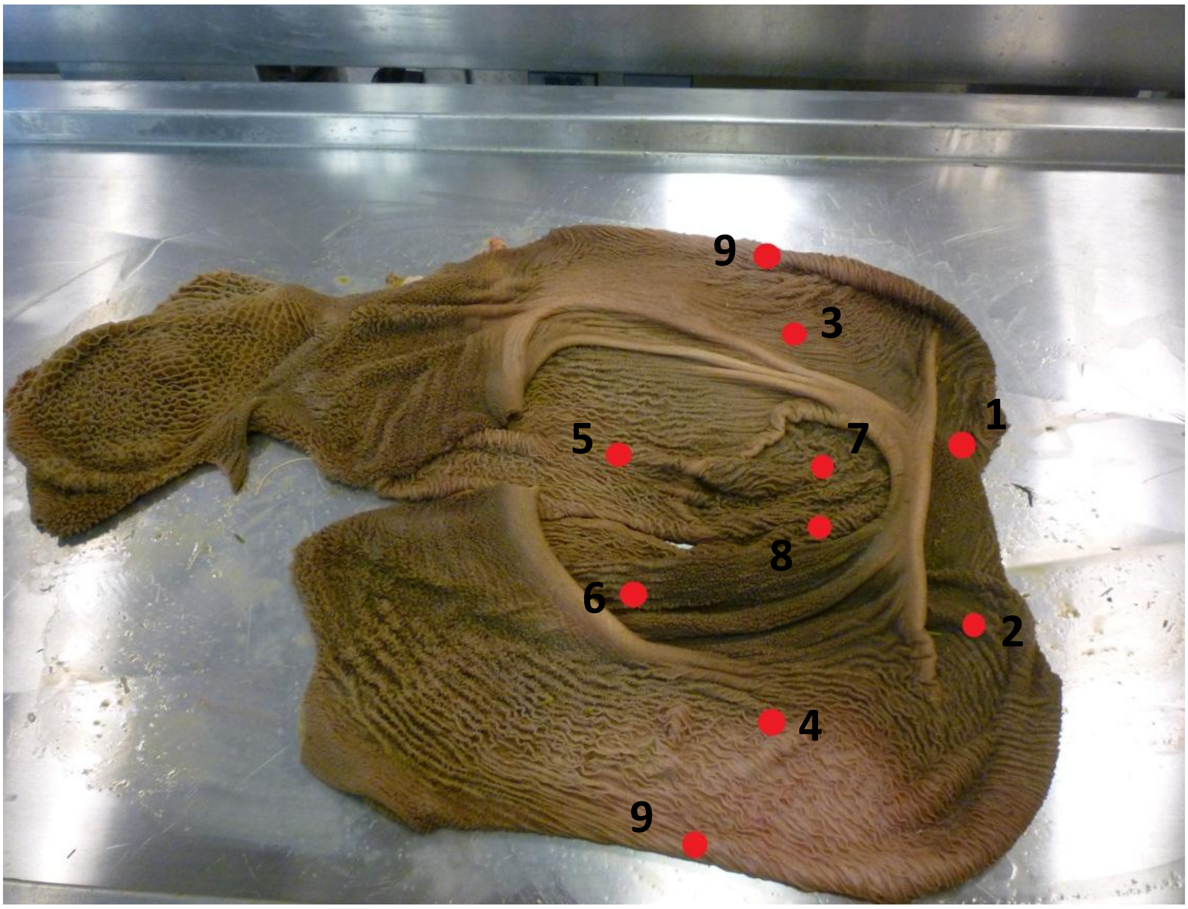
Rumen development:

All 18 calves were transported alive to the pathology department of the faculty of veterinary medicine of the University of Calgary with an age varied between 86 till 100 days. When they arrived at the faculty the calves were euthanized with euthanyl forte® intravenous injection and full body weight was recorded immediately after euthanized. After the calf is euthanized the rumen, reticulum, omasum and abomasum were removed at necropsy. Then the abomasum and omasum were removed from the rumen and reticulum. The weight of the rumen and reticulum with the contents was estimated with a scale of 0,5 kg. After that the rumen was placed on its left side with the esophageal groove facing away. For rumen sampling the method of Roth et al. (2009) was applied; a first incision was made at the end of the esophagus along the center of the reticulum to the reticulo-omasal orifice. The incision was preceded to the end of the dorsal blind sac over the dorsal sac. The caudal end of the ventral sac was the start of the second incision. The incision started at the ventral center line of the ventral sac and was preceded to the middle of the reticulum. The ventral blind sac was kept intact. The rumen was opened, emptied and flushed. The weight of the emptied and rinsed rumen was estimated again before the tissue sampling started.
From each of the 9 areas (see table 2 and figure 1) four 1 cm² tissue samples were taken. The samples were fixed on Styrofoam with pins to prevent the muscular layer of the rumen to bend. For good tissue fixation samples were put upside down in 10% neutral buffered formaldehyde solution. The tissue samples are used to measure the papillae length (PL) and papillae per square centimeter (PSC). For all samples, the 4 longest papillae were used to measure the length, resulting in 9 times 16 length observations per area. For all samples, also one square centimeter was used to count the number of papillae, resulting in 9 times 4 observations per area.
The measurements were done by using a Leica MZ 7.5 Microscope with a 0.63x magnification and an Infinity 1 camera connected to the microscope. So the samples could be analyzed with the Infinity Analyze software.

Table 2. Areas of tissue sampling in rumen (Roth et al., 2009)

Area no.	Rumen area	Exact position of sampling within area
1	Dorsal blind sac, right	One finger space of the pila coronaria dorsalis
2	Dorsal blind sac, left	One finger space of the pila coronaria dorsalis
3	Dorsal sac, right	Two fingers space to the pila accesoria sinister
4	Dorsal sac, left	Two fingers space to the pila accesoria dexter
5	Ventral sac, right	Middle of the right side
6	Ventral sac, left	Middle of the left side
7	Ventral blind sac, right	Middle of the right side
8	Ventral blind sac, left	Middle of the left side
9	Dorsal sac, middle	Middle of the connecting line of the left and right sample

Figure 1. The 9 areas of rumen sampling



Statistical analysis:

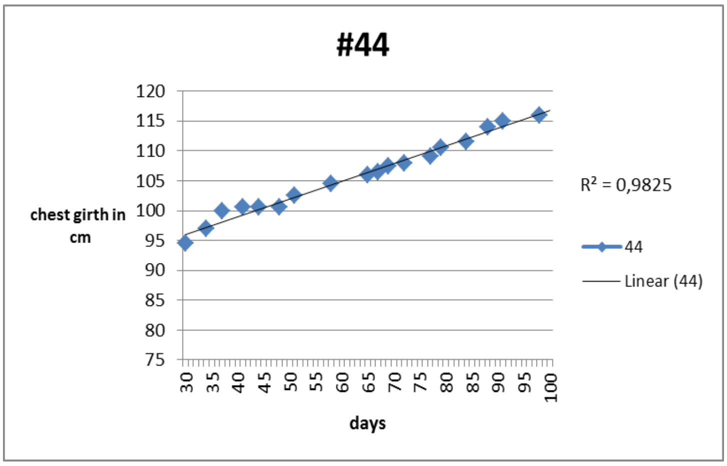
To evaluate if there was a correlation between growth and age, the chest girth and age in days were used. The weight of the empty rumen was used to determine if there was a correlation with the body weight of the calf. Further the length of the papillae and density per cm² were used to analyze if there was a correlation with the weight.
To analyze if the weaning age has an influence on rumen development a Box-and-Whisker plot was made with the length of all 18 calves together per area to compare the results of this experiment with the results of the article of Roth et al. (2009). To determine if there is a significant different between weaning at 9 weeks of age or at 12 weeks of age a one-sample T-test was done.

Results

Chest girth measurements

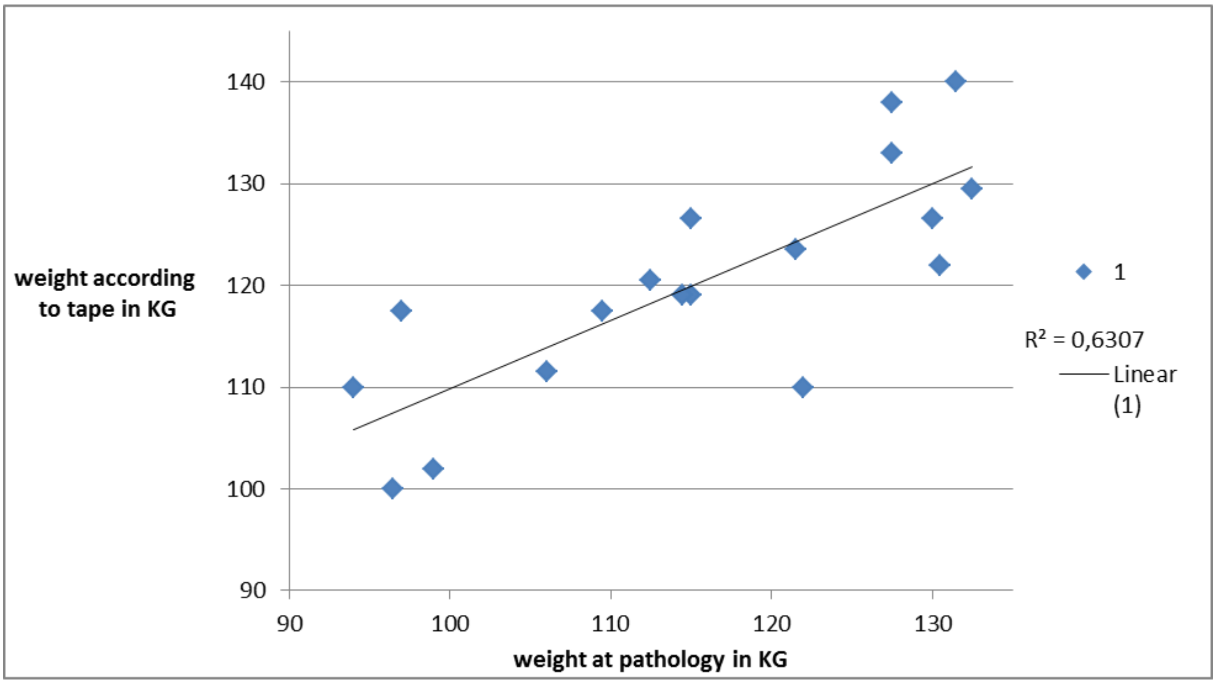
Figure 2 shows that there is a linear correlation between the size of the chest and the age of the calf. The older the calf is the bigger the chest girth is. The correlations between the chest girths and age of the calves were between 0,8355 and 0,9891.

Figure 2. Relation between age and chest girth here for example from calf #44.



To analyze if the chest girth is a good predictor for body weight, figure 3 was created. This figure shows the moderate correlation (r^2= 0,6307) between chest girth estimation right before euthanasia, and body weight according the scale of the euthanized animal.

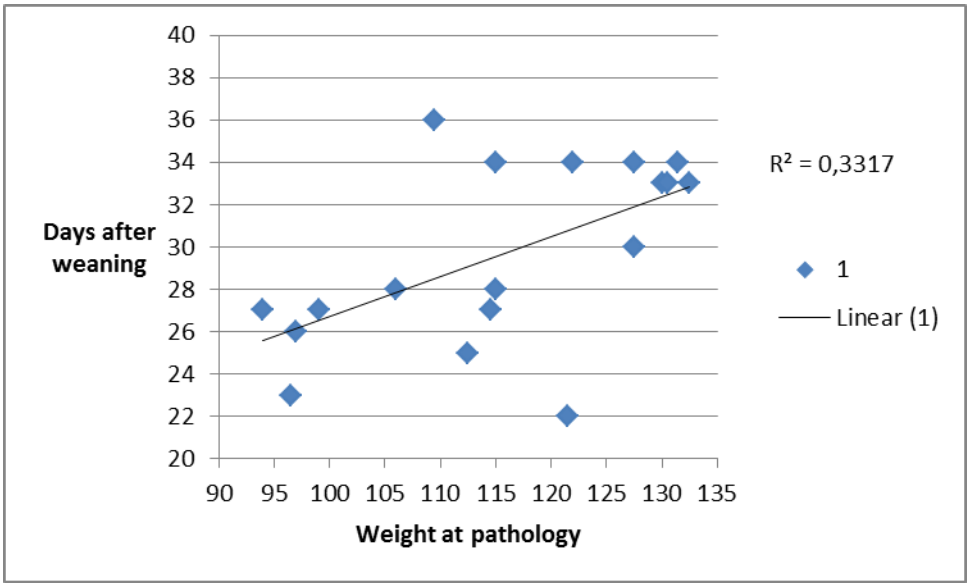
Figure 3. Relation between weight according to body weight tape measure and weight at pathology (n=18)



Rumen development

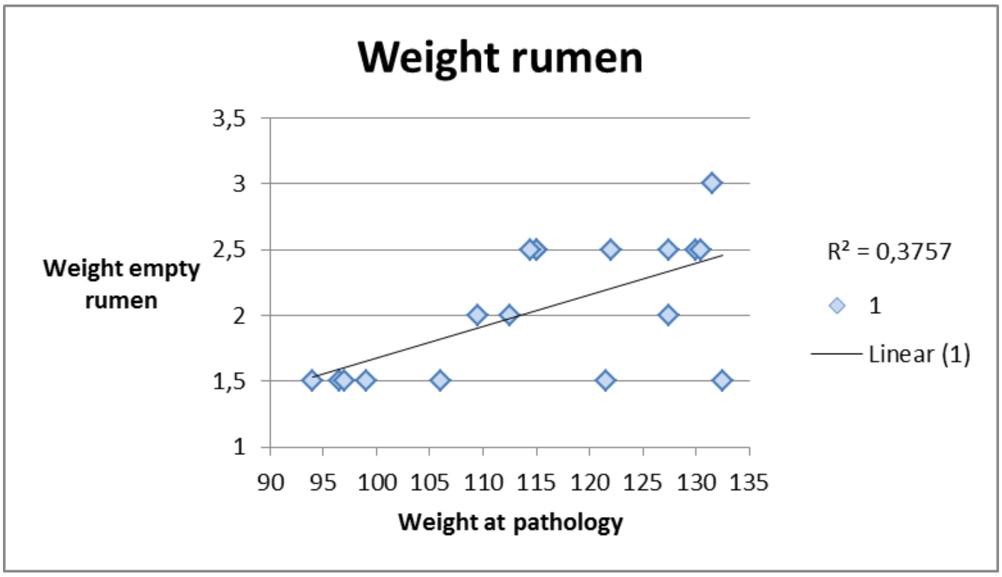
The age of the calves varied between 86 and 100 days at the moment of arrival at the pathology department resulting in a time span of 22 till 36 days after weaning. Figure 4 shows that there is a low correlation (r^2= 0,3317) between the age of the calf at euthanizing and the weight of the calf. This means that we had calves that were euthanized at 34 days after weaning and a difference of 7 kg of body weight. So the difference in age at euthanizing has no influence on the body weight of the calves in this trail.

Figure 4. Correlation between the age of the calf and the weight at the pathology (n=18)



There was a low correlation (r^2=0,3757) between the weight of the empty rumen and the body weight of the calf. Figure 5 shows that the weight of the calf is not well related with the weight of the rumen.

Figure 5. Relation between weight of empty rumen and weight at pathology, two times point (115 ; 2,5) (n=18)



In none of the nine areas a good correlation was found between the density of papillae per square centimeter and the weight of the calves. See figure 6. The correlations were between 0,0005 and 0,1906.

Figure 6 . The relation between the density per cm² and the weight of the calf as an illustration in area 4 (n=18)

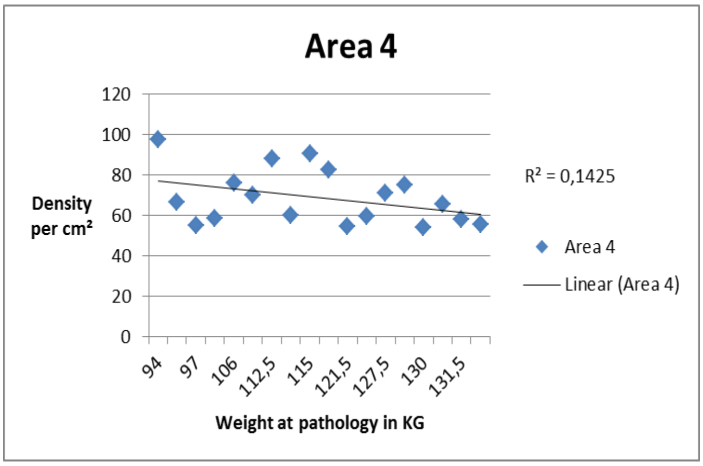


Table 3 shows the results of the density per square centimeter.

Table 3. Density per square centimeter of all 18 calves merged per area, 4 measurements per calf per area (n=4x18)

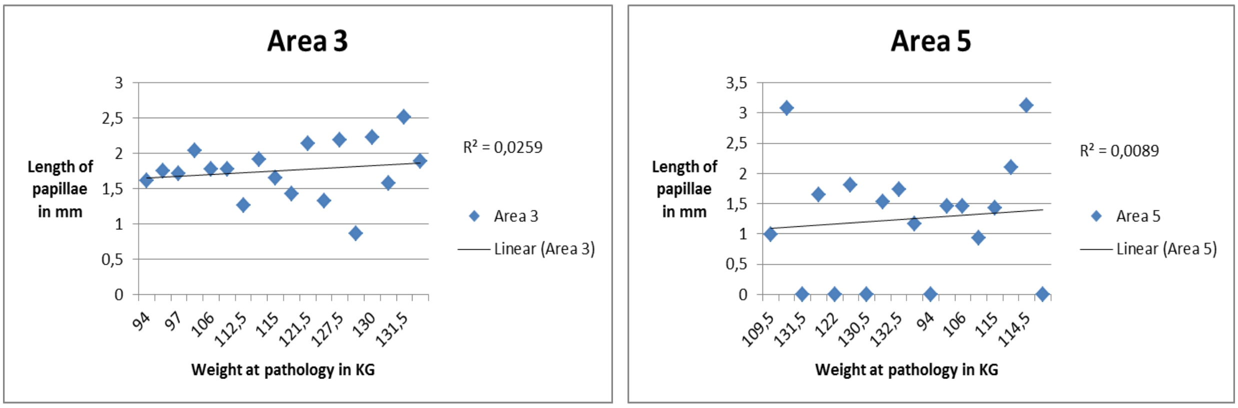
Area	Mean	Standard deviation	Median
1	62,85	11,73	60,375
2	61,94	13,73	61,125
3	66,89	13,89	66
4	68,92	13,59	66,125
5	60,69	19,59	63,25
6	64,01	18,96	56,875
7	57,11	9,84	57,25
8	55,56	11,28	54,875
9	71,92	24,08	75,875

In all 9 areas a wide spread of length of papillae was observed (see table 4). In certain areas there were papillae that were too short to measure. Especially in area 5 and 9 a lot of papillae were too short to measure and as a consequence 0 mm was reported. There was no correlation found between the length of the papillae and the weight of the calf (correlations between 0,0002 and 0,1267). In this trial were calves with a low body weight but with large papillae and there are also calves with a high weight but with small papillae (see figure 7).

Table 4. Lengths of papillae of all 18 calves merged per area

Area	Mean	Standard deviation	Median
1	2,202	0,450	2,19
2	2,251	0,673	2,11
3	1,758	0,395	1,76
4	1,794	0,607	1,79
5	1,248	0,984	1,44
6	1,854	0,799	1,93
7	2,028	0,482	2,02
8	2,306	0,633	2,19
9	0,518	0,732	0

Figure 7. Relation between length of rumen papillae and weight of the calf.

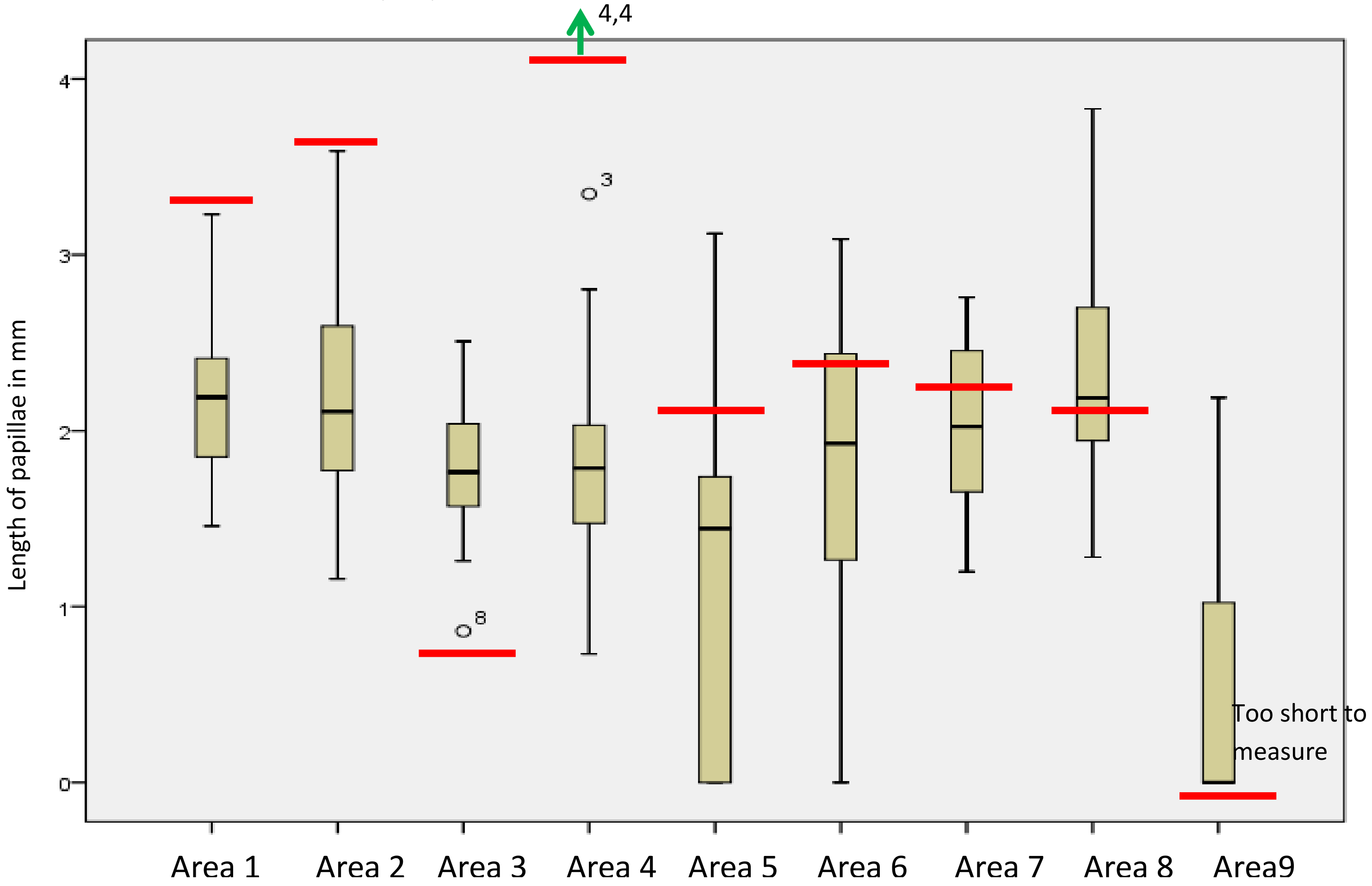


The box-and-whisker plot in figure 8 shows the length of the papillae of all 18 calves together per area. The red lines are the means of the length of the papillae from the experiment of Roth et al. (2009). We used these means to compare our results with their results. The calves of our experiment were weaned at 9 weeks of age and euthanized between 12 and 14 weeks of age. The calves in the article of Roth et al. were weaned at 12 weeks of aged and euthanized at 15 weeks of age. Table 5 shows the results of the one-sample T-test, with H_0: $\mu_{9\ weeks} \neq \mu_{12\ weeks}$ and H_1: $\mu_{9\ weeks} = \mu_{12\ weeks}$. In area 7 and 8 we have to reject the null hypothesis ($p < 0{,}05$). That means that there is no significant difference in the length of papillae in those areas.

Table 5. Results of the one-sample T-test for the length of the papillae

Area	Weaned at 9 weeks (n=16x18)	Weaned at 12 weeks (Roth et al. 2009) (n=20x23)	Significance (p=)
1	2,202	3,2	0,116
2	2,251	3,6	0,144
3	1,758	0,8	0,228
4	1,794	4,4	0,254
5	1,248	2,2	0,171
6	1,854	2,3	0,068
7	2,028	2,2	0,026
8	2,306	2,1	0,029
9	0,518	0	0,499

Figure 8. Box-and-whisker plot with the length of the papillae of all 18 calves together per area and the means per area from the article of Roth et al. (2009).



Discussion

In our study to evaluate rumen development as related to weaning age we thought that the length and density of papillae in the rumen were good parameters for rumen development because it is the best way to increase the rumen surface. But we did not found a relation between body weight and length of the rumen papillae in one of the 9 areas. There was also no correlation found between the density of papillae per square centimeter and the weight of the calf. Roth et al. (2009) found that weight gain was an easily measurable predictor of rumen development. That is a different conclusion according to our results. The lengths of our papillae were shorter in 3 of the 9 areas and in 2 of the 9 areas longer compared to the length of the papillae in the article of Roth et al. In area 3 and 4 there are big differences compared to Roth's experiment. It has been proved that the intake of concentrate and hay has a positive influence on the rumen development (Klein et al., 1987). Our calves were weaned at 9 weeks of age and euthanized between 12 and 14 weeks of age, so we expected that the papillae were longer then from the experiment of Roth et al. because their calves were weaned at 12 weeks of age and euthanized at 15 weeks of age. That means that the papillae in our calves could start develop earlier under influence of concentrate. And the calves were euthanized later after weaning then in the article of Roth et al. But the papillae were shorter. We cannot blame it on the quality of the concentrate and hay, because they were of an appropriate quality. Maybe the amounts of concentrate were too low or they were not eating all concentrate. It was impossible to determine

spill of the forage because of the protocol of the Johne's trial. We know for sure that this has nothing to do with stress because we looked for ulcers in the abomasum but we did not found any of them.
We also wanted to see if the rumen development is related to the growth of the calf. But both the length of the papillae and the density of papillae per square centimeter were not correlated with the weight of the calf. So there were calves with a good developed rumen but still a low body weight and also calves with a high body weight but with a rumen which is not well developed. That means that the weight of the calf could not be used to predict the size of the rumen and with that the calf' capacity to uptake energy and protein from its diet. This is not what we expected. We expected that the better the rumen was developed the better the calf would grow because they could absorb more energy.
Live body weight measuring using a scale could not be executed in our barn setting. As an alternative measuring the chest girth is a reliable method to calculate the weight in cows, so we tried the same in our calves. In figure 3 we can see that there is a moderate correlation between the weight according to the tape, measured just before euthanizing, and the real weight measured right after euthanizing. We can also see that there is one extreme outliner, point (122,110). This observation has a big influence on the correlation coefficient. If we could ignore that extreme outliner the correlation coefficient would we 0.78. Thus the tape is useful to predict the weight of the calf if a range of 10% is being used. It is necessary to repeat the measurements at least twice a week for a good overview in the growth of the calf. We tried to do the measurements together but we found different observations. We saw relatively big difference between the measurements, so from then on all measurements were done by one person. Figure 8 shows that in spite of the variance in measurements there is still a good correlation. All the measurements from figure 8 were done by one person; this figure only show the results of animal 47, but these results were similar to the other animals. So if the measurements are being done by one person it is a reliable method to measure the weight in calves and calculate the weight gain.
As we can see in table 5 there is a significant different in length of the papillae in most areas. So that means that early weaning has a negative impact on rumen development. But is has no relevance in practice because the length and density of papillae in the rumen are not correlated with the weight of the calf. So the calves are growing well but still have smaller papillae. For sure there will be a turning point that the weaning age and rumen development have an influence on the growth of the calves. But this point is not somewhere between nine and twelve weeks of age when started with weaning.

Conclusions

From the present study, it can be concluded that weaning at 9 weeks instead of 12 weeks of age has no significant effect on rumen development. Further there was no correlation found between the growths of the calves by using the chest girth and length or density of papillae in one of the 9 areas. The rumen papillae from calves weaned at 9 weeks of age are shorter in some of the areas compared to the rumen papillae from calves weaned at 12 weeks of age. But it has no influence on the growth of the calf. There will be a turning point at a certain age of weaning, that the calves and rumen are underdeveloped. But to find that turning point further research has to be done.
We also can conclude that the chest girth is a good predictor for the weight of the neonatal and young calf. The use of a body weight measure tape is reliable in these calves if the measurements are

done by one person and a range of 10% is being used. Same as the chest girth weight is not a reliable predictor for rumen development.

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