Dental health in piglets and its relation with piglet performance

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Abstract

This study focused on the study of the occurrence of dental health problems and its relation with piglet performance. A dental investigation soon after euthanasia was performed in 180 Maxter x Hypor piglets, which were divided over 3 groups. The age of the piglets during the dental examination was 3.5, 6.5, and 7.5 weeks for group 1, group 2, and group 3, respectively. Dental examination included the eruption and occlusion of all the teeth, the severity of black pigmentation and plaque, and the presence of caries and gingivitis of the p_3. Data on bodyweight (BW) and feed intake were collected at different time points during lifetime. Average daily weight gain (ADG), average daily feed intake (ADF), and feed efficiency (FE) were subsequently calculated. Eruption and occlusion were mainly age-dependent, as that piglets of group 1 were not having complete eruption and occlusion. A higher p^2 was associated with higher BW and ADF for piglets from group 2 and 3. In addition, eruption of the i_2 of piglets from group 2 was positively associated with ADF. Black pigmentation and plaque were found in all degrees. High severity of plaque was already found in the youngest piglets (group 1), whereas high severity of black pigmentation was not observed in those animals. Piglets from group 2 having a high severity of black pigmentation (>50%) had a lower ADG and FE. About 1/3 of the piglets from group 2 and 3 had also a red gingiva and blood upon probing. This was positively associated with BW and ADG for piglets from group 3 only. No caries were found in this study. The presence of various dental characteristics seems to play a role in piglet performance, as that associations were found. However, more research is needed for a better understanding of dental health problems in piglets and for its effect on the overall health in piglets.

1. Introduction

In human medicine it has become clear that dental health plays an important role in the quality of life. Besides its functional role in facial appearance and in speech and communication, teeth are also important in enabling consumption of a varied diet and in preparing the food for digestion (4). Dental diseases in human have, therefore, a detrimental effect on feed intake and thus on the overall health in human (48). Adverse effects on the overall health due to dental diseases may also be present in piglets, although dental health in piglets is not completely described yet. Tooth development is important for the formation of total occlusion and subsequently for consuming and mastication of food (4,55,56). Occlusion is defined by the contact between maxillary and mandible elements. Incisor occlusion plays a role in cutting food and premolar occlusion for the mastication/chewing of the food (56). Based on the functional role of eruption and occlusion, impaired tooth development (i.e. delayed eruption and occlusion, and lower elements) could led to impaired piglet performance. Effects of eruption on eating and growth was previously observed by Tucker et al. (1-3). Premolar eruption beyond 21 days of age was positively associated with the amount of time piglets spent at the creep feeder at the same time (2). They also found that piglets with erupted p_3 and p^4 at weaning had higher growth rates during the 3 weeks following weaning (3). Moreover, the same authors have found that the presence of dental staining at the time of weaning was negatively associated with future weight gain (3). Although the nature of this staining as well as the colour was not determined in that study, this finding suggests that dental staining or pigmentation may have an effect on the capacity for growth. The effect of the presence of dental health problems on feed intake is also documented in human. One of the most common dental disease, dental caries, was found to be accompanied by pain and discomfort (27), and a reduced ability to eat a varied diet in human children (26). Likewise, these adverse effects were also seen in humans with periodontitis, which is the more severed and progressive state of gingivitis (38-40). Dental plaque is known to be involved in the aetiology of dental caries and gingivitis/periodontitis (34-36,52-54). These preliminary results of studies in pigs and the knowledge of human dentistry will make studying dental health likely to provide novel insights into the growth, development, as well as overall health of pigs. Eruption, occlusion, (black) pigmentation, plaque, caries, and gingivitis are previously reported to play a role in feed intake in either piglets or humans. This makes the presence of these dental characteristics interesting when studying the effect of dental health problems on piglet performance. Therefore, the aims of this study were to determine the prevalence of dental health problems (i.e. impaired tooth development, black pigmentation, plaque, caries, and gingivitis) in piglets at the age of 3.5, 6.5, and 7.5 weeks and the relation of these problems with piglet performance.

2. Materials and methods

All procedures were performed in accordance with the European Directive (2010/63/EL, 267/33) and the Dutch Animal Experimentation Act. They were approved by the Animal Experiments Committee Utrecht (DEC). For this study animals from two studies carried out at the Swine Research Centre of Trouw Nutrition were used. This study was a double-blind experiment, where technicians and researchers from Utrecht University did not know the origin (i.e. litter group and treatment group based on different food compositions) of each animal.

2.1 Experimental design

180 Maxter x Hypor piglets, belonged to three different groups, were used. Characteristics and composition of the groups are explained below:

Group 1. This group consisted of 60 animals, both males and females were included. The birth date ranged from 28th of October until 1st of November 2015. The piglets were divided over four different test groups receiving four different kinds of creep feeding during the last week before they were weaned. The different creep feeds differed in their content and amount of probiotics and prebiotics, but detailed information is unknown because of commercial purposes. The piglets were allocated to groups and were fed *ad libitum* with the creep feed. They were euthanized just before weaning at 3.5 weeks of age.

Group 2. This group consisted of 72 animals, males and females. One piglet died during the experiment and it was therefore excluded. These piglets belonged to the same combined group as the piglets from group 1, but were kept after weaning. Birth date and creep feeding proceedings before weaning were therefore the same as group 1. These piglets were weaned at 25-26 days of age. From this moment they were housed individually and they all received the same food. 7 days later all the piglets were challenged with *Escherichia coli* (*E.coli*) for other research purposes. Euthanasia was performed 21 days after weaning at 6.5 weeks of age.

Group 3. This group consisted of 48 animals, all males. These piglets did not receive any creep feeding during the suckling period. After weaning (at 24 days of age), they were divided in six different treatment groups, receiving a control feed or one of 5 different feed containing medium chain fatty acids combined with other feed additives. The pH of the control feed was 6.8, whereas the pH of the 5 different test feeds was 6.0. Detailed information about these different feeds is unknown because of commercial purposes. These piglets were individually housed after weaning and were fed *ad libitum*. 10 days after weaning all the piglets were challenged with a *Salmonella* strain for other research purposes and 28 days after weaning they were euthanized at 7.5 weeks of age.

All the piglets were euthanized using sodium pentobarbital (Euthasol®, Virbac Laboratories, France) via intracardiac injection. Piglets from group 2 and 3 were sedated first with a combination of tiletamine and zolazepam (Zoletil®, Virbac Laboratories, France) before euthanasia. Then decapitation of the piglets was performed directly after euthanasia at the Swine Research Centre. The heads were brought to Utrecht University where a complete dental investigation was performed. A timeline of the experimental design is presented in Appendix A.

2.2 Data collection

2.2.1 Dental investigation:

For the purposes of this paper, all deciduous elements are referred by a lowercase **p**, **c** or **i** which indicates that a particular element belonged to one of the premolars, canines or incisors, respectively. Moreover, the elements are referred by a superscript or subscript indicating its position in the maxilla or mandible, respectively. Dental examination included the eruption and occlusion of all the teeth, and the presence (and severity) of black pigmentation, plaque, caries and gingivitis. Only the p_3 (and gingiva surrounding the p_3) was studied for the examination of black pigmentation, plaque, caries and gingivitis. The p_3 was examined, because it was present in the majority of the piglets. Moreover, is it a premolar type of tooth. A premolar element was preferred, because this type of element is functional for chewing the food (55) and might be more susceptible for having these dental characteristics. Although the p^3 and p_4 are also premolars and were present in all the animals, the p_3 was more accessible for its examination. A scoring system (included supporting information) for each dental characteristic is defined in Appendix B.

Eruption. Eruption is defined by the penetration of the overlying gingiva by any tooth (55). A visual examination of each tooth was performed. The deciduous teeth were recorded as being erupted or not. Emergence of teeth was considered to have occurred when any portion of the crown had penetrated the gingiva. In case of erupted elements, differentiation was obtained depending of the height of the element (i.e. lower than 3 mm or higher than 3 mm) above the gingiva. This was measured by making use of a periodontal probe with 2-4-6-8-10-12 mm markings.

Occlusion. Occlusion is defined by a match between maxillary and mandibular elements (55,56). Occlusion measurements included both the status of occlusion (total or partial) and the mode of occlusion (in case of total occlusion). For the status of occlusion a match of the i^1 to the i_1 (incisor occlusion) and a match of the p^3 and p^4 to the p_4 (premolar occlusion) were examined. Partial occlusion between premolars occurred when some or none of the cusps of p^3 and p^4 touched the cusps of the p_4. In case of total occlusion, the mode of occlusion was evaluated and classified as end-to-end, minimal underbite, minimal overbite, clear underbite, or clear overbite for the incisors and normal, mesio-occlusion or distal occlusion for the premolars.

Black pigmentation. Black pigmentation is characterized by incomplete coalescence of black spots with or without a continuous black line parallel to the gingival margin (7,8). The severity of black pigmentation was examined according to the percentage of the buccal and lingual side of the p_3 covered by the black pigmentation. The average of the percentages found on the buccal and lingual sides were taken. We used a scale ranging from 0 (no black pigmentation) to 4 (more than 75% of the surface of the tooth covered by black pigmentation) indicating the different severities of black pigmentation (see Appendix B.2). Examples of the different severities of black pigmentation are presented in Appendix C. Each piglet was assigned to one of five categories. Subsequently, high severity of black pigmentation was determined for univariable analysis. High severity of black pigmentation was considered when over 50% of the p_3 (average of the buccal and lingual side) was covered by black pigmentation.

Plaque. Dental plaque is characterized by pink staining on the p_3 in this study. The severity of plaque was scored according to the percentage of plaque that covered the buccal side of the p_3. For this purpose a dental disclosing solution (GUM®) was used to highlight dental plaque on the tooth surface. A cotton swab was used to wipe the solution on the tooth. Then we sprayed it off using water and measured the percentage of pink staining that remained on the tooth surface indicating dental plaque. We used a scale ranging from 0 (no plaque) to 4 (more than 75% of the surface of the tooth covered by black pigmentation) indicating the different severities of plaque that covered the tooth (see Appendix B.2). Subsequently, high severity of plaque was determined for univariable analysis. This was considered when over 50% of the p_3 (on the buccal side) was covered by plaque.

Caries. Caries is characterized by a brownish soft spot on a tooth surface which you cannot remove. (16). The buccal and lingual side of the p_3 were both examined for the presence of caries.

Gingivitis. Gingivitis is characterized by a 1- to 2- mm red, flared zone and bleeding upon probing (27). For the examination of gingivitis, the marginal gingiva surrounding the p_3 was checked. It included the examination of the colour of the gingiva as well as the bleeding of the gingiva after touching it slightly with the round sharp side of the periodontal probe. Periodontal probing of the p_3 was performed with a constant probing force. The examination of gingivitis was the only examination that was performed before euthanasia, directly after sedation. Therefore, mucosa was only studied in group 2 and 3. Moreover, histology samples of the gingiva were taken after euthanasia from piglet of these groups and will be used to judge gingivitis. However, investigation of these samples is not performed yet.

2.2.2 Performance measurements:

Data on bodyweight and feed intake were collected at different time points by technicians from the Swine Research Centre. Bodyweight (BW) was measured by weighting the animals individually using a scale. Creep feed intake was measured using chromium oxide as a marker in the food. A faecal sample was obtained from each piglet via a rectal swab. The colour of each sample was immediately visually assessed for the presence of chromium oxide that was added to the feed. The amount of the chromium oxide was indicative of the amount of prior ingestion of feed. Then average daily weight gain (ADG), average daily feed intake (ADF) and feed efficiency (FE) were calculated. ADG, ADF en FE of group 3 and only ADF of group 2 were measured over the last week of the experiment. ADF of group 2 was also measured over the last week of the experiment, just as was done with the ADG and FE.

Group 1. Piglets were weighed on day 0, 13, and 24 before 12 am. On day 6, 13, 19, and 22 a faecal examination was performed.

Group 2. Before weaning, bodyweight and feed intake of creep feed were obtained at the same days and in the same way as was done for piglets from group 1. After weaning these piglets were weighed on day 5, 13, and 21 and feed intake was measured daily for each piglet.

Group 3. After weaning these piglets were weighed on day 6, 13, 20, and 27. Feed intake post-weaning was measured on day 0, 6, 13, 14, 15, 16, 17, 20, and 27.

2.3 Statistical analysis

All data were analysed using R 3.2.2. software (50). Separate analyses were carried out for each of the three different groups. Descriptive analysis were used to determine the proportions of each dental characteristic. To test whether any dental condition was indicative of a specific sex or a specific side (left/right), chi-square tests were used. To examine how a specific dental characteristic affected piglet performance (i.e. BW, ADG, ADF, and FE), linear regression and logistic regression analysis were used during the univariable analysis. Results are presented with $p<0.05$ being considered statistically significant.

3. Results

3.1 Descriptive analysis

Mean BW, and weekly ADG, ADF, FE of piglets from each group are listed in table 1.
In order to examine the effect of tooth development on performance, some elements in each group were excluded from statistical analysis using three different criteria:

1. When a tooth belonged to one of the needle teeth (i^3, i_3, and c in each quadrant)
2. When a tooth had not emerged in over 90% of the population of a specific group at the time of dental investigation
3. When a tooth was erupted in all piglets of a specific group and was higher than 3 mm in the 90% of more of the piglets.

In table 2 an overview of the percentage of piglets displaying a certain stage of eruption of the elements included in statistical analysis is presented. A table presented in Appendix D shows the proportions of the modes of incisor occlusion. Proportions of piglets having high severity of black pigmentation and plaque are presented in table 3. Proportions of piglets having a red and a bleeding gingiva upon probing are also included in that table.

Table 1: Mean BW, ADG, ADF, and FE of piglets from the three groups*

Group	**BW ± SD**	**ADG ± SD**	**ADF ± SD**	**FE ± SD**
Group 1	7.98 ± 1.13	X	X	X
Group 2	13.39 ± 2.37			
Last week	X	X	512.25 ± 164.59	X
Last two weeks	X	279.68 ± 155.13	374.73 ± 107.55	0.65 ± 0.22
Group 3	16.90 ± 2.30			
Last week	X	639.35 ± 179.76	750.54 ± 220.51	0.81 ± 0.13

* BW = bodyweight in kilograms (kg) at necropsy
ADG = average daily weight gain in grams per day (g/d)
ADF = average daily feed intake in grams per day (g/d)
FE = feed efficiency in kg weight gain per kg feed intake
ADG, ADF and FE were measured over a period of 7 days (last week of the experiment) or over a period of 14 days (last two weeks of the experiment) depending of the group.
The 'X' indicates the not calculated outcome, because of missing values.
The standard deviation (SD) of the range is presented for each parameter.

Table 2: Percentages of the piglets displaying a certain stage of eruption of specific elements* for both side (left and right) in each group.

	Element	Not erupted		Lower than 3 mm		Higher than 3 mm	
		L	R	L	R	L	R
Group 1	i^1	3.3	3.3	61.7	61.7	35.0	35.0
	p^3	0.0	0.0	23.3	16.7	76.7	83.3
	p^4	66.7	66.7	31.7	33.3	1.6	0.0
	p_3	3.3	3.3	81.7	81.7	15.0	15.0
	p_4	0.0	0.0	68.3	60.0	31.7	40.0
Group 2	p^2	9.9	7.0	71.8	73.2	18.3	19.7
	i_2	32.4	32.4	32.4	28.2	35.2	39.4
Group 3	p^2	0.0	0.0	58.3	56.3	41.7	43.7
	i_2	16.7	16.7	10.4	12.5	72.9	70.8
	p_2	62.5	62.5	33.3	33.3	4.2	4.2

* The elements presented for each group are the elements that were not excluded from statistical analysis based on the three criteria: teeth belonged to one of the needle teeth (i^3, i_3, and c in each quadrant), not emerged teeth in over 90% of the group and teeth higher than 3 mm in over the 90% of the group.
The lowercase p, i and c indicate the premolar, incisor and canine elements. The superscript and subscript indicate its position in the maxilla and mandible, respectively.

Table 3: Prevalence* of a high (>50%) amount of black pigmentation and plaque that covered the p_3, and prevalence of a red gingiva and blood upon probing of the marginal gingiva of the p_3.

Dental characteristic	Group 1		Group 2		Group 3	
	L	R	L	R	L	R
>50% of black pigmentation	0.0	0.0	15.5	18.3	16.7	22.9
>50% of plaque	39.2	27.9	29.6	21.1	35.4	29.2
Red gingiva	X		31.3	37.3	44.7	44.7
Bleeding gingiva upon probing	X		25.4	38.8	31.9	40.4

* Prevalence defined as the percentage of the piglets population of the thee different groups displaying a particular dental characteristic of the p_3 (and gingiva surrounding the p_3). The two percentages in each cells indicate proportions of the left (L) and the right (R) side of the p_3
The 'X' indicates not calculated values, because the gingivitis examination was not performed in piglets from group 1.

3.1.1 Group 1:

Mean BW was 7.98 kg and ranged from 5.91 to 10.85 kg (results not shown). Weekly ADG, ADF and FE were not collected in this group (table 1).
Of the 60 piglets, all piglets had their 8 needle teeth erupted. The i_1 was erupted and higher than 3 mm in all the piglets. The i^2, p^1, p^2, i_2, p_1 and p_2 were not erupted in all piglets. These elements were therefore excluded from statistical analysis and are not included in the table. The p^3 and the p_4 are the premolars that were erupted in all the piglets. The development of the eruption of the p^3 was in a later stage than that of the p_4, as a higher proportion had the p^3 erupted with a height higher than 3 mm. A few piglets had the p_3 and the i^1 still not erupted and more than half of the population had not erupted p^4. None of the piglets had total occluded premolars and only 3.33% had total occlusion of the incisors (see Appendix D). The p_3 was in 13 piglets too small for a well examination of black pigmentation and plaque that covered this element. Nevertheless, no high severity of black pigmentation was found in the rest of the piglets. Contrary to high severity of black pigmentation, which was not observed, high severity of plaque was found in 39.2% (left p_3) and 27.9% (right p_3) of the population. None of the piglets had caries on the p_3.

3.1.2 Group 2:

As shown in table 1, mean BW was 13.39 kg and ranged from 8.3 to 19.9 kg (results not shown). The average ADG, ADF and FE over the last two weeks and ADF over the last week of the experiment are presented in table 1. One piglet loses weight over the last 2 weeks. The ADG was therefore negative, resulting in a wide range of the ADG. The ADF and FE also varied a lot between animals.
All piglets had their 8 needle teeth (i^3, i_3, and c in each quadrant) erupted. However, 2 piglets had lost their right i^3, 1 piglet its left i^3, and 2 piglets their left i_3. Since a little gap at the place where these elements should be was found. All piglets had erupted p^3, p_4, i^1, and i_1 in each quadrant (results not shown). These elements were also higher than 3 mm in more than 90% of the piglets and were therefore excluded from analysis. Moreover, it was found that more than 90% of the piglets had not erupted i^2, p^1, p_1 and p_2 (results not shown) and these elements were also excluded from statistical analysis. The p^2 and the i_2 are the elements included in statistical analysis. A higher proportion of the population had erupted p^2 compared to i_2, while a higher proportion had the i_2 higher than 3 mm compared to the p^2. Occlusion of the premolars was total and normal in 100% of the animals. 98.6% of the piglets had total incisor occlusion and subsequently 34.3% had abnormal occlusion of the incisors (see Appendix D). High severity of plaque was more often observed than high severity of black pigmentation. Gingivitis was present in approximately 1/3 of the population. Blood of the gingiva was more often observed than red gingiva. None of the piglets had caries on the p_3.

3.1.3 Group 3:

Mean BW was 16.90 kg and ranged from 12.3 to 21.9 kg. Mean values of ADG (range = 102.86 – 985.71 g), ADF (range = 100.49 – 995.0 g) and FE (0.31 – 1.06%) are presented in table 1 and these parameters were measured over the last week of lifetime.
The 8 needle teeth were erupted in all animals. The same elements that were excluded from analysis in group 2 were also in this group excluded, except for the p_2. This element was in both sides in 37.5% of the piglets erupted. The same trend that was seen in group 2 for the p^2 and the i_2 was in this group also present: a higher proportion of the population had erupted p^2 compared to i_2, while a higher proportion had the i_2 higher than 3 mm compared to the p^2. All the piglets had total occluded incisors and premolars. None of the piglets had abnormal occlusion of the premolars, whereas in 39.6% of the piglets was abnormalities in the occlusion of the incisors observed (see Appendix D). High severity of plaque was more often observed than high severity of black pigmentation. Also in this group was gingivitis present in approximately 1/3 of the population and blood of the gingiva was more often present than a red gingiva. Caries was not found on the p_3.

3.2 Univariable analysis

Association between some dental characteristics and the parameters indicating the performance of the piglets were also studied. For this univariable analysis, we determined cut-off points of the different stages of eruption of a tooth to get 2 outcomes instead of 3. Therefore, proportions with outcome 0 (not erupted element) or with outcome 2 (element higher than 3 mm) were added to the proportions with outcome 1 (element lower than 3 mm). A result of this is that we examined either the height (higher or lower than 3 mm) of a typical element and its effect on performance or the eruption (erupted or not erupted) and its effect on performance. This was only performed for the elements that were included in statistical analysis. No sex differences of any of the dental characteristics were found in each group. Moreover, there was no association between the severity of plaque and the severity of black pigmentation in each group. A lack of association was also observed between plaque and gingivitis.

3.2.1 Group 1:
Height of the right p_4 was negatively associated with body weight ($p<0.05$). Piglets having a right p_4 higher than 3 mm were 0.49 kg lighter at necropsy than piglets having a right p_4 that was lower than 3 mm. A lack of association between the other elements and performance parameters was observed. Moreover, no association between the severity of black pigmentation and plaque and the different parameters indicating the performance of piglets was found.

3.2.2 Group 2:
The height of p^2 was in both sides positively associated with BW ($p<0.01$ for left, and $p<0.05$ for right). Piglets with a p^2 higher than 3 mm were 1.99 kg (left) and 1.47 kg (right) heavier than piglets with a p^2 that was lower than 3 mm. The height of the left p^2 was also positively associated with ADF over the last 2 weeks ($p<0.05$) and over the last week ($p<0.01$) of the experiment. Piglets with a left p^2 higher than 3 mm had a higher ADF of 80.18 g and 134.75 g over the last 2 weeks and over the last week, respectively. Eruption of the left i_2 was positively associated with ADF over the last 2 weeks ($p<0.05$). ADF was 56.6 g higher in piglets with erupted left i_2 compared to piglets without erupted left i_2. High severity of black pigmentation on the right p_3 was negatively associated with ADG ($p<0.05$) and FE ($p<0.05$), indicating that piglets with a more severe status of black pigmentation on the right p_3 had a lower ADG of 95.52 g and a lower FE of 0.14.

3.2.3 Group 3:
Height of the right p^2 was positively associated with BW ($p<0.05$). Piglets having a right p^2 higher than 3 mm were 1.33 kg heavier than piglets having a right p^2 that was lower than 3 mm. No association was found between the height of the i_2 and the eruption of the p^2, and the performance parameters. A lack of association was also observed between the presence of a high amount of black pigmentation and plaque and the different parameters indicating the performance of piglets. The presence of a red gingiva and bleeding of the gingiva surrounding the right p_3 was positively associated with BW and ADG. Piglets with a red gingiva were 1.5 kg ($p<0.05$) heavier than piglets with a pink gingiva of the right p_3. Piglets with bleeding upon probing on the right p_3 gingiva were 1.4 kg ($p<0.05$) heavier and had an ADG that was 153.5 g ($p<0.01$) higher than piglets without bleeding.

4. Discussion

The presence of dental health problems may play a role in the overall health of piglets. To study this, we examined the presence of various dental characteristics and its relation with piglet performance.

Piglets from group 2 and 3 were challenged with *E.coli* and *Salmonella*, respectively. It is important to note that these interventions may influenced the results. The risk and severity of disease is dependent of the strain of the bacteria, though the strains that are used in this study are unknown to us.
E.coli is one of the most important causes of post-weaning diarrhoea in pigs (43). Also various strains of *Salmonella* can cause diarrhoea in piglets (44). Severe diarrhoea was often seen in these piglets, although it was not even better or worse after infection. Nevertheless, the presence of severe diarrhoea may influenced growth, bodyweight and feed efficacy, and might therefore have an effect on the results. Moreover, these bacteria were orally administered, what makes it interesting to investigate the impact of it on dental health. However, about the effect of these bacteria on the occurrence of dental diseases is little known. There are no specific bacterial flora that are pathognomonic for gingivitis and periodontitis in humans (29,35-37). But it is observed that *E.coli* and *Salmonella* were not part of the specific bacteria that were mostly seen in human with black pigmentation and caries (10-12). Moreover, piglets were divided over test groups receiving different kind of creep feeds before weaning (group 1 and 2) or different kind of feed after weaning (group 3). The various creep feeds differed in their content and amount of probiotics and prebiotics. These orally administered bacteria may have had an effect on the oral microbiota, and thus on the formation of plaque and black pigmentation in these piglets. The various feed given to piglets from group 3 differed in their content of medium chain fatty acids. The lower pH of the 5 different test feeds, compared to that of the control feed, may have had an effect on the formation of caries (16,52), although this was not observed in this study.

To asses delayed eruption, it is important to know the reference values of tooth eruption times established from different breeds and sexes in piglets. Tucker et al. (1) examined the sequence of tooth eruption. They performed a complete oral exam at 9 different moments (ranged from 2 days until 35 days of age) on each piglet to determine the time of emergence of each element. Although the examination of eruption was performed only once for each group in the current study, we could see a trend in sequence of premolar eruption for group 1. This sequence was the same as that was established in the study performed by Tucker et al. (1). Based on the percentage of piglets having a premolar tooth that was higher than 3 mm, we can suggest that the p^3 (>75%) erupts first, followed by the p_4 (>30%), p_3 (15%) and the p^4 (<2%) in that order. Tucker et al. (1) found a significant sex difference, in that of gilts having earlier eruption than boars from the same age. Moreover, the sequence of tooth eruption seems to vary between breeds. A different sequence of tooth eruption between Yorkshire piglets and the Pitman-Moore strain was observed (1,5). We did not find a trend in differences between gilts and boars on premolar eruption time in this study. In addition, all the piglets from our study were the same breed, so we were not able to study the effect of breed on performance.

In the current study we examined the relation between tooth development and piglet performance. For this purpose, we selected elements for statistical analysis. Elements were excluded when it belonged to one of the needle teeth, comprising the i^3, i_3, and the c in each quadrant. The reason for this was that we were interested in the effect of tooth development based on its functional role in enabling consumption of food and in mastication food for digestion (4). The effect of these needle teeth on food consumption and mastication is not described yet. Needle teeth are fully erupted at birth and angle outwards (laterally) from the jaw. The anatomy and use by delivering a damaging sideward bite of these teeth, suggest that they are used as weapons for competition among siblings (i.e. when establishing a teat order) (47). Elements were also excluded from statistical analysis because its small variation across the three groups. This was seen when a tooth had not emerged in over 90% of the population of a specific group at the time of dental investigation, but also when a tooth was erupted in all piglets of a specific group and was higher than 3 mm in the 90% of more of the piglets. The elements included in statistical analysis were used to investigate the effect of tooth development on performance.

Tooth development plays a role in consuming and mastication of food (4). Accordingly, our hypothesis was that piglets having elements at a further stage in its development, focusing on the height and eruption, would have a better performance compared to piglets having elements early in its development. Certainly, differences in the length of elements were seen. However the effects on the performance were age-dependent. We found that piglets from group 2 having a higher p^2 had a higher BW and ADF when they reached the age of 6.5 weeks. Moreover, a higher p^2 in piglets of group 3 was positively associated with BW. This positive association between the height of elements and performance parameters was absent in piglets at 3.5 weeks of age. One possible explanation of this lack of positive association can be that these piglets were not weaned yet. Besides creep feed, these piglets drank milk from their mother which may influenced growth. For consumption of this liquid diet, higher premolars were not necessary and may therefore not influenced piglet performance. Moreover, the eruption of elements was found to have an positive effect on piglet performance. Piglets from group 2 having erupted i_2 had a higher ADF compared to piglets without erupted i_2. This positive association between tooth eruption and piglet performance was also found in a study performed by Tucker et al. (3), where the eruption of certain elements (p_3 and p^4) was positively associated with weight gain at 3 weeks later (3). However, in this study we found no association between the eruption of the p^4 and piglet performance. One explanation between the observed differences between the results of this study and the results found by Tucker et al. (3), can be due to the different methods that were used for detecting associations between dental eruption and piglet performance. Although the erupted status of the p^4 was in both studies examined just around weaning, the parameters indicative for the performance were examined around weaning in this study and at 3 weeks later in the study by Tucker et al. (3).

The presence of total occlusion was age-dependent in this study. None of the youngest piglets, derived from group 1, had total occlusion of the premolars and only 3.3% had total occlusion of the incisors, in contrast to the older piglets derived from group 2 and 3, where a total occlusion of both the premolars and incisors were found. This seems logical, because total occlusion is more likely to occur when the teeth are developed, which is also age-dependent. Possible abnormalities in occlusion in older animals may have an effect on feed intake as that disability of cutting and chewing of food may occur. However, a small proportion of piglets with abnormal occlusion was observed in this study. As a result we were not able to examine the effect of abnormal occlusion on feed intake of the piglets.

Black pigmentation is described as an incomplete coalescence of black spots on an element with or without a continuous line formed by this black spots parallel to the gingival margin of that tooth (7,8). It is hard to wipe off by scratching or brushing. Only after professional scaling, black pigmentation tends to reform (51). Tooth discoloration can be divided into extrinsic, intrinsic, or internalized. In human medicine, black pigmentation is known to be an extrinsic discoloration as it is deposited on the tooth surface (25,31). Certain types of bacteria and incorporated compounds are proposed as the aetiological factors in the formation of black pigmentation (9,25,31). However, little is known about the origin or composition of this staining in pigs and other animals. In human dentistry, black pigmentation is known to be a deposit consisting of bacteria and can therefore be classified as a type of dental plaque, although it is composed of different types of bacteria (9). The predominant types of bacteria isolated from this deposit are gram-positive rods, especially *Actinomyces* spp. (10-12). The compounds that are incorporated into the tooth produce the black staining due to either their basic colour or chemical interaction at the tooth surface (25,31). Based on biochemical studies in human, it is found that iron/copper and sulphur complexes are the compounds responsible for the dark colour in black pigmentation (13).
In the present study, the youngest piglets were not affected by (a high amount of) black pigmentation compared to the older piglets (piglets from group 2 and 3). This suggests that the black pigmentation did not occur during tooth development (i.e. intrinsic) and seems to be extrinsic in the current study, likewise in human. Moreover, based on this finding, the presence of black pigmentation is age-related. The finding that the occurrence of black pigmentation increased with age is in accordance with studies performed in human (14,15). We found a negative association between the presence of a high severity of black pigmentation and ADG and FE. This suggests that this condition may reflect a reduced capacity for growth, although no associated impairment of dental health had been reported in human

(51). Further histological and biochemical research on black pigmented dental tissue in piglets is needed to determine the exact deposition and contents of the black pigmentation in these animals. This is also important to understand the exact role of black pigmentation on piglet performance.

Based on various epidemiological human studies, it is found that the presence of black pigmentation is associated with lower caries incidence (18-22). This could be an explanation why we did not find caries in the current study. It is thought that a lack of cariogenic potential in human with black pigmentation reduces susceptibility to caries. The microflora of black pigmentation consists predominantly of *Actinomyces* spp. that have lower cariogenic potential than *Lactobacillus* species and *Streptococcus mutans*, which are highly present on human teeth with dental caries (3,10-12). The latter two bacteria were, moreover, present in higher numbers in human without black pigmentation (11,12). In addition, it is thought that beneficial salivary parameters reduce the formation of dental caries. Higher calcium concentrations and higher buffering capacity are found in human saliva having black pigmentation (23). Further research investigating the microbiota and saliva in piglets with black pigmentation is needed to provide a more comprehensive picture of the effect of black pigmentation on the formation of dental caries.

In the current study, we focused only on the p_3 during the examination of caries, which may also explain why we did not found any caries. The remaining teeth may have undiagnosed, which seems to be more susceptible for caries. The needle teeth, the i^1, and the i_1, which are among the first to erupt, will be the first to experience the cariogenic challenge and will suffer the longest caries attack (49). In human infants, a higher prevalence of caries on the i^1 was found than in their remaining deciduous dentition (24). Tucker et al. (1,3) also found caries only on the i^1 in piglets at weaning (range 14-27 days). However, caries and staining were often seen together in that study. The staining might have covered the caries and therefore a misinterpretation of the presence of carious lesions may have occurred.

Likewise in human dentistry, diagnosis of gingivitis is made mostly on clinical signs and visual examination. Signs of gingivitis include redness, swelling, increased gingival exudate, sensitivity, and bleeding on gentle probing of the gingival margin (32,33). Gingival redness and bleeding upon probing were also found in miniature pigs with gingivitis (27). Histologically, the inflammatory process in the same miniature pig breed was similar to that seen in human with gingivitis (27). Although differences between species and breeds is unknown, based on those findings, we scored gingivitis according to the colour and the blood of the gingiva.
Gingivitis is a reversible disease indicating an inflammation of the marginal gingiva. In human medicine, gingivitis is rarely characterized by pain and discomfort (41). This could explain the lack of a negative association between the presence of gingivitis and the performance parameters in the current study. However, when gingivitis is left untreated, the bacteria and inflammation can spread throughout the remaining periodontal unit and can lead to periodontitis (46). Periodontitis is characterized by loss of connective tissue attachment and loss of alveolar bone and can subsequently lead to tooth loss (45,46). It is associated to physical pain and to physical disability in human, and made food uncomfortable to eat (38-40). Periodontitis in piglets could, in contrast to gingivitis, also have an adverse effect on the amount of feed intake. In this study we examine only the gingival margin for the presence of gingivitis. The remaining tissue of the periodontium (30) was not examined and periodontitis may therefore have undiagnosed. Nevertheless, periodontitis was not likely to be present in this study because of the lack of a negative association and because of previous findings where periodontitis was present in miniature pigs only after the age of 16 months (27).
It is important to note that a gold standard for the diagnosis of gingivitis and periodontitis in piglets is missing. Examination of the gingival histology samples will give us a more comprehensive picture of the value of the diagnosis of gingivitis and periodontitis which was made on visual examination in the current study.

Dental plaque is defined by a bacterial biofilm on teeth (45). Black pigmentation is also known to be a deposit consisting of bacteria (9-12). However, no association was found between the severity of plaque and the severity of black pigmentation in the current study. The lack of this association can be explained by the fact that the bacteria of black pigmentation are different than the bacteria of dental plaque (9). The dental disclosing solution, used in this study, may have not highlighted the bacteria that form the deposit of the black pigmentation.

Plaque is known to be the primary cause of gingivitis. The accumulation of a plaque biofilm cause inflammation of the gingival margin (29,34-36), which can subsequently advance in periodontitis due to an inappropriate and destructive host inflammatory immune response. However, a higher degree of plaque was not associated with red gingiva or bleeding of the gingiva in the current study. This may suggest that a low degree of plaque may also be enough to cause these clinical signs.
Dental plaque is also involved in the aetiology of dental caries. Cariogenic bacteria in dental plaque utilize sugar (monosacharides and disaccharides) in their glycolytic pathways to produce energy. During this metabolism, acid production occurs (23). As a result of this acid, the pH may become too low whereby demineralization of teeth ensues. If the diffusion of calcium, phosphate, and carbonate out of the tooth is allowed to continue, cavitation will eventually take place (4,16, 52-54).
Plaque becomes clinically interesting as it plays an important role in the development of periodontitis and in the formation of caries. These dental diseases have an adverse effect on feed intake and on the quality of life in human (26,38-41). Plaque removal, for instance by tooth brushing, is therefore fundamentally important in the prevention of these diseases (28). Although the effect of caries and periodontitis on the performance of piglets is not completely understood, plaque control in piglets may be essential for piglet health. This may be accomplished by dental hygiene chews likewise in dogs, which led significantly to less plaque in those animals (42).

Left and right differences were found when examining the effect of the dental characteristics on piglet performance. This may be explained by preferred chewing sides. The feeder and toys were arranged in a specific corner of the pen, which may influenced the preference in chewing side. Alternatively, the average result from both sides can be used for further analysis after determining that left and right are equal.

5. Conclusion

The presence of dental health problems is thought to play a role in the overall health of piglets. The observed associations between the dental characteristics and piglet performance makes this increasingly likely. However, the term ‘dental health problems’ may be incorrect, as that a negatively association was not necessarily found between the presence of the dental characteristics and piglet performance. More extended (i.e. not only the p_3) methodology and more research is required for a better understanding of the role of dentition in the overall health in piglets.

6. References

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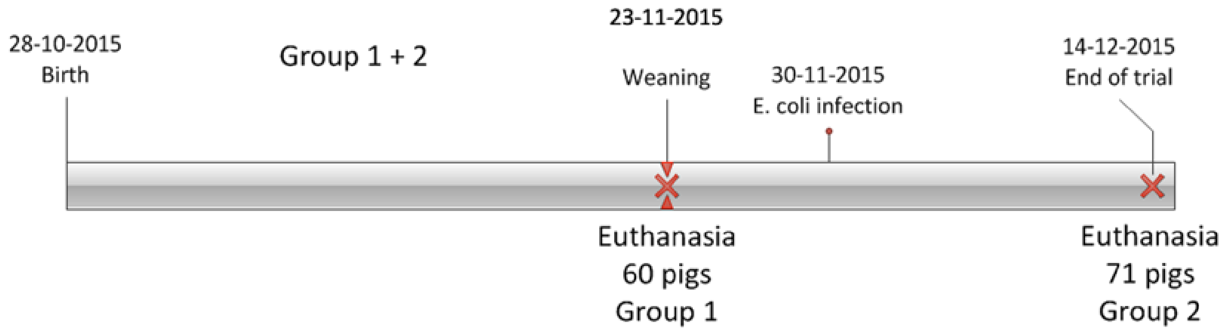
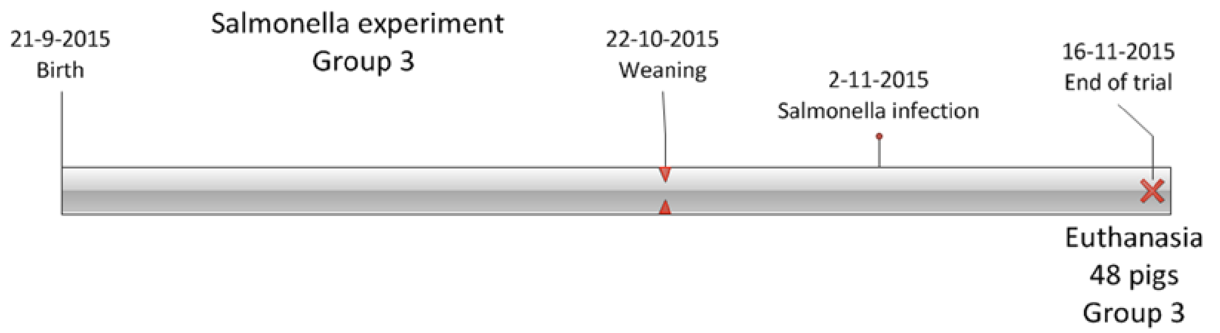
APPENDIX A:
A timeline of the experimental design performed at the Swine Research Centre.

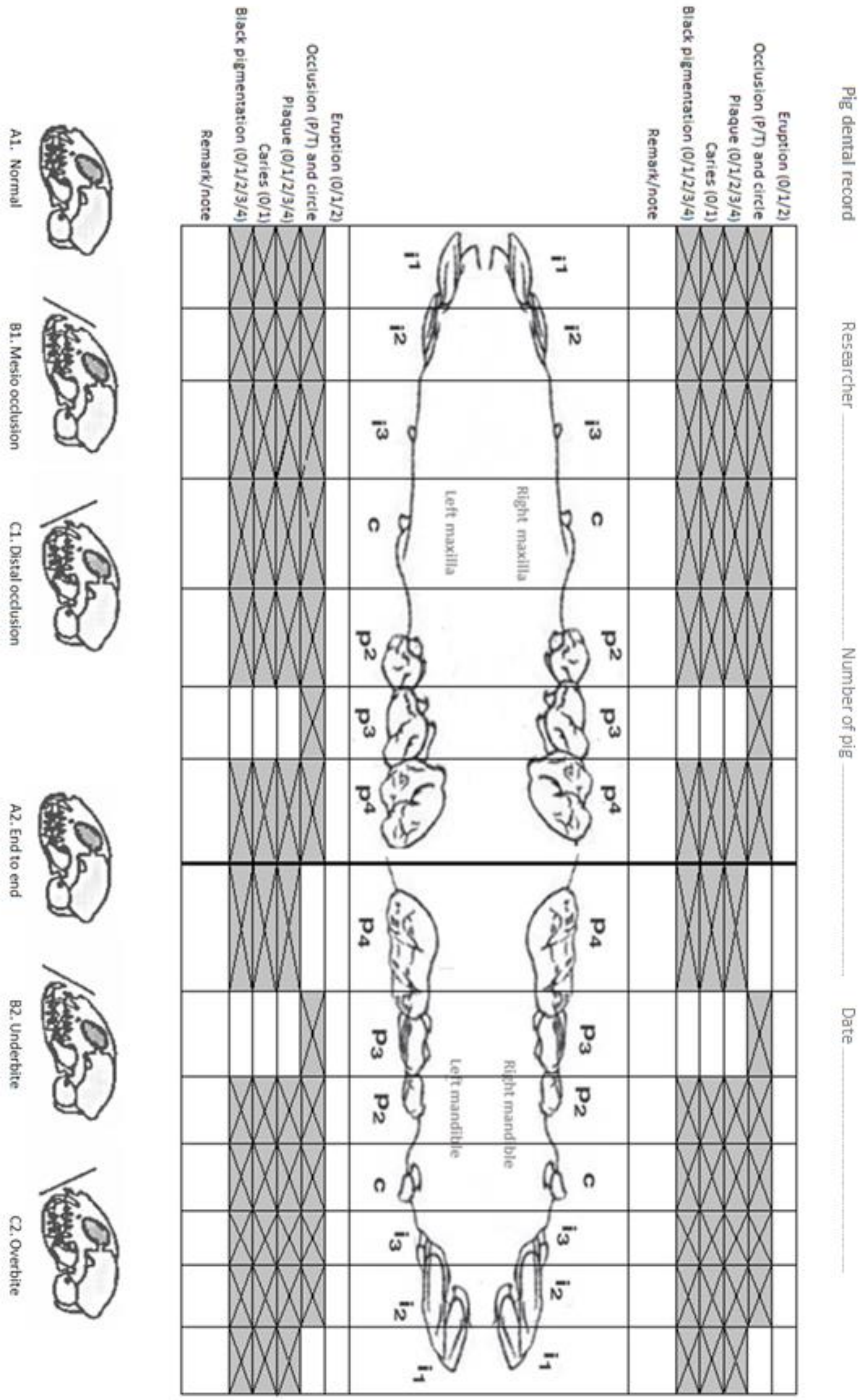




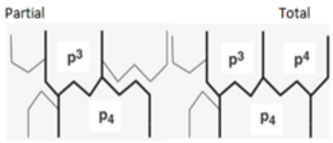
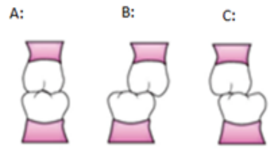
APPENDIX B:

APPENDIX B.1:

Scoring system that was used during the dental investigation.



APPENDIX B.2:
Scoring methods for dental investigation.

Parameter	Scoring teeth	Fill in	Explanation
Eruption	All teeth	0, 1, or 2	0: Not erupted 1: Erupted below 3 mm in height from gingiva 2: Erupted above 3 mm in height from gingiva
Occlusion	Incisors and premolars	P or T	***Premolars:*** Fill in **P** when partial occlusion (max. P3 and P4 are not both attached to mand. P4) Fill in **T** when total occlusion and circle one of the three options in smaller picture: A. Normal B. Mesio occlusion C. Distal occlusion  ***Incisors:*** Fill in **P** when partial occlusion Fill in **T** when total occlusion and circle one of the three options in smaller picture: A. End to end B. Underbite C. Overbite 
Plaque	P3 of the upper and lower jaw	0, 1, 2, 3, or 4	Score the percentage of plaque in the bucal area. Use 'dental disclosing solution' and put a swab/wattenstaafje in it, brush it over the tooth, spray some water over it, wait. Does it remain pink? It's plaque. 0: 0% (No plaque is found) 1: < 25% of plaque on tooth 2: 25-50% 3: 50-75% 4: > 75%
Caries	P3	0 or 1	Caries: soft brown spot on tooth Differentiation caries and food: you can remove food, you cannot remove caries 0: No caries found 1: Caries found
Black pigmentation	P3	0, 1, 2, 3, or 4	Differentiate black pigmentation from mineralised plaque/calculi 0: 0% (No black pigmentation is found) 1: < 25% of black pigmentation on tooth 2: 25-50% 3: 50-75% 4: > 75%
Remarks/abnormalities	All teeth	Nothing, Abnomality, and/ or 'MS'	Fill in: **'MS'** when a mucosal sample is taken to score gingivitis (see page 3). Abnormalities for instance: Broken teeth, during taking gingivitis score *in vivo*: when you can easily put the tool down, ...)

APPENDIX C:

Examples of the different stages of black pigmentation. The average of the percentages that covered the buccal and the lingual side of the p_3 was indicative to the final score.

Score 0: No black pigmentation
No pictures

Score 1: <25%

Buccal

Lingual

Score 2: 25-50%

Buccal

Lingual

Score 3: 50-75%

Buccal

Lingual

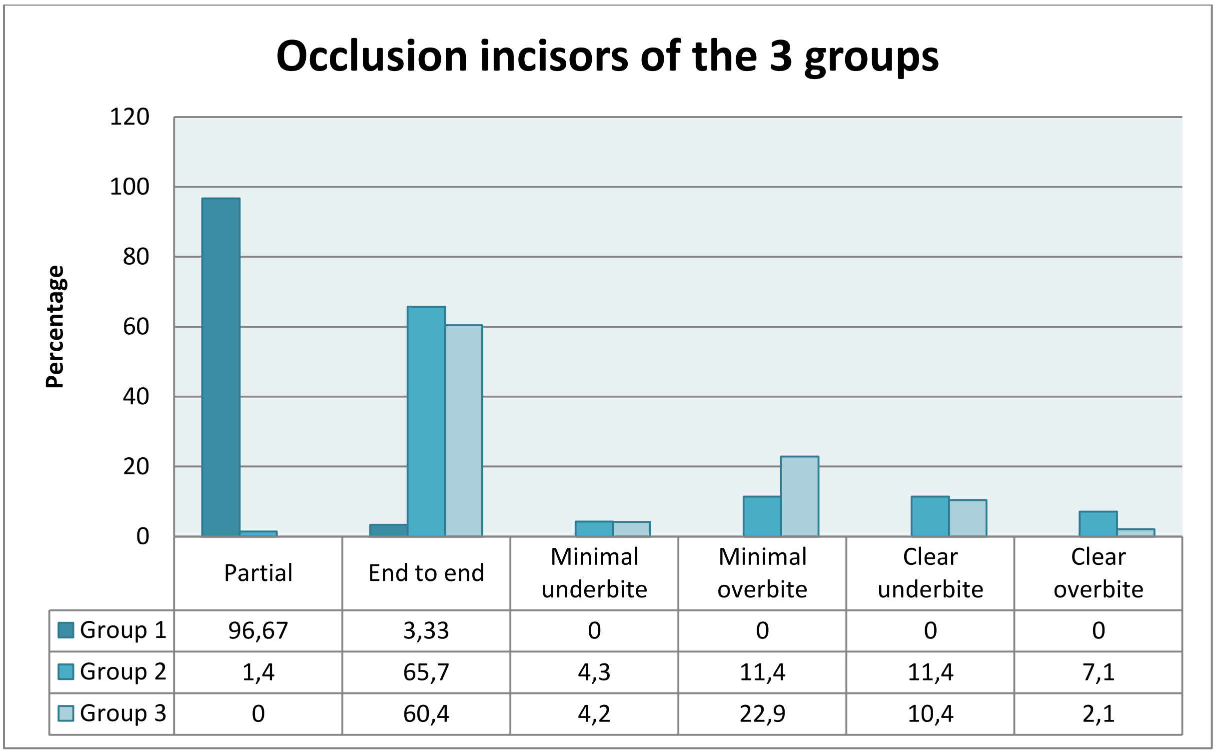
Score 4: >75%

Buccal

Lingual

APPENDIX D:

Proportions of the three groups indicating a typical formation of the occlusion of the incisors.



	Partial	End to end	Minimal underbite	Minimal overbite	Clear underbite	Clear overbite
Group 1	96,67	3,33	0	0	0	0
Group 2	1,4	65,7	4,3	11,4	11,4	7,1
Group 3	0	60,4	4,2	22,9	10,4	2,1