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Swallowing kinematics and airway protection after palatal local anesthesia in infant pigs

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Abstract

Objective—Abnormal kinematics during swallowing can result in aspiration which may become life threatening. We tested the role of palatal sensation in the motor control of pharyngeal swallow in infants.

Study Design—In eight infant pigs, we reduced palatal sensation using local anesthesia (PLA) and measured the impact on swallowing kinematics and airway protection.

Methods—The pigs drank milk containing barium while we simultaneously recorded videofluoroscopy and electromyography from fine wire bipolar electrodes in several hyolaryngeal muscles. We compared these results to control feedings and feedings following palatal saline injections (PSA).

Results—After PLA, four pigs had extreme jaw movements and abnormal tongue movement uncharacteristic of sucking. For this reason, we evaluated differences between these “Group B” pigs and the others that could suck normally after PLA, “Group A”. In the four Group A pigs, after PLA there was less hyoid elevation ($p<0.001$) but normal jaw and tongue movements. In Group B, in addition to greater jaw movement ($p<0.001$) there was more anterior and superior tongue movement ($p<0.001$) and a larger range of hyoid movement ($p<0.001$).

Conclusion—The airway was protected in all of the pigs, indicating that these changes allowed successful adaptation to the reduction in palatal sensation. However, the oral and pharyngeal phases of the swallow were functionally linked and trigeminal sensation influenced the motor control of the pharyngeal swallow.

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Keywords

Dysphagia; Electromyography; Videofluoroscopy; Neurophysiology; Greater Palatine Nerve; Nasopalatine Nerve; animal model; pig; infant

INTRODUCTION

Prematurity, neurological deficits or differences in craniofacial anatomy can cause swallowing dysfunction^{1,2}, with a sequelae of aspiration^{3,4}. While much is known about swallowing biomechanics^{5,6}, less is known about the integrated sensorimotor neurophysiology in either normal or abnormal swallowing.

Infant swallowing physiology is characterized by rhythmic suck cycles and suck-swallows, where the bolus passes past the laryngeal opening and into the esophagus. With each suck-swallow cycle there is significant rhythmic tongue movement, a lesser amount of jaw opening, and hyolaryngeal elevation. Oral sensory input from the trigeminal nerve is necessary for motor function during pharyngeal swallowing in adults⁷, but its role in infants is unknown.

Using an infant pig model, we tested the role of palatal sensation on the motor function and kinematics of the suck-swallow. We injected palatal local anesthesia (PLA) and saline (PSA) and then compared swallowing kinematics and motor function after these treatments to normal (control) feeding sessions in the same animal. We hypothesized that they would adapt to PLA with unaltered airway protection. We expected altered tongue and jaw movement since palatal stimulation elicits reflexive jaw and tongue movements in decerebrate animals⁸. The PSA treatment was designed as a sham, and we did not expect significant differences from the control feedings.

MATERIALS AND METHODS

Surgical Procedure

Eight pigs, *Sus scrofa*, a standard model,⁹⁻¹¹ were obtained from Tom Morris Farms (Reisterstown, MD) at 2-3 weeks old (3-5kg). All procedures were approved by JHMI IACUC (SW10M212).

After successfully learning to bottle feed using a pig nipple (Nasco, Fort Atkinson, WI), we implanted fine wire bipolar electromyographic (EMG) electrodes in the genioglossus (GG), thyrohyoid (TH), and cricothyroid (CT) muscles. An EMG patch electrode was sutured to the anterior surface of the mylohyoid (MH) muscle. Intraoral radio-opaque markers were inserted in the midline of the anterior hard palate, hard/soft palate junction, tongue (~10mm deep to the foramen cecum), and right and left mucogingival junction superior to the maxillary first molar (Fig. 1). Husbandry and the specifics of the methods of EMG electrode and marker implantation followed Thexton et al.¹². This methodology has been used in other pig studies and does not alter feeding^{9,13}.

Treatments

The day after surgery, experiments began and the animal was placed under 3% Isoflurane to achieve deep/surgical anesthesia for 20 minutes. A Weck Hemoclip Ligating Clip (Pilling Weck, Research Triangle Park, NC) was placed on the epiglottis ⁶.

There were two treatments: (1) saline (PSA) and (2) local anesthesia (PLA) given on separate days. The local anesthesia injections were 0.5 ml of 0.5% bupivacaine hydrochloride (Marcaine™, 5mg/ml, Hospira, Inc. Lake Forest, IL) and the saline injection was 0.5 ml of saline. A standard clinical dental injection technique and amount was used ^{14,15}. These injections were to three locations: the right and left greater palatine nerves and the nasopalatine nerve. In the process of anesthetizing the greater palatine nerve, it is likely that a small portion of the lesser palatine nerve, supplying sensation to the soft palate was also blocked. In a post-mortem study of infant pigs, this occurred in 1/3 of cases.

The day after an injection was given was a recovery day during which no experiments were conducted. The order of the treatments was randomized. After the epiglottis marker was either placed or verified, and the treatment was given, recovery from general anesthesia took 5-10 minutes. Bupivacaine hydrochloride has a minimal duration of action of 1 hour until oral reflexes are observed in infant pigs¹⁶.

Recordings of Feeding Sessions

The pigs were bottle fed while standing unrestrained in a plastic feeding box 30 minutes to 1 hour post-injection while lateral videofluoroscopy was recorded at 60 frames per second. The recording device (Allura FD20, Philips Healthcare, Best, The Netherlands) was equipped with a high-resolution digital flat-panel detector (154×154 micron pixel-pitch, 30×40 cm). The pigs were fed until 20 swallows had been recorded or at least five 15 second recordings. Bottles contained eight ounces of milk formula (Land O Lakes Solustart pig milk replacer, St. Paul, MN) with 1/3 cup powdered barium. Temperature and the amount of barium in the bottles was standardized ^{17,18}.

Videofluoroscopy Data Analysis

One judge evaluated the videofluoroscopy recordings using MaxTRAQ Version 2.2.4.1 (Innovision Systems, Inc., Columbiaville, MI). Each swallow was scored according to the Infant Mammalian Penetration-Aspiration Scale (IMPAS, Table 1) ¹⁹. The scores were tested for differences between treatments using a Kruskal-Wallis Test and a Dwass-Steel-Critchlow- Fligner Test using SYSTAT 13 (SYSTAT, 2009). Holman et al. 2012 reports intra-rater reliability for this scale with an average of 86% agreement with an intraclass correlation coefficient of 0.92¹⁹.

We calculated the total distance the hyoid marker and mandible (menton, the most anterior and inferior point on the mandible) moved relative to the palate markers. These are standard measurements in both animal and human videofluoroscopy studies²⁰⁻²². The maximum anterior/posterior and dorsal/ventral movements of tongue and hyoid were calculated relative to an x-axis between the hard/soft palate junction marker (origin) and the hard palate marker. The distances were corrected using the maximum diameter of a radio-opaque sphere

in the video. Intra-rater reliability of auto-digitizing points from infant pig lateral videofluoroscopy has shown the average variability to be 0.67 millimeters with a standard deviation of 0.83 millimeters (Unpublished data, AM Griffioen).

Electromyographic Data Analysis

Based on the results from the post-mortem dissection and the quality of the EMG recordings, one channel each for MH, GG, TH and CT was chosen for data analysis per pig. The time of the midpoint of each burst of muscle activity in the EMG recordings was identified for a maximum of 20 suck-swallow cycles per feeding session in LabChart® Pro (AD instruments, Colorado Springs, CO, Fig. 2)¹². The CT was labeled at the start of its activity due to its gradually increasing signal (Fig. 2). We evaluated timing differences during the suck cycle (MH to GG), between the suck and swallow (MH to TH), and during the pharyngeal swallow (TH to CT). When interpreting the significant differences in this data, we kept in mind that the total duration of epiglottal movement during the pharyngeal swallow is on average 208.72 ms (n=457 swallows, Unpublished data, SD Holman).

Statistical Testing

Differences between treatment and control feedings, for each dependent variable (except the IMPAS results), were evaluated using a General Linear Model ANOVA and post-hoc Tukey's HSD test with an alpha of 0.05. The sample size was approximately 480 swallows with 20 swallows per treatment per animal.

RESULTS

Two groups: Group A and Group B

The pigs were divided into two exclusive groups for statistical analyses: Group A and Group B. Group B pigs (n=4) had noticeably larger movements of the mandible, did not latch onto the nipple and had less surface deformation of the tongue during oral transport cycles after PLA (Fig. 3)^B. Group A pigs (n=4) had no noticeable change in jaw movement, latching ability or tongue movement with PLA. This clear qualitative distinction had no intermediate feeding mechanism. Group was added as a fixed factor in the ANOVA and also tested for an interaction with treatment. Individual (pig) was nested in group to account for it as a random factor.

Airway Protection

Group A, after PLA and PSA, had significantly more airway protection ($p < 0.001$ for PLA and PSA) relative to control feedings (Fig. 4). There was only marginally increased airway protection in Group B with PLA or PSA ($p = 0.068$). There were only a few incidents of aspiration across all animals and coughing was never observed.

^BSD Holman et al- In review at Journal Neurophysiology

Oral Transport

There was a significant difference in extent of jaw opening between treatments ($p<0.001$) and in the interaction between treatment and group ($p<0.001$, Fig. 5), but not between the groups. There was significantly more jaw movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$) and no difference between control and PSA.

Group A had no significant difference in jaw opening between treatments. Group B had significantly more jaw opening with PLA relative to control ($p<0.001$) and PSA ($p<0.001$, Table 2, Fig. 3, Fig. 5). No difference in jaw opening existed between control and PSA. There was significantly more jaw opening in the control feedings in Group A compared to Group B ($p<0.001$, Table 2, Fig. 5). Group B had significantly more jaw opening after PLA than Group A ($p<0.001$, Table 2, Fig. 5). One Group A pig (Pig C) was excluded from the dataset because the menton was not in view during the videofluoroscopy recording.

Significant differences existed in anterior tongue movement based on treatment ($p<0.001$) and group ($p<0.001$). Pigs had significantly more anterior tongue movement after PLA relative to control ($p<0.001$) and PSA ($p=0.004$). Group B also had more movement than Group A ($p<0.001$, Table 2).

Superior tongue movement differed between group, treatment and in interaction ($p<0.001$ for all). There was more movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$), but no difference between control and PSA. There was also significantly more movement in Group B than Group A ($p<0.001$).

In Group A there were no significant differences between treatments. In Group B had significantly more movement after PLA relative to both control ($p<0.001$) and PSA ($p<0.001$) and no difference between control and PSA (Table 2). There was also no difference between groups during control feedings or PSA feedings. There was significantly more tongue movement after PLA in Group B than Group A ($p<0.001$, Table 2).

The GG EMG activity was acceptable only for five of the eight animals in the study. In two cases the electrodes were not in the correct muscle, and in the other case the signal was not clear due to erratic tongue movements after PLA. For the remainder, the time between MH and GG activity was significantly different between treatment, group and their interaction ($p<0.001$ for all). There was less time between MH and GG after PLA compared to both control ($p<0.001$) and PSA ($p<0.001$), but no difference between control and PSA. There was also less time between MH and GG for Group B relative to Group A ($p<0.001$).

In Group A there was no difference between treatments. In Group B there was less time between MH and GG after PLA relative to control ($p<0.001$) and PSA ($p<0.001$, Fig. 2, Fig. 6, Table 2) with no difference between control and PSA. There was no difference between groups during control or PSA feedings. There was less time between MH and GG during PLA feedings in Group B relative to Group A ($p<0.001$).

Time between start of suck-swallow and pharyngeal swallow

For the lag between MH and TH activity, significant differences between treatments ($p<0.001$), groups ($p<0.001$) and in the interaction between treatment and group ($p<0.001$) existed. The differences between treatments all had $p<0.001$ with control feedings having more time, followed by PSA and then PLA with the least amount of time. Group A had a longer lag than Group B ($p<0.001$).

Within Group A there was no significant difference between control and PLA. Significantly less time existed between MH and TH muscle activity after PSA compared to control ($p<0.001$) and PLA ($p=0.024$, Table 2, Fig. 7). In Group B There was less time after PLA relative to control ($p<0.001$) and PSA ($p<0.001$, Table 2, Fig. 7). There was no difference between control and PSA feedings. There was no difference between groups during control feedings or PSA feedings. There was less time after PLA in Group B compared to Group A ($p<0.001$, Table 2, Fig. 7).

Pharyngeal Swallow

There were significant differences in range of hyoid movement between treatments ($p=0.010$) and in the interaction between treatment and group ($p<0.001$). There was significantly more hyoid movement after PLA compared to control ($p=0.007$) but no other differences between treatments.

In Group A there was less hyoid movement after PLA compared to control ($p<0.001$) and PSA ($p=0.015$, Table 2, Fig. 8), however, no difference between control and PSA. In Group B there was more movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$, Table 2, Fig. 8). There was also more hyoid movement after PSA relative to control ($p=0.006$, Table 2, Fig. 8). Additionally, there was more hyoid movement during control feedings in Group A relative to Group B ($p<0.001$, Table 2, Fig. 8). There was also more hyoid movement after PLA in Group B relative to Group A ($p<0.001$, Table 2, Fig. 8). There was no difference between groups after PSA.

There were significant differences in anterior hyoid movement based on treatment, group and their interaction, all with $p<0.001$. There was more movement after PLA relative to both control ($p<0.001$) and PSA ($p<0.001$) with no difference between control and PSA. There was also more movement in Group B relative to Group A ($p<0.001$).

In Group A no differences existed between treatments. In Group B there was more movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$) but no difference between control and PSA (Table 2). There was no difference in anterior hyoid movement during control or PSA feedings in Group A compared to Group B. There was more movement after PLA in Group B compared to Group A ($p<0.001$).

There were significant differences in superior hyoid movement based on treatment, group and their interaction, all with $p<0.001$. There was significantly more elevation after PLA compared to both control ($p<0.001$) and PSA ($p<0.001$) with no difference between control and PSA. There was also more movement in Group B than Group A ($p<0.001$).

In Group A there were no differences between treatments. In Group B there was more movement after PLA relative to control ($p<0.001$) and PSA ($p<0.001$) with no difference between control and PSA (Table 2). There was no difference between control feedings or PSA feedings in Group A and B. There was more movement in the PLA feedings in Group B than Group A ($p<0.001$).

There was a significant difference in the time between TH and CT overall with regard to group ($p<0.001$) and treatment ($p<0.001$). There was significantly more time between TH and CT after PLA relative to control ($p=0.024$) and after PSA relative to control ($p=0.021$). There was no significant difference between PSA and PLA. There was also more time between TH and CT in Group B relative to Group A overall ($p<0.001$, Table 2).

DISCUSSION

Swallowing kinematics and airway protection following palatal anesthesia

PLA affected kinematics and airway protection differently in Group A and Group B. In Group B, where the pigs did not latch onto the nipple after PLA, the increased hyoid elevation could be due to the increased range of tongue motion and jaw opening. The tongue is an elevator of the hyoid bone and its increased range of motion could have caused the hyoid bone to elevate further²³. The muscles responsible for jaw opening in Group B are also hyoid elevators²³⁻²⁵. This increased hyoid elevation and less time between the oral and pharyngeal muscle activity could have provided a slight increase in airway protection²⁶.

After PLA in Group A, who after PLA were still able to latch, despite the reduced hyolaryngeal elevation, the airway was more protected. This suggests that the response after PLA was to provide better airway protection than during control feedings. There are many other ways that mammals protect their airway during swallowing and not all of these airway protection mechanisms were measured in this study.

Swallowing kinematics and airway protection following palatal saline

Although initially intended as a sham treatment, it is not clear if the PSA was truly a sham. PSA treatment could have another effect on sensory nerves: 1) anesthesia, from pressure on these sensory nerves, or 2) pain, consistent with clinical observation. It is unknown how long either effect could last following PSA, confounding the interpretation of these results.

With PSA, Group A had significantly more airway protection which may stem from reduced time between oral and pharyngeal muscle activity. With PSA, Group B had no change in airway protection, perhaps due to increased hyoid elevation. This was a physiologically different response between the groups, not just to anesthesia, but also to saline. Yet the airway remained protected despite significant kinematic differences, demonstrating that the infant was able to adapt to both treatments. There are multiple mechanisms of airway protection, and unfortunately, not all could be measured in this study.

While the significant temporal difference in TH and CT activity with the PSA treatment were statistically significant, it was only a 5 ms difference. This small magnitude is likely not biologically significant, and may not reflect adaptation to the altered palatal sensation.

Overall Impact of Sensory Modification

Airway protection, although increased in both groups and treatments, was not abnormal prior to treatment. It is unknown how PLA or PSA could affect airway protection in an animal model with significant penetration or aspiration. Applying these treatments to a pathologic model could answer this question²⁷.

Trigeminal sensation influenced the motor output in the brainstem. Alternatives exist for the mechanism responsible for this result. The trigeminal sensory nerves that project to the nucleus tractus solitaries²⁸ could influence the nucleus ambiguus output and therefore the pharyngeal swallow. A second hypothesis is that descending input from the cortex to the swallowing CPG influences the motor output for the pharyngeal swallow²⁹. Further study is necessary to understand fully the mechanisms underlying these differences.

Limitations of the Study

We only evaluated EMG data from a few muscles of many that are active during swallowing. We also evaluated the kinematics of a few key structures, but not all that are in motion during a swallow. There are other airway protection mechanisms during swallowing that we did not measure, such as vocal fold closure and epiglottis movement.

CONCLUSION

This study is the first to document the role of sensory information as a functional link between the oral and pharyngeal phases in the infant suck-swallow. Further studies are needed to determine if there are other regions in the oral cavity where sensation is also linked to pharyngeal function. These results strongly support the use of oral sensory stimulation devices, but more research is needed to quantify the impact that sensory stimulation may have on disordered swallowing.

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Figure 1. Videofluoroscopy image with radio-opaque and anatomical markers identified
The radio-opaque markers on the hard palate, hard/soft palate junction and hyoid are clearly identified. Additionally, the radiological point of the menton is identified. The epiglottis is outlined and the nipple and snout are labeled for orientation. There are additional radio-opaque markers in the image that were not evaluated in the present study.

A. Control

B. Palatal Anesthesia

Figure 2. EMG signals in control and local anesthesia for Pig H (Group B)

A) EMG signals during a control feeding. B) EMG signals in the same pig that was not sucking following a palatal anesthesia (PLA) treatment. With PLA the mylohyoid (MH) and genioglossus (GG) are active at roughly the same time with the mid-point of GG activity actually occurring before MH. In the control feeding the MH is always active well before GG. There is also a lot less time between oral (MH) and pharyngeal (TH) muscle activity. The dashed lines indicate how the muscle was labeled. The MH, GG and TH were labeled at the midpoint of their activity while the cricothyroid (CT) was labeled at the start due to the triangular nature of its EMG signal. The time point at each label was used to calculate timing differences.

Figure 3. Tongue, hyoid and menton movements in a Group B pig during a control feeding and a feeding following PLA

Each data point represents the 2-D location of a specific anatomic marker. Each cluster of data points represents a complete record of movement for a given marker over the course of a suck-swallow cycle. The black points represent hyoid movement, the dark grey points represent tongue movement and the light grey points represent menton movement. A) The range of hyoid, tongue and mandible movements in a control feeding from one of the Group B pigs. B) The range of hyoid, tongue and mandible movements in a feeding after palatal anesthesia (PLA) in a Group B pig. The menton and hyoid markers had a larger range of motion in the Group B pig after PLA. The tongue marker had more movement in the Group B pigs after PLA.

Figure 4. IMPAS scores for Group B and Group A

A) For Group A there was more airway protection after palatal anesthesia ($p < 0.001$) and saline ($p < 0.001$) compared to control feedings. B) For Group B there was no significant differences among the three treatments ($p = 0.068$).

Figure 5. Jaw opening during the suck-swallow

On the left is the range of jaw opening movement in Group A between treatments (n=4). On the right is the range of jaw opening movement in Group B between treatments (n=4). There was significant more jaw opening in Group B pigs after palatal anesthesia (PLA, $p < 0.001$).

Figure 6. Time between mylohyoid and genioglossus muscle activity during oral phase of the swallow

On the left is the time difference between mylohyoid (MH) and genioglossus (GG) muscle activity in Group A between treatments (n=4). On the right is the time difference in Group B between treatments (n=4). There was significantly less time between MH and GG activity in Group B pigs after palatal anesthesia (PLA) compared to control and saline (PSA, $p < 0.001$). There was also less time between MH and GG activity in Group B pigs after PLA compared to Group A pigs after PLA ($p < 0.001$).

Figure 7. Time between mylohyoid and thyrohyoid muscle activity during the swallow

On the left is the time difference between mylohyoid (MH) and thyrohyoid (TH) muscle activity in Group A between treatments (n=4). On the right is time difference in Group B between treatments (n=4). There was less time between MH and TH in Group A after palatal anesthesia (PLA, $p=0.024$) and saline (PSA, $p<0.001$) compared to control. There was also less time between MH and TH activity in Group B after PLA compared to both control ($p<0.001$) and PSA ($p<0.001$). There was less time between MH and TH activity in Group B after PLA than Group A after PLA.

Figure 8. Range of hyoid movement during the pharyngeal swallow

On the left is the range of hyoid movement in Group A between treatments (n=4). On the right is the range of hyoid movement in Group B between treatments (n=4). In Group A there was significantly less movement after palatal anesthesia (PLA) compared to control (p<0.001) and saline (PSA, p=0.015). In Group B there was more movement after PLA compared to control (p<0.001) and PSA (p<0.001). There was also more hyoid movement after PSA relative to control (p=0.006). There was significantly less movement during control feedings in Group B compared to Group A (p<0.001). There was significantly more movement during PLA feedings in Group B compared to Group A (p<0.001).

Table 1

7-Point Infant Mammalian Penetration Aspiration Scale after Holman et al. 2013.

	Score	Description
normal	1	Material does not enter the airway
penetration	2	Material is in the supraglottic space, remains above the vocal folds, and passively leaves the airway before the epiglottis returns to rest position
	3	Material is in the supraglottic space, a small amount remains above the vocal folds after epiglottis in rest position
	4	Material is in the supraglottic space, a larger amount remains above the vocal folds after epiglottis in rest position
aspiration	5	New material is in the supraglottic space, then passes below the vocal folds, and is actively ejected, above the vocal folds
	6	New material is in the supraglottic space, then passes below the vocal folds and is not ejected from the trachea despite effort
	7	New material is in the supraglottic space, then passes below the vocal folds, and no effort is made to eject (silent aspiration)

Table 2

Sample sizes, means, standard deviations and ranges for the dependent variables evaluated.

		n	Mean ± SD	range
Time between oral muscle activity (MH to GG) (ms)	CON	60	67.9±27.2	17-153
	PLA	60	66.4±19.5	1-106
	PSA	60	64.3±20.0	17-124
Time between oral muscle activity (MH to GG) (ms)	CON	40	63.8±12.9	32-93
	PLA	40	-6.2±30.9	-47-65
	PSA	40	54.5±7.3	43-74
onset of pharyngeal muscle activity (MH to TH) (ms)	CON	80	125.9±44.2	56-282
	PLA	80	110.3±32.3	54-202
	PSA	80	94.4±29.8	44-194
onset of pharyngeal muscle activity (MH to TH) (ms)	CON	80	113.1±30.7	30-166
	PLA	80	64.1±55.7	6-376
	PSA	80	101.2±30.1	53-204
time between pharyngeal muscle activity (TH to CT) (ms)	CON	80	41.4±15.9	8-108
	PLA	80	43.4±18.6	10-108
	PSA	80	43.2±17.2	9-66
time between pharyngeal muscle activity (TH to CT) (ms)	CON	80	26.5±12.4	6-59
	PLA	80	30.9±13.3	0-55
	PSA	80	28.6±7.5	10-46
Range of hyoid motion (mm)	CON	77	7.4±1.7	4.8-11.0
	PLA	80	6.6±1.6	2.8-9.5
	PSA	80	7.3±2.5	2.9-13.6
Range of hyoid motion (mm)	CON	80	6.5±1.6	3.6-9.6
	PLA	60	8.5±1.5	5.5-12.3
	PSA	80	7.1±1.8	2.7-10.0
Jaw opening distance (mm)	CON	57	9.0±1.5	6.2-12.1
	PLA	60	8.3±1.9	4.2-11.8
	PSA	40	9.5±2.2	5.0-11.7
Jaw opening distance (mm)	CON	80	5.6±1.8	2.6-11.0
	PLA	58	14.5±7.9	3.1-31.7
	PSA	79	6.8±1.8	3.5-12.0
Tongue x-axis distance (A/P) (mm)	CON	79	7.1±2.0	1.0-10.5
	PLA	61	8.1±1.8	1.8-11.4
	PSA	34	7.7±1.4	2.6-10.0
Tongue x-axis distance (A/P) (mm)	CON	67	9.0±4.0	0.1-19.9

		n	Mean ± SD	range
	PLA	59	12.3±6.3	1.4-27.3
	PSA	74	8.9±3.6	3.2-17.7
Tongue y-axis distance (D/V) (mm)	CON	79	7.1±2.3	0.3-12.7
	PLA	61	7.9±2.0	1.9-12.1
	PSA	34	7.8±1.7	1.7-10.6
Tongue y-axis distance (D/V) (mm)	CON	67	8.7±4.7	2.4-28.3
	PLA	59	15.5±6.9	0.9-31.9
	PSA	74	6.8±2.3	1.7-15.7
Hyoid x-axis (A/P) (mm)	CON	79	5.7±2.3	0.4-10.3
	PLA	61	7.0±1.8	2.8-12.4
	PSA	34	5.8±1.8	2.0-8.3
Hyoid x-axis (A/P) (mm)	CON	67	6.6±3.2	0.9-16.2
	PLA	59	13.7±8.9	1.6-48.5
	PSA	74	7.5±2.4	2.6-14.9
Hyoid y-axis (D/V) (mm)	CON	79	5.6±2.2	0.6-12.9
	PLA	61	5.4±2.9	1.5-14.6
	PSA	34	4.4±2.2	1.2-8.9
Hyoid y-axis (D/V) (mm)	CON	67	5.8±3.9	0.3-26.0
	PLA	59	13.9±8.8	2.0-34.5
	PSA	74	6.0±3.0	0.7-20.9

The grey represents Group A and the white represents Group B. While we aimed to have a n of 80 for each group, sample sizes were lower if that marker was not in the frame during a swallow or if the EMG signal was not clear enough to label. The menton was not in view for one pig for all treatments. The GG signal was either not clear or not available for 3 pigs.