NOTE

Biochemistry

An efficient method for saliva collection from mature pigs to determine their enzymatic and electrolytic profiles

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ABSTRACT. In this study, a novel 'rope-assisted swab method' for the collection of saliva samples from 45 adult pigs was established and validated. This method was efficient for harvesting 2 milliliters or more of saliva from each of the pigs for subsequent analyses within two min. The amount of α-amylase and lipase in the collected saliva samples was between 38–6,655 and 2–52 U/l, respectively. For HCO_3^- and electrolytes, the range was between 9–40, 15–76, 4.3–48.5 and 7–65 mM for HCO_3^-, Na^+, K^+ and Cl^-, respectively. No significant differences in the enzymatic and electrolytic profiles were observed among sows with a high average litter size (SHA), sows with a low average litter size (SLA) and non-pregnant gilts (NPG) in this study. Our work reveals the efficiency of this collection method for mature pigs, and enzymatic and electrolytic profiling of saliva, which may be a useful reference for multiple diagnostic applications.

KEY WORDS: α-amylase, electrolytes, lipase, saliva, saliva collection method

Saliva contains a variety of hormones, drugs, enzymes, microbes, antibodies, and their associated products [19], and is therefore a readily available biological specimen for clinical investigations. The three pairs of salivary glands (parotid, sub-mandibular, and sublingual) along with the minor salivary glands of the oral mucosa account for approximately 90% of the oral fluid produced [17]. Saliva is approximately 99% water, and electrolytes, proteins, enzymes, mucins, and nitrogen-based compounds constitute the last 1% [9]. It contains a diverse array of locally synthesized and systemic proteins that can act as biomarkers [11]. The characteristics of these salivary biomarkers make them potential candidates for prognostic and diagnostic use [4]. For examples, porcine salivary components such as haptoglobin and chromogranin A have been suggested to be potential biomarkers of physical stress, although no significant differences in K^+ and Ca^+ concentrations were observed under stress conditions [10]. The amount of Na^+, K^+, and Cl^- in saliva appears to be related with the lactate anaerobic threshold during incremental test on ergometric cycle [6]. Moreover, although not measured in the current study, the salivary metabolome confer a "snapshot" reading of gene function, enzyme kinetics, and variations in metabolic processes [7]. In the past decade, extensive research has been done on metabolomic study of the saliva as a disease diagnostic, stratification, and early detection tool [16].

Saliva collection from animals does not typically require complex skills, and can easily be collected at home, at a farm, or in remote locations. Therefore, using salivary biomarkers for developing point-of-care (POC) devices, rapid test kits, and on-site clinical diagnostics would be a convenient strategy [12].

Obtaining a considerable amount of saliva for analytical purposes from a large number of animals, especially adult pigs, in a reasonable time is still a challenge. Oral fluids are usually collected from groups of animals in a pen [25], but could also be collected from individual animals to meet the study design requirements. Over the past few decades, traditional methods of saliva collection from pigs using cotton ropes (rope-chew method) [18, 24], and medical absorbent cotton tied to a string (string-assisted cotton method) [13] have been used extensively for various biochemical assays. Cotton ropes hung in a pen or stall with a solid support and medical absorbent cotton tied to a string were used in the 'rope-chew method' and 'string-assisted cotton method', respectively. However, oral-fluid collection using these two traditional methods are less effective for adult pigs since they show least interest in chewing, probably due to their higher age [18].

In the present study, 'cable lock-assisted swab method' and 'rope-assisted swab method' in addition to traditional methods ('rope-chew method' and 'string-assisted absorbent cotton method') were tested for efficient harvesting of saliva samples from adult pigs to determine their enzymatic and electrolytic profiles. A total of 45 pigs (3 groups of 15 pigs each), were included in the study. The groups were sows with a high average litter size (SHA), sows with a low average litter size (SLA), and non-pregnant gilts (NPG). The study was to develop and validate an efficient saliva collection method from adult pigs and to test the hypothesis

that there might be significant differences in the enzymatic and electrolytic profiles of SHA and SLA. If yes, it was planned to use the obtained values to correlate with the saliva components of NPG to predict their reproductive potential. However, our hypothesis was rejected by the data obtained.

On-farm collection of saliva samples was performed at PEAK Swine Genetics Inc., Clive, Alberta, a user of the Canadian National Swine Improvement Program (CSIP) managed by the Canadian Centre for Swine Improvement Inc. (CCSI). The samples were collected from individually housed pigs in the morning (8:30−9:00 am) before being fed.

In the traditional 'rope-chew method', a rope of approximately 5−15 mm thick was hung in such a fashion that it reached the shoulders of the pigs so they could easily chew on for 15−45 min, and then the fluids were tried to wrung from the ropes into sterile plastic bags. However, in 'string-assisted absorbent cotton method', medical absorbent cotton was tied to a string and placed before pigs to chew for 2 min for collection of the saliva sample. In our pilot studies, these two methods were tested and both failed to obtain sufficient amount of saliva samples for analyzing enzymatic and electrolytic profiles due to the lack of interest of adult pigs.

To overcome the recovery issue of traditional saliva collection methods, 'cable lock-assisted swab method' and 'rope-assisted swab method' were tested using two different types of synthetic swabs, SalivaBio Oral Swabs (8 × 32 mm) and SalivaBio Children's Swabs (8 × 125 mm), along with their collection tubes (Salimetrics, State College, PA, U.S.A.) (Fig. 1a). In 'cable lock-assisted swab method', a swab tied to one end of a cable lock (Fig. 1b) was placed near the buccal cavity of an adult pig (Fig. 1c), and the pig was allowed to chew on it for 2 min to ensure adequate absorption of oral fluids. Thereafter, the swab was carefully taken out from the cable knot and placed into a collection tube for harvesting saliva sample after centrifugation (10,000 rpm, 5 min, 4°C).

We developed and tested another new and efficient 'rope-assisted swab method' to further improve the harvested volume of the saliva samples. In this approach, a swab was tied with the help of a nylon thread in the middle of an undyed cotton rope (ULINE, Milton, ON, Canada) (Fig. 1d). The swab-tied rope (80 cm × 3.5 mm) was clamped with the help of a clip across the buccal cavity and behind the ears (Fig. 1e). Caution was taken to ensure that the swab was placed in middle of the buccal cavity. After 2 min of chewing, the rope was carefully taken off. The swab was placed in a collection tube for the harvesting of saliva sample, and kept at −80°C for further biochemical analyses.

To validate the newly developed 'rope-assisted swab method', quantitative analyses of salivary α-amylase, lipase, HCO_3^-, and electrolytes (Na^+, K^+ and Cl^-) was completed on a colorimetric assay with the Roche cobas 6000 c501 (Roche Diagnostics, Indianapolis, IN, U.S.A.) system, using proprietary kits as per the manufacturer's instructions.

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Differences between the groups were determined using the Student's *t*-test and considered significant if $P<0.05$.

Using 'cable lock-assisted swab method' and 'rope-assisted swab method', the recovered volume of saliva was approximately 1 and 2 m*l*, respectively, with a chewing time of 2 min for both. These volumes were achieved using the SalivaBio Children's Swabs. When the SalivaBio Oral Swabs were used, only 50% of the volumes (0.5 and 1 m*l* for 'cable lock-assisted swab method' and 'rope-assisted swab method', respectively) were obtained. This is likely due to differences in the length of the two swabs used. Therefore, the 'rope-assisted swab method' using SalivaBio Children's Swabs was chosen over the 'cable lock-assisted swab method' for saliva collection and used in this study.

The concentration of α-amylase and lipase in the saliva samples greatly varied between different animal groups. The concentration range was found to be 38−6,655 U/*l* for α-amylase and 2–52 U/*l* for lipase (Table 1). A large variation in the concentration of HCO_3^- and electrolytes was also observed among groups, and the values ranged from 9–40, 15–76, 4.3–48.5 and 7–65 mM for HCO_3^-, Na^+, K^+ and Cl^-, respectively (Table 1). There was no significant difference in the α-amylase, lipase, HCO_3^-, and electrolyte concentrations among the animal groups ($P>0.05$).

The α-amylase and lipase from saliva and blood could both be used as differential markers and have been frequently used for the diagnosis of acute pancreatitis [14, 15], though it is limited to prognostic use [14]. In our experiment, the range of amylase activity is largely variable (38−6,655 U/*l*) due to individual differences. The pig's salivary α-amylase activity was in good agreement with the findings of Fuentes *et al.* [8] (265.9−6,486.0 U/*l*), and Huang *et al.* [10] (~4,000−5,000 U/*l*). Barera *et al.* [3] also reported a similar concentration range of amylase (75−1,698 U/*l*) in serum, which is proportionally associated with the severity of celiac disease, an autoimmune disorder of human.

To the authors' knowledge, porcine salivary lipase, HCO_3^-, and electrolyte concentrations have been explored to a limited extent. Tecles *et al.* [22] reported the total esterase (hydrolyses water-soluble short acyl chain esters) activity (75.25 ± 2.80−323.65 ± 6.30 U/*l*) in the porcine saliva, a similar enzyme to that of lipase which specifically hydrolyses water-insoluble long chain triacylglycerols. Huang *et al.* [10] also reported the concentration of K^+ (1.21−1.35 mM), and Ca^{+2} (0.34−0.70 mM), in the porcine saliva. A similar range of HCO_3^- concentration (1−60 mM) was reported in human saliva under normal physiological conditions [2]. Of note, the concentrations of Na^+, K^+ and Cl^- were 75.47 ± 3.57, 17.73 ± 3.70 and 18.83 ± 4.12 mM, respectively, in human saliva [21], which is a similar range as those observed in the pigs in our current study. Secretions of salivary glands are controlled by autonomic nervous system; sympathetic activates the secretion of protein-rich saliva, whereas parasympathetic activation improves the secretion of water and mucin [20]. The submandibular glands also produce mucins, however, the secretion of parotid glands is characterized by high salivary amylase concentrations without mucins [23]. On the contrary, the sublingual and minor salivary glands produce most of the lysozyme and high molecular weight mucin responsible for viscosity of the saliva [20]. Therefore, in addition to individual differences, large variations in α-amylase and lipase concentrations could also be due to the differences in total protein content of the saliva, which is closely related to the physical and physiological conditions of the animals.
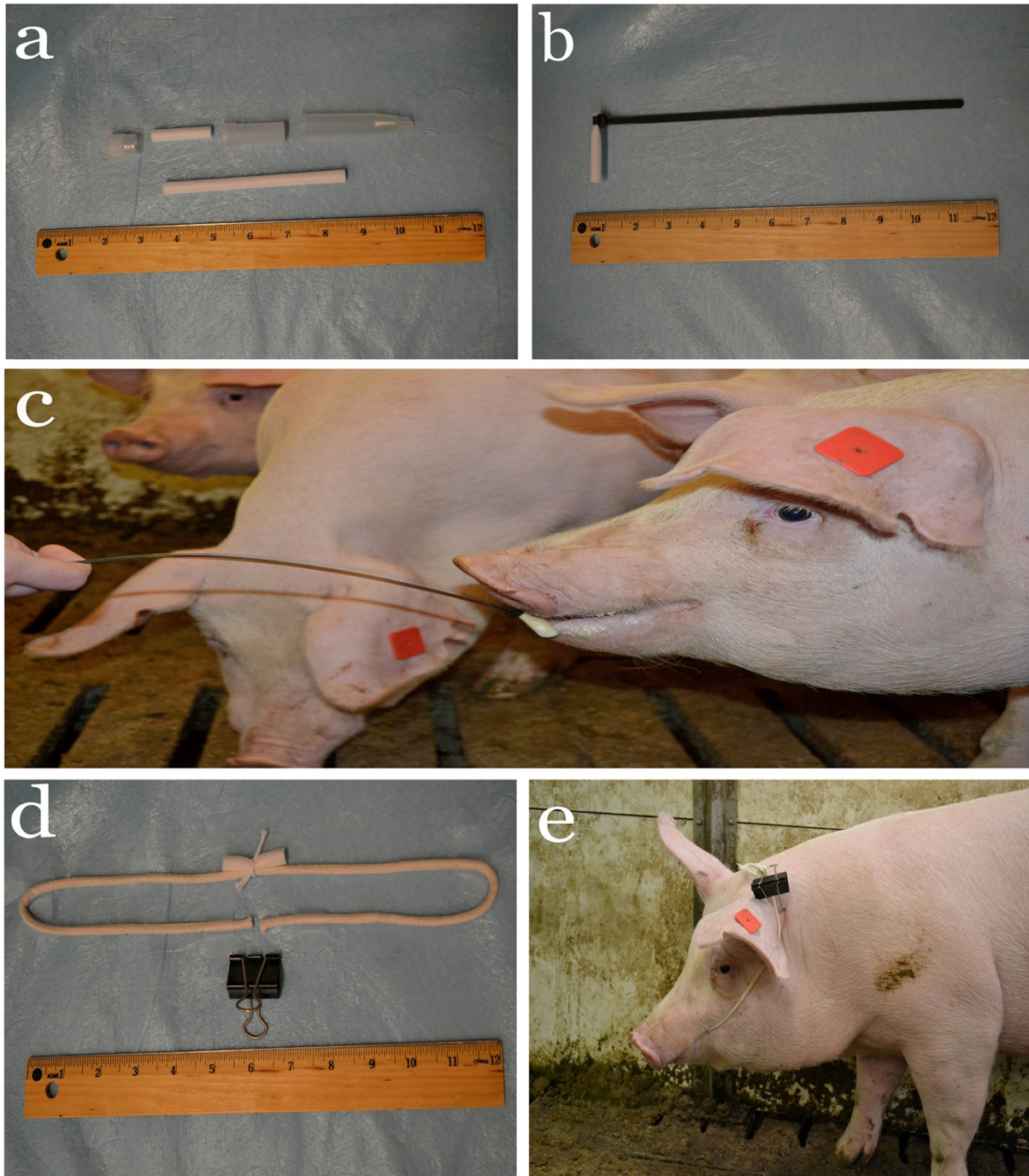
Fig. 1. Saliva collection from an adult pig using 'cable lock-assisted swab method' and 'rope-assisted swab method'. Swabs and saliva collection tube (a), swab tied to one end of a cable lock (b), cable lock- tied swab being served to a mature pig (c), a swab tied in middle of the cotton rope (d), and rope-tied swab wrapped firmly with the help of a clip across the buccal cavity and behind the ears (e).

Variation in the quantity and quality of the saliva may also be seen due to mastication, physiological stress, and physical activity [1, 5] in addition to few pathological conditions such as bleeding oral cavity, cystic fibrosis, multiple sclerosis, and epilepsy [1].

In summary, we developed and validated a novel 'rope-assisted swab method' in order to harvest a greater volume of saliva, which could be helpful when sampling large numbers of animals quickly. A high volume of saliva recovery may allow identification and utilization of multiple biomarkers for potential production traits and diagnostic purposes. This collection method may also be applicable for oral fluid collection of other species of livestock for similar purposes.

Table 1. α-amylase, lipase, HCO_3^-, and the electrolyte profile (Na^+, K^+ and Cl^-) of non-pregnant gilts (NPG), sows with a high average litter (SHA) and sows with a low average litter (SLA)

S. No.	α-amylase (U/l)	Lipase (U/l)	HCO_3^- (mM)	Na^+ (mM)	K^+ (mM)	Cl^- (μM)
NPG						
1	701	2	15	15	13.8	13
2	78	5	20	24	19.1	20
3	1,968	52	24	56	36.1	47
4	249	8	18	25	17.0	16
5	598	19	31	20	40.1	25
6	772	25	13	30	16.3	21
7	408	8	25	54	32.6	51
8	408	10	16	27	15.6	19
9	3,123	18	19	18	24.6	19
10	2,920	41	15	44	26.0	32
11	307	14	27	30	29.0	25
12	636	24	19	37	31.2	33
13	6,655	2	10	34	9.5	17
14	78	9	13	35	23.5	25
15	4,447	2	14	39	8.1	20
SHA						
16	210	13	14	32	28.7	22
17	675	5	20	46	20.6	37
18	151	9	17	30	25.5	20
19	870	7	13	33	25.9	21
20	1,428	16	22	26	21.5	19
21	528	4	21	53	22.1	42
22	621	7	22	35	26.4	24
23	104	7	24	25	32.0	25
24	100	9	24	25	32.3	26
25	189	7	25	36	31.6	30
26	1,475	50	26	38	45.1	31
27	1,675	28	24	36	38.1	26
28	745	15	31	36	39	33
29	737	14	23	52	46.1	48
30	1,634	13	29	55	36.6	49
SLA						
31	140	3	16	31	16.6	24
32	274	29	22	35	39.0	31
33	372	4	14	46	26.3	32
34	3,877	6	40	28	42.2	33
35	1,036	8	38	27	34.4	26
36	283	25	26	41	48.5	39
37	348	7	22	25	28.7	23
38	881	7	22	33	33.4	24
39	3,147	11	28	32	30.0	29
40	201	11	23	45	38.8	44
41	149	13	24	34	40.2	33
42	234	2	36	76	27.2	65
43	1,247	7	24	17	25.2	22
44	634	18	29	61	24.9	53
45	38	2	9	17	4.3	7

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