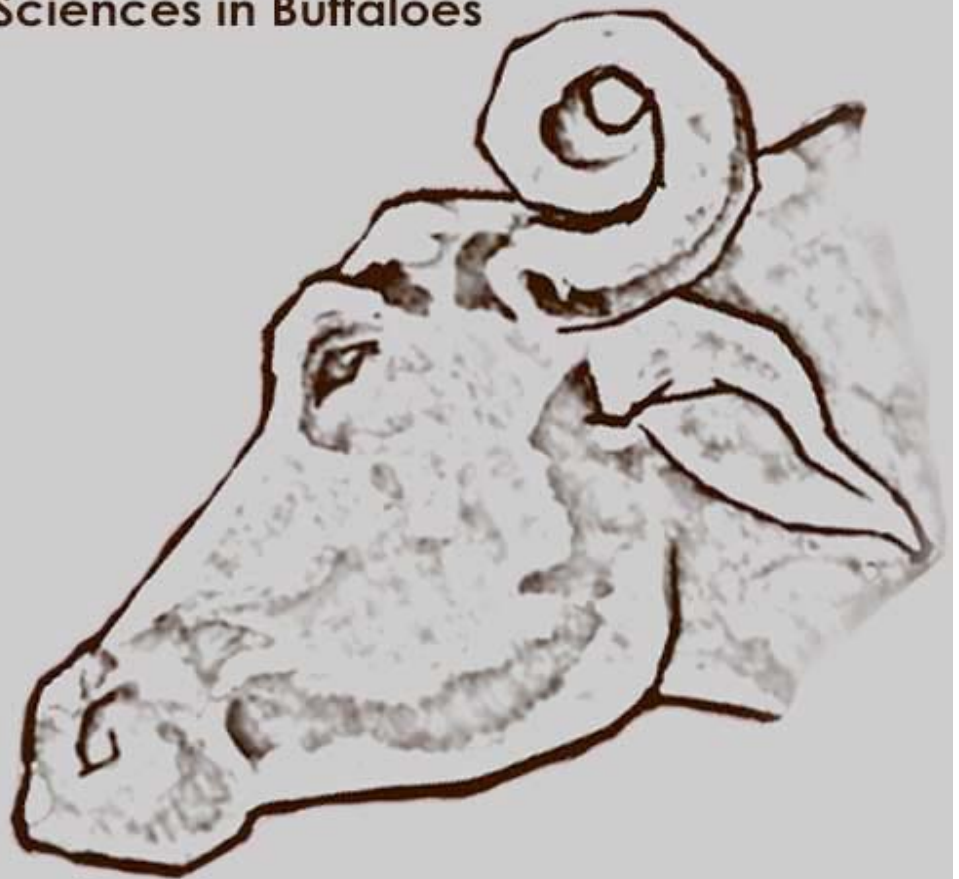


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Usage of Saliva as Alternative Biological Fluid to Serum for Minerals, Energetic and Hormones Assessment in Lactating Egyptian Water Buffaloes

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Abstract: Blood sample is the most common biological fluid utilized for diagnosis and monitoring of diseases. Saliva contains locally produced substances as well as serum component, so the aim of this study is to compare the profile of minerals, energetic and hormones in Egyptian water buffaloes. Blood serum and saliva samples were collected from 80 healthy multiparous, non-pregnant lactating Egyptian water buffaloes. Both fluids were tested for sodium, potassium, chloride, calcium, phosphorous, magnesium, insulin, cortisol, ACTH, glucose, urea, creatinine, total protein and immunoglobulin [IgA]. The results revealed that, serum concentrations of calcium, glucose, total protein, sodium, chloride, Insulin, cortisol, ACTH and IgA were significantly higher than saliva. In contrast, the concentrations of potassium and phosphorous in the saliva were significantly higher than that of serum. On the other hand no significant change in respect of urea, creatinine and magnesium was noted between saliva and serum. The relationships between saliva and serum of the estimated parameters were significantly positive except the concentrations of insulin in saliva and blood serum did not correlate. In conclusion, the saliva sample can be used in clinical practice with high level of reliability and provide non-invasive biological fluid for monitoring of different parameters in Egyptian water buffaloes.

Keywords: Saliva, serum, buffalo, minerals, energetic, hormones.

INTRODUCTION

Characteristically, ruminants secrete large volumes of saliva, which has high levels of sodium and high buffering capacity due to the presence of bicarbonate and phosphate in relatively large amounts. Buffering capacity consider the main function of ruminants saliva which has great importance to reduce drop in pH of the rumen that resulted from fermentation of relatively high grains rations [1].

Saliva shows a good correlation to blood for some analytics, including hormones, and it is collected from humans and from ruminant livestock easily [2-6]. However, there is a time lag between changes in the composition of the saliva and the changes that occur in the blood, and partitioning between the two body fluid compartments is often not simple, making correlation difficult and at times not predictable. Although saliva samples are relatively low in needed compliance, they are still not easy to collect in mid- to large-size animals without considerable restraint [7].

Serum cortisol concentrations have been used as physiological marker of stress in domestic animals [8]. However, interest in non-invasive, easier and quicker quantification method for stress markers has to investigate in saliva as an alternative physiological

medium for cortisol measurement [9] and salivary cortisol measurement is used as a practical surrogate for serum free cortisol [10].

Corticosteroid concentrations can be assayed in saliva, and measures of salivary cortisol concentrations have been used to assess stress reactions in cattle [10-12]. Several authors have demonstrated that corticosteroid concentrations in saliva are directly related to those in plasma in humans, dogs, pigs, and domestic ruminants [7, 13-17]. Others, however, have observed limitations in the extent to which salivary and plasma concentrations are related [18]. Furthermore, little information is available on the relationship between concentrations of corticosteroids in saliva and plasma of buffaloes.

The major cations in saliva are sodium [Na⁺] and potassium [K⁺] while the main anions are bicarbonate, phosphate and chloride [19]. The total phosphate concentration of saliva in most species is about twice that of plasma [20].

Recycling of phosphorous through saliva is more in ruminants than non ruminants and is stimulated by parathyroid hormone [22].

Several investigators [23-25] have shown that the saliva calcium concentration may be increased in man and dog by raising the calcium level of the blood plasma above normal. However, in clinical conditions,

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such as parturient paresis, salivary calcium was significantly decreased in ewes [26]. A significant amount of magnesium leaves the extracellular fluids to be incorporated into saliva each day. Most but not all of the magnesium in the saliva will be absorbed by the digestive tract and can reenter the extracellular pool [27].

Mucosal IgA detection in oesophago-pharyngeal fluids had potential as an indicator of infection by foot and mouth disease virus in cattle [28-30]. Salivary total protein is a vital component of saliva with salivary protein; predominately comprising proline-rich protein, mucin, amylase, immunoglobulins, satherin and antibacterial factors, and these are responsible for most of functions of saliva [31].

Blood sample is the most common biological fluid utilized for diagnosis and monitoring of diseases. However saliva is frequently studied for diagnostic purpose [32]. The objective of the present study was to make a comparison between saliva and serum concentrations of electrolytes [sodium, potassium and chloride], some hormones [cortisol, insulin and ACTH], glucose, urea, Creatinine, total protein and immunoglobulin [IgA] and some minerals [calcium, phosphorous and magnesium] and their relationships in lactating Egyptian water buffaloes.

MATERIAL AND METHODS

Eighty healthy multiparous [3-5 parturitions] non-pregnant lactating Egyptian water buffaloes [age: 6-9 years, body weight 479.6 ± 5.8 kg, daily milk production: 5.4 ± 0.2 kg] were used. Physical examination was performed immediately before sampling. Blood samples were collected *via* jugular venipuncture into 10-mL vacuum tubes and they were kept at room temperature for 30 to 60 min and were centrifuged [$1,500 \times g$ at 4°C for 15 min]. After centrifugation, serum was stored in plastic vials at -80°C until assayed. For each blood sample, a simultaneous salivary sample was collected *via* oral swab using a 4×8 cm cotton strip held in surgical forceps, which resulted in the collection of approximately 4 to 5 mL of salivary volume. Oral swabs lasting approximately 2.5 min in the mouth of the animals to be adequate to saturate the cotton strip.

Salivary samples were placed in salivette tubes [Sarstedt AG and Co., Numbrecht, Germany] and cooled in ice immediately after collection, then centrifuged [$1,500 \times g$ at 4°C for 15 min] and stored at

-80°C until assayed. Glucose, urea, creatinine, calcium, magnesium, phosphorus, sodium and potassium, chloride, total protein and immunoglobulin [IgA] were measured using commercially available kits in both serum and saliva samples, while insulin, cortisol and ACTH were measured by solid phase radioimmunoassay (RIA) using components of a commercial kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with modifications described by Kiyama *et al.* [45].

Statistical Analysis

The SAS software package [SAS Inst., Inc., Cary, NC] was used for analysis of data by means of Student's t-tests. Significance was set at $P < 0.05$, and all values were presented as the mean \pm standard error of the mean [SEM]. The relationships between the estimated parameters measured in blood serum and saliva samples were evaluated by Pearson correlation coefficients.

RESULTS

Concentrations of calcium, glucose, total protein, sodium, chloride, insulin, cortisol, ACTH and IgA in the serum were significantly [$P < 0.05$] higher than that of the saliva, while concentrations of potassium and phosphorous in the saliva were significantly [$P < 0.05$] higher than that of serum while, concentrations of urea, creatinine and magnesium were no significantly changed between saliva and serum as shown in Table 1.

The relationships between saliva and serum of the estimated parameters were significantly positive [r from 0.46 to 0.90 and $P < 0.05$] except the values of insulin in saliva and blood serum did not correlate as shown in Table 1. The total phosphorous concentration of saliva is about twice that of serum concentration while that of potassium in the saliva is about 6 folds of that in serum. The concentrations of ACTH, insulin, creatinine, total protein, IgA, glucose, chloride and cortisol in the serum are about 18,1.6,1.5,2,15, 1.7,2 and 4 folds of that in saliva respectively, while the ratio between saliva and serum in the urea, calcium,sodium and magnesium was $\sim 1 : 1$ as shown in the Table 1.

DISCUSSION

Blood sample is the most common biological fluid utilized for diagnosis and monitoring of diseases. However, whole saliva is frequently studied as an alternative for blood that can be useful even for

Table 1: Concentrations of Some Hormones, Electrolytes, Minerals, IgA, Glucose, Total Protein, Urea and Creatinine in Saliva and Serum of Egyptian Water Buffaloes with their Relationships and Ratio. * P < 0.05, ** P < 0.01, * P < 0.001, NS [Non-Significant]**

Parameters	Saliva	Serum	Relationships		Ratio
			r	P -value	Saliva : Serum
Cortisol [$\mu\text{g/dl}$]	0.4 \pm 0.03	1.6 \pm 0.2**	0.61	< 0.05	1 : 4
ACTH [pg/ml]	1.5 \pm 0.2	27.9 \pm 1.9***	0.9	< 0.01	1 : 18
Insulin [μ IU/ml]	0.3 \pm 0.02	0.5 \pm 0.04**	0.17	NS	1 : 1.6
Urea [mg/dl]	38.8 \pm 3.2	37.6 \pm 3.3 NS	0.57	<0.05	1 : 1
Creatinine [mg/dl]	0.60 \pm 0.1	0.90 \pm 0.2 NS	0.71	<0.05	1 : 1.5
Total protein [g/l]	1.8 \pm 0.4	3.7 \pm 0.3**	0.54	<0.05	1 : 2
IgA [mg/dl]	5.5 \pm 0.2	85.2 \pm 7**	0.89	<0.01	1 : 15
Glucose [mg/dl]	47.3 \pm 8.3	80.4 \pm 8.2**	0.55	<0.05	1 : 1.7
Sodium [mmol/l]	151.3 \pm 1.1	165.7 \pm 0.6*	0.58	< 0.05	~1 : 1
Potassium [mmol/l]	18.5 \pm 2.7	2.9 \pm 0.2**	0.82	<0.01	6 : 1
Chloride [mmol/l]	37.5 \pm 0.9	71.3 \pm 1.2**	0.68	< 0.01	~ 1 : 2
Calcium [mg/dl]	9.2 \pm 0.2	11.8 \pm 0.6*	0.63	<0.05	~1 : 1
Phosphorous [mg/dl]	14.9 \pm 2.6	6.8 \pm 1.1***	0.46	<0.05	2 : 1
Magnesium [mg/dl]	2.3 \pm 0.02	2.4 \pm 0.04 NS	0.67	<0.05	~1 : 1

diagnostic purposes [32, 33]. Saliva equilibrates reasonably well with many blood components, but the partitioning between the two body fluids is complex and still poorly understood [2-4]. Saliva can provide diagnostic values, particularly for compounds that are relatively stable in the blood over a reasonable period, or while the animals are at rest and concentrations are within a narrow range [7].

Significant increase in the concentration of glucose and cortisol in blood serum than that of saliva and the positive correlation between them is agreed with [7]. Significant positive correlation between salivary and serum glucose was reported previously [34-36]. Ruminants secrete a large amount of saliva into the rumen, more than 100 liters per day. The phosphorous concentration in cattle saliva is 37-72 mg/dl. These levels are considerably higher than that of bovine blood plasma, which is about 4-8 mg/dl [22] and this result is agreed with the result of the present study in Egyptian water buffaloes. Therefore, the salivary glands have an important part to play in the regulation and homeostasis of phosphorous [21, 22].

Several investigators [23-25] have shown that the saliva calcium concentration may be increased in man and dog by raising the calcium level of the blood plasma. A significant positive correlation between plasma and parotid saliva calcium and plasma calcium

concentration was significantly higher than that of saliva in sheep [26] and this result is agreed with the result of this study.

A significant amount of magnesium leaves the extracellular fluids to be incorporated into saliva each day in goats [27, 42]. Dua and Care [37] postulated that the total magnesium concentration in the mixed saliva of sheep varies between 0.20-0.30 mmol/l. The total amount of saliva secreted by sheep varies between 10-15 liter/day. Thus about 3-4.5mmol/l [about 40% of the total amount of magnesium available in the extracellular fluid] was secreted in the saliva each day. Normally the absorption rate of magnesium was about 20%. So when the animals were in tetany and the absorption of magnesium was grossly impaired and losing these much amount of magnesium through the saliva makes the animals more susceptible to hypomagnesaemia. This was the major reason for which ruminant being more susceptible to hypomagnesaemia than monogastric animals.

Lewis [38] observed urea-nitrogen concentrations from 15-36 mg/dl in orally collected saliva and this result is agreed with the finding of this study and urea also enters the rumen in saliva and the mechanism of urea transfer from blood to saliva is apparently passive diffusion, as the saliva urea concentration is proportional to BUN. Edward *et al.* [46] demonstrated

that BUN and salivary urea concentrations were highly correlated ($r = 0.96$). In this study the salivary creatinine concentrations were about 66% of serum concentrations and the concentrations were related, while salivary creatinine concentrations were 10-15% of serum creatinine concentrations in healthy populations and the concentrations were not related in healthy populations, however, a significant relationship was found in the patients ($r = 0.784$, $P < 0.001$) [47].

Characteristically, ruminants secrete large volumes of saliva, which has high levels of sodium and high buffering capacity due to the presence of bicarbonate and phosphate in relatively large amounts [1] and similarly the present study also revealed that saliva has high concentration of sodium in Egyptian water buffaloes. Relative to other nonruminant mammals, the volume of digestive secretions in ruminants is large [43]. This is mainly due to high level of continuous alkaline salivary secretion, which serves to buffer the acid products of microbial fermentation in the rumen provides a medium that facilitates the mixing and regurgitation of ingested materials. The daily volume of salivary secretion in cattle may amount to as much as 100-190 l/day and the amount of Na^+ secreted with the saliva may represent > 15 times the daily amount of Na^+ consumed with feed and > 5 times the amount in all the blood plasma [43, 44]. Potassium concentrations of saliva was significantly higher than that of serum while chloride and sodium concentrations of serum were significantly higher than that of saliva and the findings are in agreement with earlier reports [19, 39].

A significant positive correlation between salivary and serum IgA may either reflect the activity of IgA plasma cells underlying the mucosal epithelium or alternatively may occur as a result of transport of IgA molecules from the blood circulation [40]. The cortisol concentration in saliva is a direct reflection of the free fraction in the blood [13]. Furthermore, under stress conditions, the binding capacity of cortisol-binding protein becomes saturated, resulting in a disproportionate increase in free cortisol, the fraction that is biologically active [4]. These facts partly explain the differences between concentrations of plasma and salivary cortisol.

Cortisol concentration was higher in serum than in saliva, and a significant positive correlation between salivary and plasma concentrations was in agreement with the earlier reports in sheep in response to introduction of stress [12, 14]. Yates *et al.* [9] also

found a similar relationship between blood serum cortisol and salivary cortisol concentrations in sheep. However, it has been demonstrated that salivary cortisol levels have a steady and predictable relation to the free, unbound cortisol levels in serum and salivary levels accurately reflect serum levels regardless of the degree of stimulation of the salivary glands [39]. Previous studies have found correlation coefficients between cortisol in saliva and cortisol in serum ranging from $r = 0.71$ to $r = 0.96$ [48]. Little information is available on the relationship between concentrations insulin, ACTH and total protein in saliva and serum of buffaloes and needed further investigations.

According to the results of this work and the significant relationships between salivary and serum concentrations of the estimated parameters, it is possible to use the saliva sample in clinical practice as alternative fluid to serum and provide non-invasive biological fluid for monitoring of different parameters in Egyptian water buffaloes.

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