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RELIABILITY AND ACCURACY OF DETERMINING MINERALS AND ELECTROLYTES IN GOAT URINE USING A DRIED FILTER PAPER METHOD

P. J. D. C. Bagasse

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RELIABILITY AND ACCURACY OF DETERMINING MINERALS AND ELECTROLYTES IN GOAT URINE USING A DRIED FILTER PAPER METHOD

by

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Submitted in partial fulfilment of the requirements for the degree MSc (Master of Science) in the Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria

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SUMMARY

The lack of facilities for veterinary services, such as analytical laboratories, which are distant from the field and at immense distances are aggravatted by the constraint of transporting and preserving the samples. A method where a certain amount of the urine sample is absorbed onto filter paper, but dried in the field and then sent to the laboratory (Dried Filter Paper Method, DFPM), has been used in human medicine, but never applied in the veterinary field. The practice of expressing various urinary minerals/electrolytes relative to the concentration of creatinine has recently become generally accepted. A single sample obviates the need for collection of urine over a 24-hour period. Fractional excretion (FE) of minerals and electrolytes (which is the mineral or electrolyte to creatinine ratio), is a simple, inexpensive measurement and a reasonable indicator of the renal clearance of minerals/electrolytes, using a single urine sample.

The investigation was divided into two phases, The first was conducted entirely *in vitro*, using simulated urine (artificial and goat urine) dried on filter paper, manipulated in various ways under laboratory conditions, and the second phase was conducted *in vivo* and *in vitro*, using urine (collected from water-deprived goats) dried on filter paper and manipulated in various ways under laboratory conditions.

Determination of mineral/electrolyte to creatinine ratios using the dried filter paper method consisted of impregnating a specific filter paper with a specific volume of artificial/goat urine, diluted in a specific eluent, and then analysed for the analytes (creatinine, phosphate, chloride, magnesium, calcium, sodium, and potassium).

Due to the wide range of filter papers, which could have been used for the trial, it was necessary to run an experiment to identify the best for the purpose. The experiment used filter papers from 2 different brands (Whatman® and Scheicher & Schuel filter papers). The following filter paper were compared: Whatman® no 2, 3, 5, 6, 42, 43, 44, and 542 and 860, 593, 595, 597, 598 for Schleicher & Schuell (very high quality). There were few

significant differences. Whatman filter paper number 6, was chosen, because of the constant and uniform mineral/electrolyte to creatinine ratios.

The very high variability of urine mineral/electrolyte concentrations in ruminants, and the limited linearity range of routine analytical equipment, constrains the routine use of laboratory analysers in urine mineral and electrolyte assessment. One of the approaches is to use a diluent, with a particular mineral/electrolyte concentration near to the lower end of the linear/standardised range. Therefore, "mixing" a small volume of urine with the diluent will result in a final mineral/electrolyte concentration, that falls into a linear and controlled/standard range for the particular mineral and electrolyte. A diluent was tested and the results for analytes show a high interclass correlation (R_I >0.75) between the expected and the calculated values of this ratio.

The stability of mineral/electrolyte to creatinine ratio (E/Cr) in artificial and goat urine specimens using the dried filter paper method stored at 2 different temperatures during ten days was also evaluated experimently. While it has been found that P/Cr ratio, Mg/Cr ratio, K/Cr ratio are stable for the 10 days, the Cl/Cr ratio, Ca/Cr ratio, and Na/Cr were found to be less stable during the same period of time. The average results do not differ significantly from the control in either the artificial urine or the goat urine.

Experiments were conducted to evaluate the reproducibility of artificial urine and artificial urine diluted 1:5 to simulate reproducibility of mineral/electrolyte to creatinine ratios with higher and lower concentrations, respectively. The results indicate a relatively good reproducibility of the method, because the variation, as measured by standard deviation, is small relative to the mean, except for Cl/Cr ratio and Ca/Cr ratio, where the results presented showed a relatively low reproducibility.

In theory, since ratios can be obtained, and should be unchanged by taking measurements at different dilutions even if the amount of specimen is unknown, there should be no need to absorb a fixed amount of urine onto the filter paper when urine is collected, using this method. However, there appear to be limits to this in reality, dilution of urine below a 1:10

dilution and/or the volume impregnated onto the filter paper below 0.525 ml on Whatman® number 6 filter paper.

An experiment with goats on the relationship between the influence of water deprivation on mineral/electrolyte to creatinine ratio over a period of time demonstrated that concentrations and excretion of electrolytes vary from animal to animal, but the mineral/electrolyte to creatinine ratios by DFPM hardly vary, even if the goat is deprived of water.

Using goat urine to determe mineral/electrolyte to creatinine ratio with the dried filter paper method gives high interclass correlation for mineral/electrolyte to creatinine ratio between the control (fresh urine sample, preserved in freezer) and the dried filter paper method on goats given water *ad libitum*. Interclass correlation agreement for the two methods was R_I >0.75.

On the basis of the results, the method is robust for use as a urine sample preservation and transportation method for the determination of mineral/electrolyte to creatinine ratio with an added advantage of not needing either preservative or refrigeration.

SAMEVATTING

Die tekort aan fasiliteite vir veeartsenykundige dienste, soos analitiese laboratoria, wat dikwels groot afstande van die boerderye geleë is, word vererger deur die beperkings wat deur die vervoer en bewaring van monsters teweeg gebring word. 'n Metode waar 'n hoeveelheid uriene monster op filtreerpapier geplaas en gedroog word en dan na die laboratorium gestuur word vir die ontleding van sekere stowwe (Droë Filtreer Papier Metode, DFPM), is al in menslike geneeskunde gebruik maar nog nooit in die veeartsenykundige bedryf nie.

Die gebruik waar die urinêre mineraal of elektroliet konsentrasie in verhouding tot die konsentrasie van urinêre kreatinien uitgedruk word, word nou nogal algemeen. Dit laat die gebruik van 'n enkele uriene monster toe wat baie voordelig is in vergelyking met die versameling van 'n 24-uur monster. Fraksionele uitskeiding (FE) van mineraale en elektroliete, synde die mineraal/elektroliet tot kreatinien verhouding, is 'n eenvoudige, ekonomiese meetinstrument asook 'n redelike indikator van die renale klaring van minerale en elektroliete, met die gebruik van 'n enkele uriene monster.

Hierdie ondersoek is in twee fases uitgevoer. Eerstens, 'n uitsluitelike *in vitro* fase met die gebruik van gesimuleerde, (kunsmatige) uriene, op filtreerpapier gedroog en dan op verskye wyses, onder laboratorium omstandighede, gemanipuleer. Die tweede fase was beide *in vitro* sowel as *in vivo* en het die versamel van uriene van bokke wat aan verskye vlakke van waterweerhouding onderwerp was, en die verdere manipulasie daarvan in die laboratorium behels.

Die bepaling van urinêre mineraal/elektroliet tot kreatinien verhoudings met die gebruik van die gedroogde filtreerpapiermetode behels die toediening van 'n sekere volume uriene (kunsmatig en bok) op 'n gespesifiseerde tipe filtreerpapier, uitweek met 'n spesifieke oplosmiddel en dan die bepaling van mineral, elektroliet (fosfaat, chloride, magnesium, kalsium, kalium en natrium) en kreatinien konsentrasies.

As gevolg van die wye verskeidenheid van filtreerpapier tipes wat vir hierdie doel gebruik kon word, was dit nodig om 14 tipes van twee vervaardigers te vergelyk. Daar was min betekenisvolle verskille maar op grond van die laagste variasie van mineraal/elektroliet tot kreatinien verhouding, was Whatman #6 uitgeken vir verdere gebruik in die ondersoek.

Die groot verskeidenheid in urien konsentrasie van mineraale en elektroliete by herkouers asook die beperkte lineariteitsbestek van analiseerders in gebruik in roetine kliniese laboratoria, beperk die gebruik van die toerusting in die bepaling van die stowwe in urien. Een van die benaderings om die probleem die hoof te bied is om 'n "verdunningsvloestof" met voorafbepaalde mineraal/elektroliet konsentrasies, naby die onderste afsnypunt van die metode se lineariteits/standardisering bestek, te gebruik. Meng van 'n klein volume urien monster met hierdie "verdunningsvloeistof" veroorsaak dan dat die finale mengsel se konsentrasie dan wel binne die instrument/metode se lineêre en gestandardiseerde bestek val. Hierdie ondersoek rapporteer dan die suksesvolle evaluasie van so 'n "verdunningsvloeistof" op die vermoe om mineraal/elektroliet tot kreatinien verhoudings herhaalbaar oor 'n wye konsentrasiespektrum te lees. Die metode het 'n hoë, gemiddelde intraklas korrelasie ($R_1 > 0,75$) tussen die verwagte en berekende waardes getoon.

Die stabiliteit van die DFPM vir mineraal/elektroliet tot kreatinien verhouding, in kunsmatige sowel as natuurlike bok urien monsters wat teen twee verskillende temperature (22 en 30 grade Celcius) vir tot tien dae gehou is, is bepaal. Dit is gevind dat Fosfaat, Magnesium en Kalium tot Kreatinien verhoudings die grootste stabiliteit getoon het. Daar was egter geen betekenisvolle verskille tussen die DFPM en kontrole, nog in kunsmatige, nog in bok uriene monsters, nie.

Die herhaalbaarheid van die DFPM metode op kunsmatige urien en kunsmatige urien wat 1:5 verdun is, (om herhaalbaarheid teen hoë en lae konsentrasies te vergelyk) is bepaal. Die resultate toon dat, algemeengesproke, is die herhaalbaarheid goed. Die KV van Cl/Cr en Ca/Cr was egter nie so goed as die ander nie.

In teorie, aangesien verhoudings bereken kan word ongeag die verdunning en selfs sonder kennis van die volume, behoort daar geen rede te wees om 'n bepaalde volume urien op die filtreerpapier te sit, met die gebruik van hierdie metode, nie. Daar blyk egter beperkings te wees in die werklikheid. Verdunning van die urien onder 1:10 en/of toediening van 'n volume kleiner as 0,525ml op die Whatman #6 filtreerpapier, veroorsaak afwykings vanaf die verwagte resultate.

Die gebruik van bok urien om die mineraal/elektroliet tot kreatinien verhouding met die DFPM te bepaal gee hoë intraklas korrelasie (>0,75) tussen die kontrole (vars uriene wat in 'n vrieskas bewaar is) en verhoudings wat met die DFPM op bokke, op *ad libitum* water, bepaal is. 'n Eksperiment op bokke op die verhouding tussen die invloed van water weerhouding op die mineral/elektroliet tot kreatinien verhouding oor 'n periode van tyd, het gedemonstreer dat die konsentrasies en uitskeiding van minerale en elektroliete in uriene baie varieer. Die mineraal/elektroliet tot kreatinien verhouding, egter, bepaal met die DFPM toon baie min variasie, al is die diere ontwater.

Op die basis van hierdie resultate kan daar tot die gevolgtrekking gekom word dat die metode "robust" en betroubaar is vir die bewaring en vervoer van urien monsters vir die bepaling van mineraal/elektroliet tot kreatinien verhoudings met die bykomende voordeel dat daar geen bewaringsmiddel of verkoeling nodig is nie.

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GLOSSARY OF ABBREVIATIONS

1.	A.U	=	Artificial Urine
2.	DDDW	=	Double Distilled De-ionised Water
3.	$MgCl_26(H_2O)$	=	Magnesium chloride hexahydrate
4.	$CaCl_2$	=	Calcium Chloride
5.	KCl	=	Potassium Chloride
6.	NaCl	=	Sodium Chloride
7.	$NH_4H_2PO_4$	=	Ammonium Phosphate
8.	$NaHCO_3$	=	Sodium Bicarbonate
9.	$C_3H_2N_3O$	=	Creatinine
10.	Mg	=	Magnesium
11.	Ca	=	Calcium
12.	PO_4	=	Phosphate
13.	Na	=	Sodium
14.	Cl	=	Chloride
15.	K	=	Potassium
16.	Cr.	=	Creatinine
17.	Conc.	=	Concentration
18.	Elect.	=	Electrolyte
19.	D. Rate	=	Dilution rate
20.	mmol/l	=	Millimoles per litre
21.	g/l	=	Grams per litre
22.	M.M.	=	Molecular Mass
23.	C l	=	Concentration of a Substance
24.	E.C.	=	Electrolyte concentration in the diluent
25.	OVAH	=	Onderstepoort Veterinary Academic Hospital
26.	ISE	=	Ion Selective Electrode
27.	FCx	=	Ratio of clearance of substance X
28.	GFR	=	Glomerular Filtration Rate
29.	Fex	=	Fractional Excretion of substance X

30. Ux	=	Concentration of substance X in Urine
31. Ucr.	=	Concentration of Creatinine in urine
32. Ecf	=	Extracelullar Fluid
33. SID	=	Strong Ion Difference
34. A.A.	=	Atomic Absorption
35. DFPM	=	Dry Filter Paper Method
36. Exp.	=	Expected Value
37. Calc.	=	Calculated Value by referring formulae
38. Avg.	=	Average
39. Std. D	=	Standard Deviation
40. Stad E.	=	Standard Error
41. C.C.	=	Coefficient Correlation
42. Interc.	=	Intercept

1. BACKGROUND

Transporting liquid samples (blood, saliva, and urine) over long distances from rural areas has been a major disincentive to the provision of laboratory health services to these areas by urban-based institutions.

Mozambique is a country of 778,895 km^2 in extent and 2500 km of coast line. It has a mean temperature of 25^{0}C (maximum of 34^{0}C and minimum of 16^{0}C) and humidity around 70%-80% (FAO, 1984). Mozambique is an developing third world country, which thus means there is a distinct lack of financial resources. This implies a lack of facilities for veterinary services, such as analytical laboratories, which are also distant from the field. One must consider the immense distance, and thus the constraints in transporting and preserving samples, where the laboratories are as far as 100 km from the field. Mozambique is not unique as far as third world countries go regarding such problems.

Furthermore 85% of the livestock belongs to smallholders, which is the poorest community layer in the country. In addition, they do not possess the pasture management knowledge that is necessary nor the adequate knowledge of livestock nutrition related to the high utilisation of natural pasture, low soil nutrient and poor management. The mineral deficiency evidenced in animals has become a problem, and could be a reason for the low growth rate.

An approach used in man by some authors (Elnagar *et al,* 1997; Portal *et al, 1971;* Takemori, 1980), suggests that using filter paper to transport liquid samples may be beneficial. Liquid samples are dried on filter paper, which is then sent by post, reconstituted with a nominal volume of water in the receiving laboratory, and analysed to provide an estimate of the same constituent present in the original fluid sample. This result is then related to a "standard" constituent like creatinine. The technique may be especially useful, economical and practical for processing large screening studies, such as surveys of animal populations for nutritional studies. Another advantage of this technique is that the sample can be kept for long time.

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2. LITERATURE REVIEW

2.1. INTRODUCTION

Metabolic monitoring of livestock was initiated in United Kingdom laboratories during the 1970's (Payne *et al,* 1970). The system/method never quite achieved its primary goal of serving as an early warning system for nutritional problems. It may be assumed that this was caused by the fact that blood (a homeostatically extremely well controlled environment) was used exclusively for sampling.

Electrolyte concentrations in plasma are kept in homeostatic equilibrium (Lackey *et al,* 1995). Blood concentrations of electrolytes tend to remain within normal limits until the onset of clinical signs, making early detection and prevention of electrolyte imbalances difficult (Lobingier and Zinkl, 1992).

In the kidneys, a fluid that resembles plasma is filtered through the glomerular capillaries into the renal tubules. As this glomerular filtrate passes down the tubules, its volume is reduced and its composition altered by tubular reabsorption and tubular secretion to form urine (Ganong, 1995; Randall *et al,* 1997). The kidneys alter the composition of the plasma so as to eliminate waste products while the water and important electrolytes and metabolites are maintained within normal limits in the body. Furthermore, the composition of urine can be varied, and many homeostatic regulatory mechanisms minimise or prevent changes in extracellular fluid (ECF) by changing the amount of water and various specific solutes, including electrolytes, in urine (Ganong, 1995; Randall *et al,* 1997; Rose and Renke, 1994). The kidneys are responsible for maintaining normal blood concentrations of a large number of electrolytes and would be expected to modify urinary electrolyte concentration by tubular excretion and retention (Lobingier *et al,* 1992).

Since mineral content of the urine reflects whole-body mineral status better than mineral concentrations in blood, urine should be used in any assessment of mineral nutrition status (Caple *et al,* 1982; Vrzgula, 1990). Urinary electrolyte concentration is difficult to

interpret unless dietary intake, water consumption, urine electrolyte and serum electrolyte concentrations are known. Using renal excretion or clearance of electrolytes could aid in the detection of impending mineral metabolic imbalances allowing institution of preventative measures (Lunn and McGuirk, 1990). Consequently, if the urine and serum electrolyte concentrations are known and water consuption can be estimated (from creatinine in urine, see below), then dietary intake can be assessed.

Changes in renal excretion of electrolytes in response to metabolic diseases would be expected to precede the onset of clinical signs, because the net balance of electrolytes necessitates appropriate renal conservation or excretion and results in increased or decreased total electrolyte content in urine (Fleming *et al,* 1992). Any dietary deficiency of essential electrolytes and inadequate correction of metabolic disorders could also be detected by monitoring the renal excretion of electrolytes (Fleming *et al,* 1992).

Renal excretion of electrolytes has been measured by expensive, laborious, and time-consuming 24-hour urine collection techniques. Fractional excretion of electrolytes is a simple, inexpensive measurement that has been shown to be a reasonable indicator of renal clearance (Ng *et al,* 1984) and has been used for diagnosing potential dietary mineral problems in ruminants (De Morais and Chew, 1992; Fleming *et al,* 1991; Itoh, 1989; Ng *et al,* 1984). Fractional electrolyte excretion has been used in the horse (Traver, *et al* 1976) dog (Carrillo *et al,* 1979; Grossmann and Brobst, 1982) and in ruminants (Garry, 1987; McKinnon, 1998) for the diagnosis of metabolic and renal diseases. It is important for the early detection and diagnosis of malnutrition and mineral deficiencies in dairy cows (Jonas, 1971). Fractional electrolyte excretion is used to determine if there is an increase or decrease of the urinary excretion of an electrolyte (Neiger and Hagemoser, 1985).

Normal values of minerals in ruminant urine vary greatly due to the species, type of feed, water balance, and state of production. Due to the high variability, setting up a reference range is a difficult task. However, reference values, are available in the literarure (Altman and Dittner, 1972).

2.2. ELECTROLYTE FRACTIONAL EXCRETION

Urinary electrolyte determination is diagnostically important in various clinical settings:

(1) The measurement of urinary electrolyte excretion is helpful in determining the route of electrolyte loss (Oken, 1981).

(2) The measurement of the electrolyte concentration in urine is important in differential diagnosis of acute renal failure (Oken, 1981).

(3) The simple determination of urinary electrolyte concentration in a herd of ruminants can be used to diagnose electrolyte imbalances (Olson, 1989).

In the situations mentioned above, amongst others, a random urinary electrolyte concentration sample can rapidly supply valuable diagnostic information.

Clinical assessment of renal tubular function through the determination of electrolyte and creatinine concentrations in concurrently obtained samples of serum and urine is commonly performed in veterinary medicine (Constable, 1991). Confusion exists regarding the correct name for this index, since it has been defined as both fractional excretion (FEx) and fractional clearance (FCx). On a definition basis FEx and FCx can represent entirely different mean values (Constable, 1991). FEx is the ratio of excretion of substance x to the excretion of another substance, which is usually creatinine (Constable, 1991). FEx provides no information about tubular function. However, it provides information on the absolute amount of substance x and does not need a 24-hour urine collection for creatinine measurement (Neiger *et al,* 1985). FCx of substance x represents a ratio of clearance of substance x to clearance of another substance, usually creatinine. This provides information concerning the action of tubular transport mechanisms on substance x and the degree of conservation or excretion of that substance (Hays and Sweenson, 1993). FCx determination requires urine and serum creatinine concentrations as well as substance x urine and serum concentrations (Constable, 1991; Itoh, 1989; Neiger *et al,* 1985). Normally, it is recommended that the urine creatinine concentration be calculated from a 24-hour urine sample. Fractional clearance depends on tubular reabsorption or secretion of electrolytes. The calculation is thus dependent on creatinine

clearance equalling glomerular filtration rate (GFR) (Lunn *et al,* 1990). This could be useful in determining the clearance rate of other substances, assuming that creatinine is constantly excreted, by using the substance x to creatinine ratio.

The potential value of fractional clearance determinations in ruminants is dependent on creatinine excretion (Lunn *et al,* 1990). Some reports suggest that creatinine excretion in ruminants is constant (Fleming *et al,* 1992; De Groot and Asfjes, 1960; Farningham, 1996; Fleming *et al,* 1991). However, there is sufficient daily variation to make an interpretation of FCx data in individual cattle unreliable (Fleming *et al,* 1991; McKinnon, 1998). However, used within groups of animals, this parameter is still considered valuable (Farningham, 1996; Fleming *et al,* 1992; McKinnon, 1998). In similar studies with sheep, where particular care was taken to avoid excitement associated with sample collection, it was found that creatinine excretion was constant (Farningham, 1996), and that there is a good correlation between total creatinine and excretion of electrolytes in normal animals (Fleming *et al,* 1992; Garry, 1987; Garry *et al,* 1990). Thus, this important finding, reflecting the ability of using creatinine concentration in urine to determine mineral excretion in urine, which could reflect their balance in body. Therefore, urinary electrolyte concentrations can be used to diagnose mineral deficiency on a herd basis (Olson *et al,* 1989). The potential value of FCx determinations in ruminants is dependent on creatinine excretion (Lunn *et al,* 1990).

Determining the excretion of metabolites by total urine collection (24-hour urine sample) over extended periods is laborious, and in some types of experimentation a lengthy collection procedure is undesirable or impossible (Hodgen *et al,* 1967). It is necessary to catheterise the animal, keep it in a metabolic cage or install a urine collection device. Disadvantages associated with this include secondary urinary tract infection and urine contamination and may, therefore, yield erroneous results (Lane and Merritt, 1983).

An additional parameter worthy of consideration is the calculation of mineral/electrolyte to creatinine ratio (E:C ratio) according to the formula:

$$FEx = \frac{Ux}{Ucreat.} \times 100 \quad (1)$$

Where:

FEx = Fractional excretion of substance X

Ux = Concentration of substance x in urine

Ucreat = Concentration of creatinine in urine

The measurement of the FEx of certain electrolytes has been reported to detect mineral imbalances, undetected by serum Chimical studies (Itoh, 1989; Neiger *et al,* 1985; Traver *et al,* 1976). This may allow recognition of the problem before it has progressed to an extreme or severely debilitating degree, but on the other hand, it may not provide information about tubular function, which is seen as a constraint (Neiger *et al,* 1985; Traver *et al,* 1976). Because, tubular secretion can make a significant contribution to the urine concentration of ions, it should not be neglected.

The approach, mentioned, requires the collection of a single urine sample, and, in the case of electrolytes, was found to be a satisfactory indicator of excretion in ruminants in comparison with FCx. (McKinnon, 1998). However, the variability of creatinine excretion in ruminants made this parameter more suitable for assays within groups of animals as opposed to individual cases. Therefore, regardless of this constraint, using this parameter could make field work easier by collecting a single urine sample, as well as providing an indication of mineral status in a herd.

2.3. ANALYTES

2.3.1. Creatinine

2.3.1.1. Metabolism and function

Creatinine is a waste product of the nonenzymatic cycling of creatine within the body (Fuller and Elia, 1988; Jones and Burnett, 1974; Tietz, 1976). Creatine is spontaneously converted into creatinine at the rate of about 2% per day (Tietz, 1976). The amount of creatinine produced is relatively constant within an individual and is largely proportional to muscle mass (Albin and Clanton, 1966; Fuller *et al,* 1988). Water and creatine intake (De Groot *et al,* 1960) and the rate of synthesis (Tietz, 1976) can also be important determinants.

Enteric bacteria degrade some creatinine, but most is excreted from the body in urine (Jones and Burnett, 1974; Tietz, 1976). Creatinine is neither reabsorbed nor excreted as it traverses the renal tubules and renal pelvis (Finco and Barsanti, 1982; Tietz, 1976). Thus, measurement of creatinine in urine is important as a measure of glomerular filtration rate (GFR). Renal clearance of creatinine has been evaluated as a method for measuring GFR in several species (Finco and Groves, 1985), including horses (Gronwall, 1985), dogs (Finco, 1980; Finco *et al,* 1981), cattle (Albin and Clanton, 1966), and sheep (Farningham, 1996; Nawaz and Shah, 1984). Furthermore, the rate of creatinine excretion has been used as a reference for evaluating urinary excretion rates of minerals (Field, 1964) and in nutrient balance studies in man (Bistrian *et al,* 1975) and other ruminants (Butcher and Harris, 1957). Because it's daily excretion rate is nearly constant in the animal (Albin and Clanton, 1966; Fuller and Elia, 1988), a reliable measure of creatinine could be very useful for "standardising" the concentration of other urine constituents (such as minerals and electrolyte) for clinical or research purposes.

2.3.1.2. Sample stability

A reliable measurement of urinary creatinine concentrations is important in determining FEx due to the relationship of urinary creatinine to other substances found in urine. Although modern analysers can process large numbers of urine samples at a rapid rate, there will be occasions when the samples need to be stored for a long period. Therefore, by evaluating the stability of constituents allows to know the conditions of the proper storage as well as the time needed between complete collection and analysis of urine specimen (for example, mailing).

Hydrochloric acid has been recommended for preservation of urine samples in which urea and ammonia were to be analysed (Varley *et al,* 1980), but creatinine was unstable using this method (Soliman *et al,* 1986). Thymol has been found to be a satisfactory preservative for a wide range of substances including creatinine (Naftalain and Mitchell, 1958). Specimens kept for analysis of creatinine were best stored at refrigerator temperature if they had to be analysed within a few days; for longer periods, freezing was recommended (Spierto *et al,* 1997; Tietz, 1976).

Creatinine and minerals in urine are stable at temperatures of 4°C to –20°C, for up to three months (Lobingier *et al,* 1992; Penafiel *et al,* 1983; Spierto *et al,* 1997; Tietz, 1976). More recent studies (Spierto *et al,* 1997) show that creatinine is stable in fluids at room temperature (25°C) and even under moderately abnormal storage conditions (55° C), if it is kept no more than 2 days. However, in urine samples from farm animals, which are invariably contaminated, it seems to be less stable under the same conditions.

Creatinine in dried urine samples kept on filter paper for later analysis produced very good results (Portal *et al,* 1971; Takemori, 1980). This suggests that it might be possible to test large populations of animals in remote areas with tropical or subtropical climates. Those results are similar to other reports on filter paper preservation studies in man, which show only slight variation of creatinine over time and no significant variation of minerals during the same period of time (Lobingier *et al,* 1992; Portal *et al,* 1971;

Soliman *et al,* 1986; Takemori, 1980). The latter is to be expected because the minerals are stable in any sample or environment, unless the sample is influenced by other factors such as dilution volumes and other substances present in the samples.

2.3.1.3. Analytical method for creatinine

Currently, the most widely used technique for determination of creatinine concentration is the kinetic Jaffé reaction (Murray, 1984). This reaction is based on the formation of the reddish complex, creatinine-picric acid, (Javonski complex) under alkaline conditions. This method was modified from the original to decrease the interference caused by Jaffé reactive pseudocreatinine or noncreatinine chromogens (Smith *et al,* 1983). However, improved methods, such as the enzymatic method, further reduce interference compared with the kinetic Jaffé reaction. The enzymatic technique yields result, which are directly proportional to the kinetic Jaffé reaction and deals effectively with all interference except bilirubin. Both methods have the same precision (Jacobs *et al,* 1991). This suggests that the enzymatic method could be used in place of the kinetic Jaffé reaction for routine work. However there are 2 main constraints which could limit the enzymatic method: (1) the cost is 25% greater and (2) the shelf life, which is half of the kinetic Jaffé test (Jacobs, *et al* 1991). For these reasons, the kinetic Jaffé reaction is still widely used, in most clinical laboratories (60.47%) in comparison with the enzymatic method (15.76%). This was based on data from the Murex Biotech, quality assessment programme, report for cycle13 (Murex Biotec, 1999).

2.3.2. Calcium

2.3.2.1. Metabolism and function

Most calcium is in the skeleton and can be mobilised to maintain a constant concentration in blood. It plays a vital role in muscle contraction, membrane stability (Payne, 1977; Tietz, 1976; Vrzgula, 1990), maintenance of cardiac rhythmicity (Payne, 1977; Vrzgula, 1990), and as co-factor in many enzyme reactions (Payne, 1977; Tietz 1976; Vrzgula,

1990), and as co-factor in many enzyme reactions (Payne, 1977; Tietz 1976; Vrzgula, 1990). Consequently, maintenance of calcium homeostasis is most important in preserving normal physiology. Homeostasis is maintained by the interaction of two hormones, parathyroid hormone and calcitonin, together with the action of metabolites of vitamin D which preserve the concentration of calcium in the blood within very narrow limits (Payne, 1977; Payne and Payne, 1987).

A particular problem for the interpretation of profile tests is that blood calcium exists in two forms (Payne and Payne, 1987). Total calcium, the form usually measured, contains a soluble ionised portion (46%) and a non-ionised portion which is bound to serum proteins, mainly albumin (47%). This fraction is not filtered at the glomerulus, because of high molecular mass of the proteins, therefore, the fraction is not excreted in the urine. The remaining 5% forms complexes with other ionised elements (citrate, bicarbonate or phosphate), the above percentages varying, depending on blood pH (Hays and Sweenson, 1993; Payne *et al,* 1974).

Most of the filtered calcium is reabsorbed actively from the proximal tubule and passively at the medullary portion of the loop of Henle (Hays and Sweenson, 1993). Urinary calcium concentration is a sensitive way to detect calcium deficiencies in horses, since the urine is the most important route of calcium excretion in this species (Caple *et al,* 1982; Jonas, 1971). About 90% of the calcium in the ruminant's urine is of endogenous origin (Payne, 1977; Payne *et al,* 1970; Payne and Payne, 1987). Therefore, increasing dietary calcium intake does not affect the calcium content in urine according to Vrzgula (1990). Therefore, only small amounts of calcium are excreted in the urine (Boehncke, 1976; Massry, 1973; Vrzgula, 1990). The calcium content in the urine only increases in intensive demineralisation of the bones or in enhanced parathyroid activity, in chronic metabolic acidosis and in nephrosis (Jonas, 1971; Vrzgula, 1990). This is not in agreement with Massry *et al,* (1973) who found that the slight increase in absorption that occurs on a high-calcium diet is reflected in an increased renal excretion. However, studies, comparing evaluation of minerals status in ruminants by a different methods (Jonas, 1971), found that the evaluation of the calcium concentration in urine does not make too

and others routes of calcium excretion such as milk and faeces (Jonas, 1971; Horst, 1986). Therefore, an average secretion of more than 5mg/dl (1.25 mmol/l) of calcium can be assumed in ruminants. In addition, approaches in humans, using calcium/creatinine ratio as a predictor of preeclampsia, have been found not to be sensitive enough to be used as a screening test for the emergence of pre-eclampsia (Saudan *et al,* 1998). Meanwhile, if combined with the evaluation of phosphorus in the serum, the urine calcium concentration can provide useful information related to the state of calcium supply of the animal. Calcium and phophorus secretion are related, due to the same hormone controlling mechanism (Jonas, 1971).

2.3.2.2. Calcium imbalance

An insufficient intake of calcium or vitamin D will result in rickets in young animals (Hays and Sweenson, 1993; Payne, 1977). In adults, calcium deficiency may cause osteomalacia, a generalised demineralisation of bones, and osteoporosis (Vrzgula, 1990).

Parturient paresis, or milk fever, in cows is also associated with abnormal calcium metabolism and normally occurs at the onset of lactation. The most consistent abnormality in calcium balance is acute hypocalcemia (Vrzgula, 1990), which can lead to a dramatic decrease in milk production.

2.3.2.3. Analytical method for calcium

Most colourimetric calcium analytical methods are based on the formation of colour complexes between calcium and organic molecules such as θ-cresolphtalein, one of the most commonly used for routine calcium analysis. However, to reduce interference by magnesium the method was modified by including 8-hydroxiquinolone (Walmasley and Flower, 1981) and to overcome protein interference the Technicon® autoanalyser uses the principle of dialysis (Farrell, 1984).

Atomic absorption is more accurate and sensitive than other methods, and because of this is used as the reference method. It is, however, more technically demanding and labour intensive, not lending itself to automation. The θ-cresolphtalein method, which is commonly used, is reported to give higher values than the atomic absorption method (Farrell, 1984c). However, instrument problems may present an impediment for smaller and high-throughput laboratories in terms of the atomic absorption method (Farrell, 1984c). Hence, smaller and less well equipped laboratories usually use the θ-cresolphtalein method. This is supported, by the Murex Biotech cycle report 13 (1999), which shows that a majority of laboratories world-wide, obtaining a "full" set of calcium assay results, uses θ-cresolphtalein (47.62), while others use either other colorimetric (27.25%) or indirect ion selective electrode (3.71%). There are very few clinical laboratories using other methods, like atomic absorption for which there is even no code number, indicating how rarely it is used for routine analysis in clinical laboratories (Murex Biotec, 1999).

2.3.3. Inorganic phosphorus

2.3.3.1. Function and metabolism

Inorganic phosphorus is combined with calcium and carbonate to form compounds that lend rigidity to bones and teeth and is vitally concerned in many metabolic processes such as buffering of body fluids. Every form of energy exchange inside cells involves the formation and breaking of high energy bonds that link phosphorus (Hays and Sweenson, 1993). Phosphorus has many physiological functions, which are related to those of calcium (Payne, 1977). In ruminants, in particular, phosphorus in saliva has an additional and highly important function, serving firstly as a buffer to control pH of the rumen, and secondly as a source of phosphorus for the multiplication of the rumen flora and fauna (Payne, 1977; Payne and Payne, 1987). Dietary phosphorus and calcium are absorbed in the upper small intestine (duodenum). The amount absorbed depends on the source and body needs (Hays and Sweenson, 1993). The calcium and phosphorus are absorbed from the intestine by the portal route, circulate through the body and are readily withdrawn

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from blood for use by the bones and teeth during periods of growth (Hays and Sweenson, 1993; Horst 1986; Morse *et al,* 1992).

Parathyroid hormone and thyrocalcitonin regulate the plasma phosphorus level (Payne *et al,* 1970). The plasma phosphorus level is inversely related to the blood calcium (Hays and Sweenson, 1993). Thyrocalcitonin decreases plasma calcium and phosphate levels while parathyroid hormone increases them. Maintenance of the serum inorganic phosphate concentration is achieved largely through renal control (Massry *et al,* 1973). About 80 to 95 percent of the filtered phosphate is reabsorbed, and most reabsorption occurs in the proximal tubule (Ganong, 1995; Randall *et al,* 1997; Reece, 1993). Virtually no phosphate is excreted with low dietary phosphate load, and this is believed to be due to an increase of Na^+-phosphate symport activity (Reece, 1993). With high dietary phosphate load, urinary excretion increases because of a direct effect of phosphate concentration. In addition, increasing parathyroid hormone secretion, metabolic acidosis (Reece, 1993), demineralisation of bone tissue due to calcium or vitamin D deficiency and renal diseases (Vrzgula, 1990), also promote excretion of phosphate.

In ruminants, only a small amount of phosphorus is excreted in the urine (Morse *et al,* 1992; Vrzgula, 1990), due to a relatively alkaline urine, and because their diet involves the intake of large quantities of potassium bicarbonate, this would render calcium and phosphate insoluble, and thus, rule out their excretion in the urine (Payne and Payne, 1987). Salts of calcium and phosphorus are relatively insoluble under alkaline conditions and if present would precipitate and cause obstruction of the urinary tract (urolithiasis) (Payne, 1977; Payne and Payne, 1987). Thus major routes of excretion of endogenous and exogenous phosphorus in ruminants are faeces and milk (Horst, 1986; Morse *et al,* 1992; Vrzgula, 1990). The situation is reversed in ruminants on intensive husbandry and feeding, when energy-rich diets induce acidosis; the urine then becomes acidic and the kidney actively excretes large quantities of phosphorus. Fortunately this extra output seldom causes hypophosphataemia, because energy-rich diets usually contain cereals with high phosphorus content (Payne and Payne, 1987).

2.3.3.2. Inorganic phosphorus imbalance

There are many areas of the world where phosphorus deficiency limits production, and this aspect of nutrition comes to the fore when efforts are made to improve productivity by modern techniques. For instance in Botswana, Cooper reported (Cooper, 1974) that although clinical aphosphorosis was rare, inorganic phosphorus levels in serum reflect deficiency in the grazing (Cooper, 1974); another report, on blood inorganic phosphorus levels in cattle in Botswana, showed that 45% of traditionally farmed cattle are hypophosphataemic (Reed *et al*, 1974).

The ratio of calcium:phosphorus has an important effect on availability of phosphorus (Horst *et al*, 1963). A relative deficiency of inorganic phosphorus occurs with any excess of calcium, which in its turn suppresses the hormonal mechanism stimulating calcium mobilisation (Theiler and Green, 1932; Wise *et al*, 1963). Phosphorus deficiency is related to botulism in ruminants, through the consumption of contaminated carcasses, which inevitably leads to their death (Theiler and Green, 1932). Pica, due to phosphorus deficiency has been described (Clark, 1974), with signs of poor condition, malformed joints, lameness, a depressed appetite and an overwhelming desire to chew bones (Clark, 1974).

There has been controversy concerning a link between phosphorus deficiency and infertility. Phosphorus deficiency in dairy heifers was verified on grounds of both nutrition and blood chemistry with supplementation from 3.9 mg/100 ml (1.26 mmol/l) to 6.6mg/100ml (2.13 mmol/l) the fertility improved very slightly (Payne and Payne, 1987).

Excess of phosphorus and calcium intake appears to be associated with diets containing too much cereal, causing urolithiasis in sheep (Lamprecht *et al*, 1969), urinary calculus in goats (Sato and Omori, 1977) and osteodystrophy in other ruminants (Payne and Payne, 1987).

2.3.3.3. Analytical method of phosphorus

Because elemental phosphorus does not occur to any appreciable extent in the body, methods have been directed toward analysis of two phosphate anions, which interchange rapidly depending on pH (Farrell, 1984b). Monovalent ($H_2PO_4^{-1}$) and bivalent (HPO_4^{-2}) anion forms are present in a ratio of 100:1 at pH 4.5 in urine, making it impossible to say with any certainty what is the molecular mass of inorganic phosphate (Farrell, 1984b). The potential error, however is very small; of the order of 1%.

The most commonly used analytical methods are based on the reaction of phosphate ions with molybdate to form a blue complex structure. Since this method was developed it has been improved to make the method faster, simpler and the reagents more stable. The most improved method is the reduced phosphorus-molybdate complex, read at 340 nm, which is used by the majority of laboratories (Farrell, 1984b). However, problems encountered in this type of assay include instability of the reducing reagent (stannous chloride) (Daly and Ertingshausen, 1972). The introduction of stannous chloride-hydrazine sulphate results in better linearity (Farrell, 1984b). In addition the Murex Biotec cycle report 13, indicates that the majority of clinical laboratories use the unreduced phosphomolybdate complex method (50.82%), whereas 24.26% use phosphomolybdate blue methods (Murex Biotec, 1999).

Urine samples may contain large quantities of organic phosphates that can decompose on exposure to elevated temperature (Farrell, 1984b). When acidifying with HCl, urine phosphate is stable for 6 months (Daly and Ertingshausen, 1972; Farrell, 1984b; Tietz, 1976). Urine samples are usually diluted 1:10 with normal saline before analysis (Farrell, 1984b; Tietz, 1976). However, this could alter sodium and chloride concentration or will need additional dilution calculation, which can induce to an error, so the procedure must be avoided.

2.3.4. Magnesium

2.3.4.1. Function and Metabolism

Magnesium (Mg) is the second most important intracellular cation in quantity, after potassium (Salem *et al,* 1992). Mg is involved in various metabolic processes including oxidative phosphorylation, ion transport, deoxyribonucleic and ribonucleic acid metabolism and protein synthesis (Salem *et al,* 1992), and plays an important role as activator for a large number of important enzymes, such as pyruvic acid carboxylase, pyruvic acid oxidase and creatine kinase (Hays and Sweenson, 1993; Wester, 1992). These metabolic associations make Mg essential to basic energy-demanding processes such as neuromuscular excitation, muscular contraction and cell membrane permeability (Wester, 1992). Mg enters the body by ingestion. The principal sites of Mg absorption are the rumen and reticulum (Hoffsis *et al,* 1989) by a complex mechanism related to Mg^{+2}-K^{+}-Na^{+} interrelationship (Brown *et al,* 1978; Martens and Rayssiguier, 1980; Poe *et al,* 1985). Small amounts of Mg are absorbed in the intestine (Marten, 1983; Smith, 1959; Tomas and Potter, 1976). Mg absorption is depressed in ruminants consuming elevated quantities of potassium (Poe *et al,* 1985), but if animals are fed a diet with adequate sodium the effect is minor (Hays and Sweenson, 1993; Martens *et al,* 1980; Poe *et al,* 1985).

Primarily dietary absorption, renal excretion, and diffusion among the tissues control the concentration of Mg in serum. Most Mg is intracellular and while approximately 70% of the Mg in the animal is in bone and relatively unavailable, it cannot be mobilised in time of need (Hays and Sweenson, 1993; Payne *et al,* 1970). Only 1% exists in the extracellular compartment. Approximately 45% of extracellular Mg is bound to protein or complexes as salts, and the remaining 55% is free (ionised) (Marten, 1983; Walser, 1961). The proximal tubule accounts for 20-30% of its reabsorption and most of the remainder is reabsorbed from the loop of Henle (Hays and Sweson, 1993; Marten, 1983).

Hormones known to affect Mg balance are parathyroid hormone, aldosterone, thyroxin, and vitamin D (Cohen *et al,* 1970; Hays and Sweenson, 1993; Todd, 1969). Parathyroid hormone increases reabsorption of Mg from the kidneys and bones (Hays and Sweenson, 1993; Todd, 1969). Aldosterone increases Mg losses from the kidneys, but Mg has no role in the induction of aldosterone secretion (Todd, 1969). Vitamin D, given long-term to animals, decreases serum Mg by increasing Mg losses in urine (Frank, 1977). Plasma Mg increases in animals after vitamin D therapy because of increased Mg uptake from the gastrointestinal system (Schneider *et al,* 1998).

It is believed that the input of Mg into extracellular fluid is almost entirely from alimentary absorption (Payne, 1977). Dietary Mg deficiency or limited absorption from digestive organs is reflected in a decrease in the Mg level in urine within a short time (Vrzgula, 1990). Therefore, urine Mg excretion could be indicative of the amount and availability of Mg in ruminant feed. There is a close correlation between serum Mg concentration and the excretion of Mg in urine (Hidirogou *et al,* 1981). Urine Mg levels (especially ones corrected for creatinine) reflect the dynamic status of Mg in dairy cows better than serum levels (Alexander, 1985; Payne and Payne, 1987).

2.3.4.2. Magnesium imbalance

A form of Mg deficiency is called grass tetany, and can be corrected by administration of Mg salts (Hays and Sweenson, 1993; Tietz, 1976). The clinical signs of hypomagnesaemia are often precipitated by stress (Hidirogou *et al,* 1981). Ruminants with hypomagnesaemia usually demonstrate clinical signs manifested by inappetence, alert expression and excitability (Hoffsis *et al,* 1989). Neuromuscular hyperirritability increases with the continuation of the deficiency and may be followed eventually by cardiac arrhythmia, generalised tremors, and death (Hays and Sweenson, 1993). Grass tetany is not the only untoward effect of hypomagnesaemia, retained placenta is another (Krupnik and Marcinkwski, 1983). An additional disadvantage of magnesium imbalance is that it leads to poor digestion in the rumen, with low production as a secondary consequence (Wilson, 1999). This was confirmed in sheep, when magnesium concentration fell below 1

mg/dl (0.411 mmol/l) it caused a decline in milk fat, protein, and lactose in ewes (Scholz and Ismail, 1977).

Hypomagnesaemia imposes deleterious effects on calcium metabolism, which may be why cows with low magnesium status are especially liable to milk fever at calving (Payne and Payne, 1987). Studies showed that, when magnesium concentration fell below 0.75 mmol/l, calcium mobilisation rates fell drastically so that cows could not mobilise skeletal reserves sufficiently (Sansom *et al,* 1982).

Excessive Mg intake may reduce calcium and phosphorus availability (Payne, 1977). It is thought that Mg not only competes with calcium for absorption in a common pathway, but might also combine with phosphorus to form relatively insoluble salts, which fail to be absorbed (Payne, 1977).

2.3.4.3. Analytical method of magnesium

Several Mg analytical methods have been developed and automated and are currently in widespread use. Mg analysis has provided many problems for the analyst because samples are easily contaminated and there is a problem of binding to chelators, which could interfere with Mg determinations in urine (Daly and Ertingshausen, 1972). The Mg reference method is atomic absorption because it has excellent accuracy and precision, but is not a popular method (Farrell, 1984a; Murex Biotec, 1999). The recommended method is the methylthymol blue method since it correlates well with atomic absorption (Farrell, 1984a). This method is based on a reaction between Mg ions and methylthymol blue, giving a coloured complex, which can be read at 515 and 600 nm. However, the method has fallen out of favour and is largely replaced by the xylidyl blue method. According to the Murex Biotec cycle report 13, 23.94% of clinical laboratories uses the colorimetric method, whereas 22.78% of clinical laboratories use Xylidyl blue method. The second most commonly used method reported by Murex Biotech cycle report 13 is calmagite (15.83), followed by atomic absorption (5.79%) and methylthymol blue (5.79). It has been recommended that for determining the magnesium concentration in a urine sample, it

should be acidified to pH 1 by adding concentrated HCl (Daly and Ertingshausen, 972). However, this procedure would alter the concentration of chloride.

2.3.5. Chloride

2.3.5.1. Metabolism and function

Chloride is an essential element required for normal metabolism. It is a principal anion in extracellular fluid. Chloride is a chief anion of the gastric juice and is accompanied by hydrogen ions in nearly equal amounts. The chloride of the gastric secretion is derived from blood chloride and is normally reabsorbed during later stages of digestion in the lower intestine (Hays and Sweenson, 1993; Payne *et al,* 1970). Chloride is involved in the regulation of extracellular osmotic pressure and makes up more than 60% of the anions in this fluid compartment. Thus the chloride ion is important in acid-base balance (Lunn *et al,* 1990).

A close relationship exists between chloride and sodium ions (Hays and Sweenson, 1993). The concentration of chloride is subject to more variation than that of sodium, since other anions, especially bicarbonate, can exchange for chloride (Sabatini and Kurtzman, 1984). Most chloride is removed by glomerular filtration and reabsorbed by the proximal tubules accompanied by sodium reabsorption and excretion of potassium or hydrogen to preserve electroneutrality (Lengemann *et al,* 1952; Lunn *et al,* 1990; Rose and Renke, 1994).

Chloride is excreted in the faeces, sweat and urine, primarily as sodium or potassium chloride, although it may be accompanied by ammonium ions when base needs to be conserved (Payne, 1977).

Sodium and chloride are electrolytes with strong effects and both represent the largest quantity of cation and anion fraction in extracellular fluid respectively. Measuring their concentration can be used for an indirect approach to acid-base status referred to as the strong ion difference approach (Leith, 1990; Olson *et al,* 1989). Strong ion difference

(SID) is the difference between the sum of strong cations and strong anions. It is important because it provides an indirect measure of the amount the H^+ in acid-base balance (Leith, 1990). SID is not directly measured, because measuring all strong cations and strong anions is difficult. Excess of chloride and a constant level of sodium represents an acidosis, whereas an excess of sodium and a constant level of chloride represents an alkalosis (Leith, 1990; Lunn *et al,* 1990). On a chloride–deficient diet, the excretion of chloride in urine or perspiration is markedly reduced (Hays and Sweenson, 1993; Lunn *et al,* 1990; Payne, 1977). Urine chloride, and particular Cl/Cr (chloride/creatinine ratio) should therefore provide an important indicator of chloride deficiency.

2.3.5.2 Chloride imbalance

Chloride deficiency in cattle is not a common nutritional problem, because the concentration of this element in forage and sodium chloride ration supplementation is usually adequate (Blackmon *et al,* 1984). However, chloride deficiency has been reported to present various clinical signs in calves (Blackmon *et al,* 1984). Excessive depletion of chloride ions through losses, or deficiency in the diet, may lead to alkalosis due to excess of bicarbonate, since the inadequate level of chloride is partially replaced or compensated for by bicarbonate (Blackmon *et al,* 1984; Hays and Sweenson, 1993). Lactating dairy cows given a 0.10% chloride diet exhibited lethargy, decreased feed intake and milk production, loss of body weight, cardiovascular depression, and mild dehydration (Fettman *et al,* 1980). Naturally occurring disease states in cattle, including displaced abomasum, abomasal impaction, high intestinal obstruction, (Whitlock, 1976) and nephrosis, (Brobst *et al,* 1978) are characterised by hypochloraemia, which could aggravate or potentiate chloride deficiency.

2.3.5.3. Analytical method of chloride

Chloride analysis has been improving since 1953 (Miller, 1984b; Schoenfeld and Lewellen, 1964; Scribner, 1950; Zall *et al,* 1956). The most commonly used colorimetric method is the mercuric/ferric thiocyanate method, which has good accuracy and precision

(Caple *et al,* 1982), and is used by 32 out of 348 (9.2%) clinical laboratories world wide (Murex Biotec, 1999). Another method the ion selective electrode (ISE) which provides the same accuracy and precision is used by 76.7% of the 348 clinical laboratories reported by Murex Biotech cycle report 13 (1999) as compared to the 9.2% clinical laboratories which use mercuric/ferric method. However, particularly the trial will analyse chloride by using mercuric/ferric method, since the accuracy, precison and coefficient of variation of the method (1%-1.5%) does not differ from the ISE method (1%) (Miller, 1984b).

2.3.6. Sodium and Potassium

2.3.6.1. Function and metabolism

Sodium is the main cation found in extracellular fluid and is present largely as the sodium ion. The major function of sodium concerns regulation of crystalloid osmotic pressure, acid-base balance, maintenance of membrane potentials, transmission of nerve impulses and absorption of many compounds (Hays and Sweenson, 1993). The role of sodium in regulation of osmotic pressure may be typified by consideration of the electrolyte distribution of blood plasma (Hays and Sweenson, 1993), and changes in osmotic pressure are largely dependent on the sodium concentration (Payne, 1977; Payne and Payne, 1987).

Normally sodium comes from the diet in the form of the sodium chloride (salt). Sodium is readily absorbed as the sodium ion and circulates throughout the body. The mechanism for sodium absorption is so effective that faecal sodium can, if necessary, be reduced to nearly zero (Payne, 1977). Sodium is freely filtered through the glomerulus and is almost completely (90%) actively reabsorbed by the proximal tubules in the form of sodium chloride. The other 10% is reabsorbed by the distal and collecting tubules under the influence of aldosterone. Sodium reabsorption is very important because it affects the regulation of several other electrolytes, such as passive reabsorption of chloride and bicarbonate as well as water (Hays and Sweenson, 1993).

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Plasma sodium concentration in sheep is remarkably unaffected by high intake of sodium chloride (Meintjes and Engelbrecht, 1993), whereas daily urinary sodium excretion fluctuates widely according the dietary intake, thereby keeping the body sodium content remarkably constant.(Harrington, 1982) Changes in sodium deficiency include decreased salivary, urinary, ruminal and faecal sodium concentration and increased aldosterone concentration (Morris, 1980).

Potassium is a major intracellular cation and is largely responsible for the maintenance of intracellular volume. About 95 % of all body potassium is intracellular, with only 2%-5% of body potassium in the extracellular fluid (Rose and Renke, 1994). The cell maintains a high potassium and low sodium concentration by means of Na-K ATPase located in the cell membrane (Alexander and Perrone, 1987).

Potassium serves the same general functions relating to osmotic pressure, regulation of acid-base balance and cellular membrane potentials, within the cells, as does sodium in extracellular fluid (Hays and Sweenson, 1993). The kidneys regulate total body potassium concentration because the intestines non-selectively absorb potassium, although in some cases there is an increased intestinal excretion. All potassium is filtered and is unique among substances that it can be either reabsorbed or secreted, and is ordinarily reabsorbed at a rather constant rate (Hays and Sweenson, 1993), in the distal tubules, principally under the control of aldosterone (Reece, 1993; Suki, 1976). Two mechanisms, which control the net potassium excretion are operative in the distal tubule. The first depends on the balance of the amount of potassium in the cells as well as the potassium ingestion. High potassium intake increases intracellular potassium as well as potassium excretion. The second mechanism regulates potassium balance via aldosterone, which, besides regulating sodium reabsorption, simultaneously enhances potassium secretion in the distal tubule (Gonick *et al,* 1971).

Changes in serum potassium concentration are associated with potassium losses by the gastrointestinal tract or by the kidneys (Willard, 1989). During a low intake of potassium, there is net reabsorption with minimal secretion, which could affect the transcellular

potassium redistribution. Acidosis can occur due to increased excretion of hydrogen ions in exchange for potassium, to maintain electroneutrality (De Morais *et al,* 1992; Harrington, 1982). With high potassium intake and sodium deficiency, secretion exceeds reabsorption and potassium is excreted in urine in order to maintain a balance in extracellular fluids (De Morais *et al* 1992; Hays and Swenson 1993). Excess blood potassium might be caused by an increase in potassium intake or in the face of decreased potassium excretion due to renal failure (De Morais *et al,* 1992).

Urinary potassium levels are therefore, helpful in the evaluation of patients with unexplained hypokalaemia (Harrington, 1982). Fractional clearance has been used to assess disorders of potassium (Fleming *et al,* 1991; Fleming *et al,* 1992; Kamel *et al,* 1990). Unfortunately, FCx can not be expressed without the knowledge of GFR (Kamel, *et al* 1990), but using fractional excretion instead could be useful for potassium assessment (Fleming *et al,* 1991).

2.3.6.2. Sodium and potassium imbalance

Normally, a slight reduction in serum sodium concentration is accompanied by reduction in effective plasma osmolality and can cause polydipsia and polyuria. Clinical signs are related more to the rapidity of onset than to the severity of associated plasma hyposmolality (DiBartola, 1989). Central nervous system manifestations, such as mental confusion, disorientation and seizures are the most common signs (DiBartola, 1989). Sodium deficiency affects production adversely. Many of these effects are mediated by behavioural changes, which become dramatic and violent as the deficiency progresses. Payne (1977) commented on pica, in which cows drink each other's urine and lick deep excavations in hedgerows to find even meagre salt supplies. Similar pica and poor growth rate in beef cattle was observed on low sodium pasture (Murphy and Plasto, 1972). Sows on sodium-deficient diets suffered low weight gains, difficulties in parturition and high incidence of foetal death (Horing, 1979).

Potassium deficiency leads to increased cellular sodium levels as a means of maintaining cation-anion balance (Hays and Sweenson, 1993). When the potassium is low, transmission of nerve impulses becomes impaired, and muscle paralysis develops. Cardiac arrhythmias, such as increased QRS duration, and abnormal QRS complexes can occur in potassium deficiency (Hays and Sweenson, 1993).

Excessive intake of sodium chloride with inadequate consumption of water may result in salt toxicity (Scarratt *et al,* 1985). Hypernatraemia results in an accumulation of sodium ions in tissues (including the brain) which could result in cerebral oedema and cell dysfunction (Scarratt *et al,* 1985).

2.3.6.3. Analytical method of sodium and potassium

Often sodium and potassium are analysed simultaneously. There are 3 main analytical methods for sodium and potassium. The flame photometer is used in most laboratories and electrolyte research programmes as a reference method (Miller, 1984a; Murex Biotec, 1999). Another method is Ion Selective Electrode (ISE) direct and indirect. "Direct method" measures the ion activity in an undiluted sample, whereas the indirect one measures the ion activity in a prediluted samples (Miller, 1984a).

The only significant error of flame photometry and the indirect ion selective method is that attributable to alteration to the water volume fraction of urine in some diseases (Miller, 1984a). Both sodium and potassium can be individually quantified by atomic absorption spectrophotometry. (Miller, 1984a). However, although, it has the advantage of higher sensitivity, it is less precise and can not be recommended for a routine work load (Miller, 1984a). Both flame photometer and ISE methods are satisfactory for clinical use with typical coefficients of variation (1%) for sodium and 1,5% for potassium at normal serum levels (Miller, 1984a). The indirect ISE method is used by the majority of clinical laboratories (55%-61%), whereas the direct ISE is used by 15%-27% of clinical laboratories for routine analysis of sodium and potassium (Miller, 1984a; Murex Biotec, 1999).

Attention should be paid to the variation of urine mineral concentrations, because of the diet mineral composition (Fleming *et al,* 1992) and amount of water in the body (Meintjes and Engelbrecht, 1993). Therefore, one should be take into consideration the dilution system. It is important to have final electrolyte concentrations, which fall into the range of linearity, however some errors are expected to occur during the dilution process.

2.4. Filter Paper Method

Filter paper is usually used for filtering. Filter paper has been used for a variety of similar studies which have demonstrated its usefulness as a way of preserving and transporting various types of samples. The majority of the studies that use filter paper mainly involve transport of human plasma (Morton and Kelley, 1990), blood (DuBey and Caplan, 1996; Elnagar *et al,* 1997; Ng *et al,* 1984; Sharma *et al,* 1994; Stibler and Cederberg, 1993), saliva (Hashida *et al,* 1996), and urine (McInnes *et al,* 1983; Takemori, 1980; Takemori, *et al* 1993a; Takemori *et al,* 1993b) samples for the detection of viruses (Hashida *et al,* 1996), hormones (Ng *et al,* 1984), and cerebrospinal fluid for the diagnosis of meningitis (Whittle and Greenwood, 1976). Recently the use of filter paper as a sample preservation method has become more common (Takemori, 1980; Takemori *et al,* 1993a; Takemori *et al,* 1993b). Results of studies using the dry filter paper method for the preservation of urine samples and storage in various conditions, to determine the stability of some drug (DuBey *et al,* 1996), creatinine stability and recovery (Portal *et al,* 1971; Takemori, 1980), minerals (Takemori, 1980), and some E:Cr ratios (mineral and electrolyte/creatinine ratio) (Takemori, 1980; Takemori *et al,* 1993a; Takemori *et al,* 1993b), showed that the method does not affect the drug, creatinine, mineral and mineral recovery. However, in the cited studies hydrochloric acid had to be added to obtain more precise results. Those studies using filter paper for different purposes have demonstrated its usefulness as a way of preserving and transporting various types of samples.

Trials using urine dried on filter paper have generally given excellent results and some compounds analysed remain stable enough for the sample to be mailed in an ordinary envelope to a central laboratory (Narisawa *et al,* 1983; Portal *et al,* 1971; Takemori,

1980). It therefore seems apparent, that filter paper should be useful for transporting and conserving urine specimens.

However, for this type of application, there are a few constraints concerning the filter paper type, mainly:

1. The properties of various grades - speed (relative mean flow) and retention characteristics (refers to the type of precipitate the grade will retain) (Tietz, 1976). This is because filter paper is made for filtration or separation purposes and most of its properties are related to that use: Whatman®[a] no 3, thick, medium speed paper with high retention, was used successfully (Fleming *et al,* 1992) compared to Whatman® no 1 filter paper for testing drugs in different urine specimens diluted with saline solution (Fleming *et al,* 1992). It was concluded that using simple filter paper for developing a method probably could give acceptable results. So, attention should be paid to the implications of this method for the urine specimen, if it is intended to use this method as urine specimen storage or transportation (Stibler abd Cederberg, 1993).
2. The length of time, which the filter paper should be placed in eluent to recover 90-100% of the original analytes varies from 10–30 min in specific diluent (distilled water) for serum samples (Morton and Kelley, 1990; Sharma *et al,* 1994), to 45-60 min for urine (Narisawa *et al,* 1983; Takemori, 1980), and 1-3 hours in an experiment done with saliva (Hashida *et al,* 1996); Based on results from studies that preserved urine on filter paper (Portal *et al,* 1971) a minimum of 30 min should be considered.
3. For transport purposes the wet filter paper has to be dried. Results of studies showed that instead of drying the filter paper with hot air, such as with hairdrier (Portal *et al,* 1971) it should be dried in open air (Takemori, 1980).

Experiments using filter paper for transporting and analysing transferrin in serum, demonstrated that the reliability of the results is highly dependent on the duration of storage (Stibler and Cederberg, 1993). However, it should be noted that in these

[a] Merck™, Laboratory Supplies, Midrand, Gauteng, South Africa

experiments, the samples were stored for more than 10 years and then analysed (Elnagar *et al* 1997). Certainly, the analyte in serum dried on filter paper, lose their capacity or activity, which could be the reason for the unreliability of the results.

A method for collecting and transporting urine samples on filter paper used for field surveys was designed and applied to epidemiological surveys (Takemori *et al,* 1993a; Takemori *et al,* 1993b). Under the conditions of large-scale field testing, the filter paper preservation method gave a significantly better conservation of creatinine and minerals than temperature control methods (Portal *et al,* 1971; Takemori, 1980). This is probably due to the fact that refrigeration or chilling is difficult to achieve during the transportation to the laboratory of samples collected in distant areas (McInnes *et al,* 1972; Portal *et al,* 1971).

Using the filter paper method may eliminate various problems before analysis, such as the centrifugation for clarifying samples, the need for sterile equipment, cold storage, and the freezing of samples. In developing countries, where sample preservation facilities are limited, dried filter paper samples could be reliable and inexpensive (Sharma *et al,* 1994) and easy to transport (even if the analytical field laboratory is far form the sample collection site). Therefore, using filter paper could provide a simple and cheap alternative for transportation and preservation of specimens.

There is a definite need to develop the filter paper technique in order to make it useful for monitoring animal population health in rural Mozambican areas, specifically in the investigations of mineral imbalances.

2.5. ASSESSING THE MINERALS USING A DILUENT

Mineral and electrolyte concentrations in plasma tend to remain within fairly narrow normal limits (constant) regardless of total body status (Lackey *et al,* 1995; Lobingier *et al,* 1992). The kidneys are responsible for maintaining normal blood concentrations of many electrolytes by modifying the urinary electrolyte concentration through renal

excretion and retention (Lobingier *et al,* 1992) which justifies the used urine to assess the electrolyte/minerals status (Butcher and Harris, 1957; Caple *et al,* 1982). However, the effect of this is to create extremely wide ranges of electrolyte concentration in urine.

Flame photometer and atomic absorption (A. A.) methods, as they are linear over large concentration ranges, are the most commonly recommended methods for the assessment of minerals in urine, except for phosphorus. Because these instruments are expensive and the methods laborious, they are not routinely used for mineral and electrolyte analysis by most clinical laboratories. The vast majority of clinical laboratories (medical and veterinary) use Ion Selective Electrode directs measurement (ISE) and photo-colorimetric methods for detection of minerals and electrolytes in plasma (Murex Biotec, 1999). Urine minerals and electrolytes assays are very infrequently requested in routine clinical laboratory diagnosis. However, the limited linearity range and very high variability of urine mineral concentration, are constraints to the use of ISE and photo-colorimetric methods in urine mineral assessment (Christopher *et al,* 1996). Therefore, an adaptation of the standard methods for serum assay has to be made (Christopher *et al,* 1996). One of the approaches is to mix the urine with a "diluent", designed to have a final mineral concentration, which falls into the linear and controlled/standardised range of the ISE and photo-colorimetric method for the particular electrolyte/mineral. Such a urine diluent is usually made up to contain mineral concentrations near the lower end of this range, so that the contribution of minerals from the urine tends to push the final concentrations to the middle of this range (Christopher *et al,* 1996).

3. SYNOPSIS OF THE BACKGROUND TO THE PROBLEM

Deficient intake of electrolytes/minerals (Ca, Na, K, Cl, Mg, PO^{-}_{4}) or increased losses due to metabolic diseases can lead to poor production and even overt clinical disease in cattle and small ruminants.

Consequently, in situations where the adequacy of minerals and electrolytes intake is unknown and/or difficult to control (e.g. animals grazing/browsing natural vegetation without supplementation), it would be advantageous for making nutrient management decision, to be able to obtain an index of the mineral/electrolyte status of such animals.

Previously published data shows, even in the face of severe dietary deficiency or metabolic losses, that the plasma mineral/electrolyte concentrations remain within or very close to the normal (or reference) range until the onset of clinical signs (Fleming *et al,* 1992), mitigating against early detection or correction (Payne, 1977; Payne and Payne, 1987).

The homeostatic mechanisms responsible for this persistence of normal plasma levels of minerals and electrolytes act principally through renal control (McInnes *et al,* 1972). Furthermore, the composition of urine can be varied, and many homeostatic regulatory mechanisms minimise or prevent changes in extracellular fluid by changing the amount of water and various specific solutes, including electrolytes, in urine (Duncan *et al,* 1994; Ganong, 1995).

As a consequence of this role of the kidney in homeostic control leadind to plasma mineral and electrolyte stability, urine electrolyte concentrations vary widely, from unmeasurably low during dietary deprivation to concentrations far in excess of plasma values when the body load is in excess of requirement rather than plasma or serum (Christopher *et al,* 1996; Lackey *et al,* 1995).

Consequently, any dietary deficiency of essential minerals and electrolytes and inadequate correction of metabolic disorders could be better detected by monitoring the renal excretion or retention of minerals and electrolytes rather than plasma or serum (Christopher *et al,* 1996; Lackey *et al,* 1995; Payne and Payne, 1987).

Urinary electrolyte concentration is difficult to interpret unless water consumption/hydration status is known (Christopher *et al,* 1996; Payne, 1977; Payne and Payne, 1987). Published data confirms that this problem can, to a large extent, be resolved by relating the concentration of a mineral and electrolyte to the concentration of creatinine in the urine, i.e. the mineral and electrolyte:creatinine ratio (E/Cr or FEx). This ratio has been shown to be fairly stable, in the face of constant mineral/electrolyte intake, because the production (and therefore excretion) of creatinine is very constant throughout the day and is not influenced by changes in water intake, diet or non-renal diseases (Fleming *et al,* 1992; McKinnon, 1998).

However, in the field, mainly for herds which are located far from the clinical laboratories, storage and transportation of urine specimens can become a problem. Particularly when urine collection should be continued over a long period of time. Using the filter paper method and sending the sample by normal mail, could prove to be a reliable method of storage, transport, and preservation for creatinine analysis as well as minerals and electrolytes in urine.

4. PROBLEM STATEMENTS

4.1. It is not known what type of filter paper gives the most consistent and accurate results for assessing analyte concentration and mineral/electrolyte to creatinine ratio in artificial and goat urine by the dried filter paper method (DFPM).

4.2. It is not known whether a single diluent would permit accurate assay of urine mineral concentration, using both the Technicon RA 1000® analyser (Calcium, Magnesium, Creatinine, Chloride) and Novabiomedical™ ISE (Sodium and Potassium) methods.

4.3. It is unknown what the reproducibility is of mineral/electrolyte to creatinine ratios from the DFPM.

4.4. It is not known

4.4.1. Whether the storage temperature influences the dried urine analyte concentration (minerals/electrolytes and creatinine) using the DFPM.

4.4.2. Whether the time period of storage influences the dried urine analyte concentration (minerals/electrolytes and creatinine) using the DFPM.

4.5. It is not known

4.5.1. Whether the volume of goat urine applied to the DFPM could influence the mineral and electrolyte to creatinine ratio.

4.5.2. Whether the different volumes of eluate used for reconstituting goat urine used on the DFPM could influence mineral and electrolyte to creatinine ratio.

4.6. It is not known

4.6.1. If the DFPM mineral/electrolyte to creatinine ratios remain constant for animals on a particular ration.

4.6.2. If the DFPM mineral/electrolyte to creatinine ratios remain constant in the goats on a constant diet subjected to different levels of water deprivation.

4.7. It is not known how the DFPM will be accepted and useful among veterinarians for the provision of health services to rural areas. (Note: This problem is seen as one to be addressed at a later stage)

5. OBJECTIVES

5.1. To determine which type of filter paper gives the most consistent and accurate results for assessing analyte concentration and mineral and electrolyte:creatinine ratio in artificial and goat urine by DFPM.

5.2. To determine if a single diluent permit a accurate assay of urine mineral concentration on filter paper method, using both Technicon RA® 1000 analyser (Calcium, Creatinine, Chloride, Magnesium, Phosphate) and Novabiomedical™ ISE (Sodium and Potassium) methods.

5.3. To determine the reproducibility of the analyte concentrations and mineral/electrolyte to creatinine ratio in artificial urine and goat urine by DFPM.

5.4.1. To determine if the storage temperature influences the dried urine analyte concentration (minerals/electrolytes and creatinine) using the DFPM.

5.4.2. To determine if the time period of storage influences the dried urine analyte concentration (minerals/electrolytes and creatinine) using the DFPM.

5.5.1. To determine the effect of different volumes of urine in the applied DFPM on the recovery of mineral/electrolyte to creatinine ratio.

5.5.2. To determine the effect different volumes of eluate used for reconstitution in the DFPM on the recovery of mineral/electrolyte to creatinine ratio.

5.6.1. To determine if the DFPM mineral/electrolyte to creatinine ratios remain constant in goats on a constant ration.

5.6.2. To determine if the DFPM mineral/electrolyte to creatinine ratios remain constant in goats subjected to different levels of water deprivation.

5.7. To determine the acceptability of the DFPM for assessing electrolyte concentrations in urine and nutritional status of domestic animals for the provision of health services to rural areas. Emphasis on broadcasting advertisements for the information of Veterinarians about the usefulness of the method and contact with other institutions to promote the method. (Note: This objective will be addressed in a separate investigation).

6. RESEARCH QUESTIONS

6.1. What type of filter paper will give the most consistent and accurate results, for assessing mineral/electrolyte to creatinine ratio in artificial and goat urine by the DFPM?

6.2. Would a single diluent permit an accurate assay of urine mineral concentration, using both Technicon RA 1000® analyser (Calcium, Magnesium, Creatinine, Chloride) and Novabiomedical™ ISE (Sodium and Potassium) methods?

6.3. Will the DFPM give reliable reproducibility of mineral and creatinine or mineral/electrolyte to creatinine ratio, to be of use as the transporting method of urine from the field without any preservation?

6.4.1. How does the storage temperature affect the mineral and creatinine concentration and mineral/electrolyte to creatinine ratio in artificial and goat urine when the DFPM is used?

6.4.2. How does the period of storage affect the mineral/electrolytes and creatinine concentration and mineral/electrolyte to creatinine ratio in artificial and goat urine when the DFPM is used?

6.5.1. Does the volume of applied urine on the DFPM affect the mineral/electrolyte to creatinine ratio recovery?

6.5.2. Does the volume of eluate used in the reconstitution in DFPM affect the mineral/electrolyte to creatinine ratio recovery?

6.6.1. Do constant diet ration in goats affect the DFPM mineral/electrolyte to creatinine ratio?

6.6.2. Do different levels of water deprivation in goats affect the DFPM mineral/electrolyte to creatinine ratio?

7. MATERIAL AND METHODS

(Note – The study was divided into two phases as set out below 7.2.)

7.1 Procedures Common To Both Phases

7.1.1. Solutions, materials, and formulae

a) Artificial urine

In an attempt to find a means of accurate diagnosis, which is applicable to urine collected from animals in rural areas, it was intended to test an artificial urine containing specific amounts of Na, K, Cl, Mg, Ca, P, and creatinine. Based on data from the literature (Altman and Dittner, 1972), as well as some in-house records of the concentration of minerals and electrolytes in the urine of ruminants, a set of artificial urine samples were made up to represent the expected range of mineral/electrolyte concentrations as follows.

a) Creatinine (µmol/l) – 2873 – 9500
b) Calcium (mmol/l) – 0.625 – 4.99
c) Chloride (mmol/l) – 7.05 – 141
d) Magnesium (mmol/l) – 2.06 – 12.3
e) Sodium (mmol/l) – 2.18 – 63.5
f) Potassium (mmol/l) – 0.02 – 293
g) Phosphorus (mmol/l) – 0.09 – 2.96

A stock solution (Artificial Urine, A.U.1) was formulated to contain minerals/electrolytes at levels that are regarded as "high" concentrations (Table 1). The Analytical reagents were weighed and dissolved in double distilled de-ionised water (DDDW) as presented in Table 2.

Table 1: Constituent concentration and the compounds weighed out in order to prepare the Artificial Urine solution 1 (A.U. 1)

	$MgCl_26H_2O$[a] (2.13424 g/l)	$CaCl_2$[b] (0.38848g/l)	KCl[a] (3.69098g/l)	NaCl[a] (3.71089 g/l)	$NH_4H_2PO_4$[b] (0.2875 g/l)	$KHCO_3$[c] (24.37494 g/l)	$C_4H_7N_3O$[a] (1.07464 g/l)	Total (mmol/l)
Mg	10.5 mmol/l							10.5
Ca		3.5 mmol/l						3.5
PO_4^-					2.5 mmo			2.5
Na				63.5 mmol/l				63.5
Cl	21 mmol/l	7 mmol/l	49.5 mmol/l	63.5 mmol/l				141
K			49.5 mmo/l			243.5 mmol/l		263
Cr							9.5 mmo/l	9.5

Artificial urine (A.U.1) was diluted with DDDW, as indicated in Table 2 to yield a further eight solutions of decreasing concentration and each identified (A.U.2 to A.U.9).

The concentration range of the artificial urine, therefore, represented two orders of magnitude (100 fold), this range being consistent with that found in urine samples in the laboratory's in-house experience.

Table 2: Constituents concentration (mmol/l) made up with different dilution ratios of A.U. and DDDW

A.U.	*A.U.1* (mmol/l)	*A.U.2* (mmol/l)	*A.U.3* (mmol/l)	*A.U.4* (mmol/l)	*A.U.5* (mmol/l)	*A.U.6* (mmol/l)	*A.U.7* (mmol/l)	*A.U.8* (mmol/l)	*A.U.9* (mmol/l)
D. rate	*1*	*1:1.5*	*1:2.5*	*1:5*	*1:10*	*1:15*	*1:30*	*1:50*	*1:100*
Mg	12.3	8.2	4.92	2.46	1.23	0.82	0.41	0.246	0.123
Ca	4.99	3.33	1.996	0.998	0.499	0.333	0.166	0.0998	0.0499
PO_4^-	2.96	1.97	1.184	0.59	0.296	0.197	0.0987	0.059	0.0296
Cl	141	94	56.4	28.2	14.1	9.4	4.7	2.82	1.41
Na	63.5	42.3	25.4	12.7	6.35	4.2	2.12	1.27	0.635
K	293	195.3	117.2	58.6	29.3	19.53	9.767	5.88	2.93
Cr	9.57	6.38	3.83	1.91	0.957	0.638	0.319	0.191	0.0957

Where:

Conc. = Concentration; Mg = Magnesium; Ca = Calcium; PO_4^- = Phosphate; Na = Sodium; Cl = Chloride; K = Potassium; Cr = Creatinine; A.U. = Artiificial urine; D.Rate = dilution rate

[a] Univar®, Saarchem-Holpro (Pty), Krugersdorp, Gauteng, South Africa
[b] Merck™ NT Laboratory Supplies (Pty) Ltd, Midrand, Gauteng, South Africa
[c] Sigma-Aldrich, Vorna Valley 1686, Gauteng, South Africa

b) Diluent

By weighing out analytical reagents and dissolving them in DDDW as set out in Table 3, a diluent was made containing minerals and electrolytes and creatinine at the lower end of the physiological range experienced in serum.

Table 3: Constituents concentrations and pure Chimicals to prepare the diluent

	$MgCl_26H_2O$ (0.10163 g/l)	$CaCl_2$ (0.19979 g/l)	KCl (0.26098 g/l)	NaCl (5.37057 g/l)	$NH_4H_2PO_4$ (0.0575 g/l)	Na_2HCO_3[a] (4.04054 g/l)	$C_4H_7N_3O$ (0.00905 g/l)	Total (g/l)
Mg	0.5 mmol/l							0.5
Ca		1.8 mmol/l						1.8
PO_4^{-2}					0.5 mmol/l			0.5
Na				91.9 mmol/l		48.1 mmol/l		140
Cl	1.0 mmol/l	3.6 mmol/l	3.5 mmol/l	91.9 mmol/l				100
K			3.5 mmol/l					3.5
Cr							0.8 mmol/l	0.8

Where:
Electr. = Electrolytes; D. Rate = dilution rate; AU = Artificial urine minerals concentration; Conc. = Concentration; Mg = Magnesium; Ca = Calcium; PO^-_4 = Phosphate; Na = Sodium; Cl = Chloride; K = Potassium; Cr = Creatinine

Each compound was weighed out using a balance (Mettler, AT201, Mettler Products®, Microsep[b] (PTY) LTD, RSA), calibrated by W & TS, RSA[c]. Both diluent and artificial urine solutions were prepared by dissolving different compounds in water at 25^0C, and then kept in volumetric flasks, as indicated in Tables 3, 4, and 5. The pH of both the diluent and artificial urine was 8.6, approximately the same as ruminant urine. Because the artificial urine is relatively alkaline, containing a large amount of potassium carbonate, the calcium and phosphates become insoluble (Payne and Payne, 1987). Thus, to solubilise these compounds, both diluent and artificial urine solutions were acidified to pH 5.2 with the addition of 0.5 ml of sulphuric acid in 350 ml of diluent.

[a] Univar®, Saarchem-Holpro (Pty), Krugersdorp, Gauteng, South Africa
[b] Mettler, AT201, Mettler Products®, (Pty), Ltd, Bramley, eastgate, Johannesburg, South Africa
[c] Weighing & Technology services, P.O. Box 49248, Hercules, 0084, Pretoria, South Africa

c) Calculations and formulae

- The concentration (calculated concentration) of the minerals/electrolyte and creatinine of the eluted solution (after assay) was calculated using the following formula:

$$\mathbf{Conc_A.=(50*Reading)-(49*EC)} \quad \textbf{(2)}.$$

Where:

$Conc_A$ = concentration of each analyte in eluted solution.

Reading = Reading values obtained by analysis of elution solution for each analyte in artificial urine and/or diluent as well as goat urine.

EC= concentration of each analyte in the diluent (eluent).

- For calculation of analyte concentration from the reagents/compounds the following formulas were used (De Freiras 1985):

1. For calculation mineral and electrolyte concentration for artificial urine and diluent solutions:
   a. For calculation of the concentration of the mineral and electrolyte (x) in reagent/compound:

$$mmol/l(x) = \frac{mg/l(x)}{MM(x)} \quad \textbf{(3)}$$

   b. For calculation of minerals/electrolyte (x) mass in a specific reagent/compound (y):

$$X_{(g)} = \frac{MM_{(y)}}{MM_{(x)}} \quad \textbf{(4)}$$

2. For calculation of creatinine mass from the reagents/compound and concentration for artificial urine and/or diluent, following formulae was used:

   a. $$mmol/l = \frac{mg/l}{MM} \quad \textbf{(5)}$$

   b. $$\mu mol/l = mmol/l * 1000 \quad \textbf{(6)}$$

3. For calculation of different volumes of Artificial Urine 1 (A.U.1) for each analyte, used to make up other Artificial Urine (A.U.2 to A.U.9) solution, consider that the concentration is known:

$$C_1V_1 = C_2V_2 \qquad (7)$$

Where:

MM (y) = Molecular mass of the compound

MM (x) = Molecular mass of the analytes

C_1= Concentration of substance y in Artificial Urine 1

C2 = Concentration of substance y in Artificial Urine 2 to 9

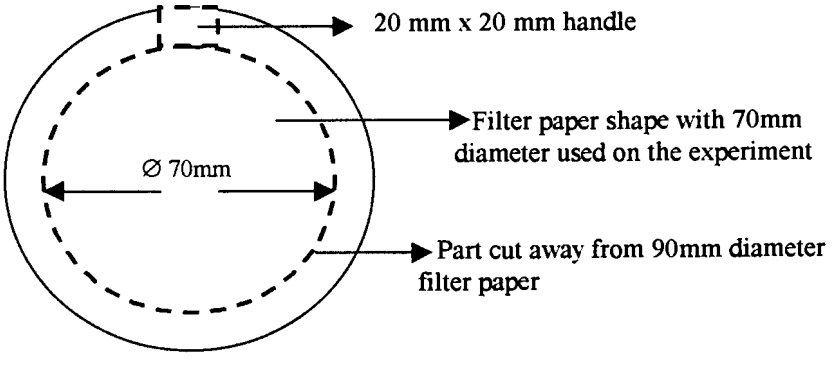
V1 = Volume of Artificial Urine 1

V2 = Volume of Artificial Urine 2 to 9

d) Filter Paper

A trial to evaluate the analyte recovered from different types of filter paper from different brands was conducted (**see results section**). This was the basis for choosing Whatman®[a] filter paper number 6 as this filter paper type gave rather more reliable and consistent results than the others. The Whatman® round filter paper number 6, had been cut in a circular shape with 20 mm handle on the top side and 70 mm in diameter, to be used in all experiments, see Figure 1:

Figure 1: Schematic drawing of the filter paper used in the experiments



[a] Merck™, Laboratory Supplies, Midrand, Gauteng, South Africa

e) Procedures in General

The filter papers were impregnated with 0.5 ml of **artificial/goats urine** (except in the experiment designed to evaluate the impregnation volume). After the filter paper was impregnated, it was hung by clipping the "handle" onto a cord using a paper clip. They were placed, side by side at fixed intervals, in such a way that each filter paper did not come into contact with another. The samples were left to dry at room temperature (22^0C) (room, 3-18, Clinical Pathology Laboratory, 3rd floor, Onderstepoort Veterinary Academic Hospital, with central air conditioning control). After the filter paper was dry (approximately 24 hours), it was stored in a 120mm x 35mm, plastic, disposable petri dish[a]. After each interval of time (**depending on each experiment**), the dried filter paper was eluted with 25 ml of diluent (eluent) in a petri dish. The filter paper was left in this diluent for 60 minutes, while agitating the petri dish by hand, approximately every 15 minutes. The filter paper was then removed from the petri dish and shaken well to remove excess solution. The eluted solution was then transferred to a Technicon® autoanalyser cup[b] for assay. Artificial urine and/or goat urine was used "wet" as control, in each experiment. The control sample of each of artificial/goat urine was kept in a freezer (-18^0C), for the same period of time as the sample dried onto filter paper. The condition of storage, namely, freezing would guarantee the stability of the analytes during the period of time, by decreasing bacterial degradation of creatinine and changing the pH of the urine (Lobingier *et al,* 1992; Soliman *et al,* 1986; Spierto *et al,* 1997). The control was then thawed in the laboratory environment, mixed and 0.5 ml was diluted with 25 ml of diluent, to make up the same volume as used for the filter paper method. The solution was transferred to a Technicon® autoanalyser cup for assay. The analyte concentrations in artificial urine and/or goat urine were calculated using the formula (**2**), see above.

[a] Concord Group Markenting (Pty), Ltd, Londgdale, Johannesburg, South Africa
[b] Technicon RA® 1000, Bayer (Pty) Ltd, Health Care Division, Business Unit, Isando, Gauteng, South Africa

7.1.2. Analytical Methods

A Technicon RA 1000™[a] (random access wet-chemistry analyser) for creatinine, phosphate, chloride, magnesium, and calcium, and a Novabiomedical™[b] (Ion Selective Electrode analyser) for sodium and potassium for blood/serum assay, from the Section of Clinical Pathology of the Department of Medicine of the Faculty of Veterinary Science were used.

a. **Phosphate (PO^{-}_{4}):** The method of phosphorus analysis is based on the principle of reduction of phosphomolybdate to molybdenum blue by stannous chloride-hydrazine sulphate (Amador and Urban, 1972; Daly and Ertingshausen, 1972). The method is based on the fact that the unreduced phosphomolybdate complex absorbs ultraviolet light, which can be read at 340 nm (Amador and Urban, 1972). The stannous chloride and aminonapthosulfonic acid are used as reducing agents (Technicon RA® systems, 1994). Of 305 clinical laboratories on the Murex Biotech, quality assessment programme (QA programme) cycle report 13, only 7 do not use the phosphomolybdate method (Murex Biotec, 1999), which means that the method is widely used and representative of routine assays. The reagents used were Bayer diagnostic®[c] USA, catalogue T01-1303-05, method no SM4-0144E94 (Technicon RA® systems, 1994), for the Technicon RA 1000™ analyser.

b. **Chloride (Cl):** The reagent, and equilibrium solution of mercury, ferric, and thiocyanate (Schoenfeld and Lewellen, 1964; Skeggs and Hochstrasser, 1964; Zall *et al*, 1956), when combined with chloride ions, undergoes a double displacement reaction forming a yellow-brown chromophore (ferric thiocyanate). The quantitative end point method, is read at 500 nm. The following conditions or compounds interfere significantly with this method:

[a] Technicon RA® 1000, Bayer (Pty) Ltd, Health Care Division, Business Unit, Isando, Gauteng, South Africa
[b] Novabiomedical™, ISE, Waltam, electrolyte Analyses, The Scientific Group, Lynnwood, Pretoria, South Africa
[c] Bayer, Healthcare Division, Laboratory Business Unit, Isando 1600, South Africa

systems, 1994). This method is used by 32 out of 346 clinical laboratories world wide (Murex Biotec, 1999). Other methods such as ion selective electrode (ISE), which is the most common by used method reported by Murex Biotech cycle report 13 (1999). Due to the analyser used in this Clinical Pathology Laboratory and applicability to the DFPM, the mercuric/ferric method was chosen as a routine method. The reagents used were Bayer diagnostic®, USA, catalogue T01-1474-03, method no SM4-0162E94 (Technicon RA® systems, 1994), for the Technicon RA 1000™ analyser.

c. **Magnesium (Mg):** Xylidyl blue in an alkaline medium forms a red chelate with magnesium (Mann, 1957; Mann and Yoe, 1956). The reagent was modified to eliminate the use of organic solvents and thereby enable this method to be performed on the Technicon RA 1000™ (Technicon RA® systems, 1994). The change in absorbance at 500 nm is directly proportional to the magnesium concentration (Technicon RA® systems, 1994). This method has been reported as the most commonly used by clinical laboratories as a routine method. Of 259 clinical laboratories 62 used xilidyl blue, whereas 41 clinical laboratories use calmagite and 15 used colorimetric methods. Only 15 of 259 laboratories use atomic absorption (Murex Biotec, 1999). On the basis of these numbers, the method represents routine analysis in most clinical laboratories. The reagents used were bioMérieux, SA[a], catalogue 61411 adapted to method no SM4-0162E94, (Technicon RA® systems, 1994) for the Technicon RA 1000™ analyser.

d. **Calcium (Ca):** The method is based on using an alkaline medium where θ-cresolphthalein complexone forms a coloured complex with calcium ions, which is measured at 550 nm (Kessler and Wolfman, 1964). The 8-hydroxyquinoline in the reagent binds the free magnesium, minimising interference from magnesium (Gitelman, 1967). Reports show that of the 378

[a] bioMérieux, Marcy l'Etoile, France

interference from magnesium (Gitelman, 1967). Reports show that of the 378 laboratories world-wide, with a "full" set of calcium assay results, 180 use θ-cresolphtalein, while 14 use indirect selective electrode, 5 use other methods including atomic absorption, and 103 use other colorimetric methods (Murex Biotec, 1999). Taking these numbers into consideration, θ-cresolphthalein complexone is the most commonly used method for routine analysis. The reagents used were Boehringer Mannheim, GmbH[a], Germany catalogue Sys 1 1489216, adapted to method no SM4-0162E94, (Technicon RA® systems, 1994), for the Technicon RA 1000™ analyser.

e. **Sodium (Na):** The indirect Ion Selective Electrode method measures potentiometric changes as a function of ion concentration, which quantifies the Chimical activity of the ion. Sodium was analysed using the ISE-indirect method. Supporting the option to choose the ISE-indirect method, is the fact that of 368 clinical laboratories 204 use the indirect ISE method for sodium analysis, wheres 102 use direct ISE (Murex Biotec, 1999). The reagents used were catalogue 15646, Nova Biomedical™ ISE analyser, Waltham®, Novabiomedical, USA, MA 02254 (Novabiomedical™, 1993).

f. **Potassium (K):** Potassium was analysed using the ISE-indirect method. Supporting the option to choose the ISE-indirect method, is the fact that of 369 clinical laboratories 209 use the ISE-indirect method for routine analysis for potassium, whereas 103 use ISE-direct (Murex Biotec, 1999). The reagents used were catalogue 15646, Novabiomedical™ ISE analyser, Waltham®, Novabiomedical, USA, MA 02254 (Novabiomedical™, 1993).

g. **Creatinine (Cr.):** Creatinine reacts with picric acid in an alkaline medium to produce a red coloured complex (Kinetic Jaffé reaction method) (Jaffé, 1886; Rossignol *et al*, 1984). The rate of production of this complex, in a selected time interval, is proportional to the original creatinine concentration. The

a Boehring Mannehein, Germany, Representative Laboratory Diagnostic, Randburg, Sourh Africa

systems, 1994). In support of using this method, 234 clinical laboratories out of 387 clinical laboratories used the kinetic Jaffé reaction method for creatinine assay, whereas 20 used buffered picrate and 61 used the enzymatic method (Murex Biotec, 1999). The reagents used were Bayer diagnostic® USA, catalogue T21-1929-02, method no SM4-0160E94 (Technicon RA® systems, 1994), for the Technicon RA 1000™ analyser.

7.1.3. Statistical Methods

Statistical processing was performed using Sigmastat[a] and Excel 97[a]. Because the data obtained in most instances were either discrete variables, or not distributed normally, non-parametric methods of data analysis were used. With all tests, significance was accepted if $P<0.05$. The Mean, Standard deviation, and coefficient of variation, significant difference between control and filter paper method; interclass correlation between the method and wet control; slope and intercept for recovery of the analyte concentration and the mineral/electrolyte to creatinine ratio were used as a criteria for the analysis. Excel 97 was used for Graph generation. Futher, specific statistical methods are mentioned for each experiment (*vide infra*).

7.2. Experimental Design and Model System

The investigation was divided into two phases:

Phase 1, was conducted entirely *in vitro*, using simulated urine (Artificial urine) dried on filter paper, manipulated in various ways under laboratory conditions.

Phase 2, Involved certain *in vivo* manipulation of goat hydration status, using urine (from water deprived goats) dried on filter paper, to determine variation of the mineral/electrolyte to creatinine ratio.

The goats were part of a separate trial and the urine was to be collected as part of that trial.

[a] SigmaStat for windows Version 3.2, Jandel Corporation, San Rafael, CA, USA

The goats were part of a separate trial and the urine was to be collected as part of that trial.

Therefore, 24-hour urine collection was avoided, making the method simpler, more practical and applicable to field conditions.

7.2.1. PHASE I *(In vitro)*.

7.2.1.1. – TESTING DIFFERENT TYPES OF THE FILTER PAPER

Objectives: To determine which type of filter paper gives the most consistent and accurate results for assessing analyte concentration and mineral/electrolyte to creatinine ratio in artificial and goat urine by DFPM.

7.2.1.1.1. Model

The experiment was conducted entirely *in vitro*, using simulated urine (Artificial urine) dried on filter paper. There was a wide range of filter papers available from different brands. For this particular experiment the filter papers were chosen from two manufactures (Whatman® and Scheicher & Schuell). During the trial seven types of Whatman® filter paper were selected, (numbers 2, 3, 5, 6, 42, 44, 542,) and five types of Scheicher & Schuell, (numbers 860, 593, 595, 597, 598).

7.2.1.1.2. Experimental Design and Procedures

Ten replicates of each filter paper type were impregnated with artificial urine, as described in 7.1.1.e. A matching sample of each of artificial urine was kept in a freezer (-18^{0}C), for the same period of time to be used as a control. After ten days the filter paper replicates were processed by the same procedure as described in 7.1.1.e.

7.2.1.1.3. Statistical Methods

Mean and standard deviation of mineral/electrolyte to creatinine ratio results were performed. A t-test compared statistical differences between the reference (control) and filter paper.

7.2.1.2 – TESTING THE DILUENT

Objective 1: To determine if a single diluent permits an accurate assay of urine mineral concentration using the filter paper method, using both Technicon RA® 1000 analyser (Calcium, Creatinine, Chloride, Magnesium, Phosphate) and Novabiomedical™ ISE (Sodium and Potassium) methods.

7.2.1.2.1. Model

The experiment was conducted *in vitro*, using a simulated urine (artificial urine). The diluent made for this purpose should have a final mineral concentration which falls into the linear and controlled/standardised range for the particular electrolyte/mineral applied to the analysis of plasma/serum. Such urine diluent is made up to contain mineral concentrations near the lower end of this range, so that the contribution of minerals from the urine tends to push the final concentrations to the middle of this range, in order to get sample readings in the linear range.

7.2.1.2.2. Experimental Design and Procedures

Statistical evaluation of agreement between two methods for measuring a quantitative variable (Lee *et al* 1989) was the design used in this experiment which attempted to measure the closeness of linear relationship between the expected analyte concentrations against calculated analyte concentrations of artificial urine diluted with a specific diluent (eluent). Five samples of each artificial urine solution (**Table 2**) were diluted 1:50 with

the diluent and analysed for magnesium, calcium, phosphate, chloride, and creatinine. The readings obtained from the analysis were used to calculate the concentration of each mineral in the artificial urine. The analyte concentration was calculated by the formula (**2**).

7.2.1.2.3. Statistical Methods

Descriptive statistics of the mean and standard deviation of mineral/electrolyte to creatinine ratio were recorded. To measure the agreement between the calculated and assayed results, interclass correlations (Lee *et al,* 1989) were determined for each analyte. To measure the agreement between calculated and assayed results, bias plots were constructed in which the difference between the methods is shown in Figures (1-8).

7.2.1.3. – DETERMINATION OF THE REPRODUCIBILITY OF THE METHOD

Objectives: To determine the reproducibility of the analyte concentrations and mineral/electrolyte to creatinine ratio in artificial urine and goat urine by the DFPM.

7.2.1.3.1. Model

The experiment was conducted entirely *in vitro*, using artificial urine dried on Whatman® number 6 filter paper (see procedures in 7.1.1.d.). A series of dilutions as described in 7.1.1.b with DDDW of artificial urine was made, from a high concentration solution. After ten days the filter paper and control sample were analysed as described in 7.1.1.e.

7.2.1.3.2. Experimental Design and Procedures

The experimental design specified in this experiment attempts to determine the coefficient variation of analytes within the samples, which will show the degree of reproducibility of artificial urine impregnated in filter paper. A total of 40 samples (10 for each artificial urine concentration (normal and dilution 1:5) for the filter paper method and 10 for a

control), (**Table 4**) were used for this trial. Ten days was considered a suitable period of time to simulate "field" conditons.

Table 4: Two different artificial urine concentrations used for testing the reproducibility and recovery of analytes from the dried filter paper method

	Artificial Urine (A.U.1)		Dilution 1:5 of A.U. 1	
	Concentration (mmol/l)	E:Cr ratio	Concentration (mmol/l)	E:Cr ratio
Analytes				
Phosphate	2.556	0.02664	0.5132	0.02702
Chloride	126.6	1.31930	25.32	1.3319
Magnesium	9.652	0.10058	1.9304	0.10164
Calcium	2.794	0.02912	0.5588	0.02942
Sodium	54	0.56273	10.8	0.56866
Potassium	262.2	2.73239	52.44	2.76116
Creatinine	9.596		1.8992	

Where:

E:Cr = mineral/electrolyte to creatinine ratio

7.2.1.3.3. Statistical Methods

Descriptive statistics (mean and standard deviation) and coefficient of variation of samples were performed.

7.2.1.4. - TESTING THE EFFECT OF TEMPERATURE AND STORAGE TIME ON THE METHOD

Objectives 1: To determine if the storage temperature influences the dried urine analyte concentration (minerals/electrolytes and creatinine) on the DFPM.

Objective 2: To determine if the time period of storage influences the dried urine analyte concentration (minerals/electrolytes and creatinine) on the DFPM.

7.2.1.4.1 Model

The experiment was conducted *in vitro* using artificial/goat urine at two different temperatures, controlled laboratory temperature (22°C) and high ambient temperature typical of Southern Africa (30^0C). An incubator was used to obtain a temperature of 30°C. In order to determine the effect of time, daily intervals, up to Ten (10) days, were used. This attempted to simulate the environment that the filter paper will be subjected to during transport by regular mail. However, using just artificial urine does not express the real situation. As the target animal model for application of the DFPM is the goat; it is incumbent on the investigators to evaluate the method using goat urine, if at all possible.

The urine samples were collected from a pool of urine from three goats clinically evaluated, which presented no renal disease. The clinical status of the animals was evaluated by Dr. H. Zulch (Department of Physiology, Veterinary Science Faculty, University Of Pretoria). The goats were kept in metabolic crates at OVARU (Onderstepoort Veterinary Academic Reasearch Unit). The goat urine analysis followed the same procedure described above.

7.2.1.4.2. Experimental design and Procedures 1

Testing the effect of temperature and period of time on the Dry Filter Paper Method for mineral/electrolyte to creatinine ratio in artificial urine

Comparing two independent samples of equal size was the design used for this trial. A total of 120 samples of artificial urine (6 replicates for each temperature, for each day) were used for this trial. A matching sample of the artificial urine was kept in a freezer (-18^0C), for the same period of time as a control. On each day the six replicates for that day were analysed and compared with the results of the frozen (control) sample of that day.

7.2.1.4.3. Experimental Design and Procedures 2

Testing the effect of temperature and period of time on Dry Filter Paper Method for mineral/electrolyte to creatinine ratio in goat urine.

Comparing two independent samples with equal size was the design used for this trial. A total of 120 samples of three different goat urine (6 replicates for each temperature, for each day) were used for this trial. A matching sample of each of three different goat urine samples was kept in a freezer (-18^{0}C), for the same period of time and used as a control. On each day the six replicates for that day were analysed and compared with the results of the frozen (control), sample of that day.

7.2.1.4.4. Statistical Method

Descriptive statistics (mean and standard deviation) were performed. The t-test was used to compare statistical differences between the reference (control) and temperature (22^{0}C and 30^{0}C). Regression analysis was used to compare the variaton of mineral/electrolyte to creatinine ratio over the period of time (10 days).

7.2.1.5. - TESTING THE EFFECT OF DIFFERENT VOLUMES OF IMPREGNATION AND DIFFERENT URINE DILUTIONS ON THE METHOD

Objective 1: To determine if different volumes applied to the DFPM would influence the mineral/electrolyte to creatinine ratio.

Objective 2: To determine if different urine dilutions used in the DFPM, would influence the mineral/electrolyte to creatinine ratio.

The volume of urine in fieldwork is difficult to measure, mainly if this work is related to animals which are grazing normally. This is particularly difficult when the location of urine collection is far from the laboratory, as well as storage and transportation. Consequently, the assumption has been made that any volume of urine can be used, as a sample for urine minerals analysis, as the mineral/electrolyte to creatinine ratio should not vary. The goat urine samples were collected as mentioned above 7.2.1.4.1.

7.2.1.5.2. Experimental Design and Procedure

The urine pool was diluted (1:5 and 1:10 dilution respectively) with DDDW, to give different concentrations of analytes. The volumes are shown in **Table 5**, where the volume of 0.7 ml was the maximum volume which the filter paper could absorb. This specific volume was considered as the dipping volume. The dipping volume means submerging the filter paper into a container completely with the available amount of urine until it was completely soaked. Five filter paper samples for each volume of urine were prepared. An overall of 120 samples were prepared and analysed for this experiment. The controls for each volume of the 3 different urine samples (**Table 5**), were placed in small glass vials (capped with plastic lids) at the same time as impregnation of the filter paper, and kept in the refrigerator during the same period of time (1 week). A diluent which was slightly different form the diluent in 7.1.1.b was used, Appendix Table 16.

Table 5: Different urine concentrations and volume of urine impregnated on each filter paper

Goat Urine	Urine volume (ml)			
U1 (normal)	0.175	0.35	0.525	0.7
U2 (Dilution 1:5)	0.175	0.35	0.525	0.7
U3 (Dilution 1:10)	0.175	0.35	0.525	0.7

Where:

U.1-3 = Three different goat urine concentrations (normal; Dilution 1:5; Dilution 1:10)

7.2.1.5.3. Statistical Method

7.2.1.5.3. Statistical Method

The statistics were performed using Sigmastat. Comparison of the control results and the DFPM (method) results was performed by using the t-test. The Mann-Whitney rank sum test was performed for results, if the test for normality of data failed.

Two way ANOVA's were used to compare statistical differences between different volumes of impregnation and different goat urine dilutions on the Dry Filter Paper Method. If differences were found between the groups means, the Tukey test for all pairwise multiple comparisons was used to determine which group differed.

7.2.2. PHASE II (*in vitro and in-vivo*)

7.2.2.1. - TESTING THE EFFECT OF DIFFERENCES IN WATER INTAKE ON MINERAL/ELECTROLYTE TO CREATININE RATIO IN GOATS URINE BY USING THE METHOD

Objectives : To determine if different levels of water deprivation in goats will affect the DFPM mineral/electrolyte to creatinine ratio.

7.2.2.1.1. Model

It is assumed that the mineral/electrolyte to creatinine ratio (E/Cr.) of a one-off urine sample is a reliable indicator of the 24-hour mineral excretion. This is based on a further assumption, that the use of creatinine concentration as the denominator "protects" the data from variation due to the concentration of urine. As these goats were water-deprived it provided a unique opportunity to test this assumption.

The *in-vivo* component of this experiment was conducted in conjunction with research project NR 36.5.369 of the Department of Physiology of Faculty of Vetrinary Science. The goats, housed in metabolic crates at OVARU were used with the permission of the

principal investigator of the project mentioned above. Urine was naturally voided into collecting containers. The collecting containers were placed within a general–house controlled cooling system.

The goats were divided into 3 groups of 5 goats each. All the goats were fed a diet of the same composition prior and during the sample collection process and were subjected to the following water intake regime:

1. Group 1 (Water *ad libitum*).
2. Group 2 (Restricted 50 % of water – water intake every 2 days).
3. Group 3 (Restricted 30 % of water – water intake every 3 days).

Both temperature and humidity were controlled in the metabolic rooms where the animals were housed. Urine samples were collected every morning between 7:30 – 8:30, for 6 days.

7.2.2.1.2. Experimental Design and Procedures

A latin squares model was used as experimental design where each treatment appeared once with each subject and an equal number of times in each order of presentation. A single urine sample for each goat of each day (collection lasted for 6 days) was collected and five filter paper samples for each single urine sample were prepared. The samples of the group 1 were divided in two sets, one set for comparison between control (urine) and the method (DFPM) and other set was used to achieve the objective of this experiment. Overall, 450 samples were prepared and analysed for this experiment. A matching sample of each urine sample was placed in a small plastic-capped vial at the same time as impregnation of filter paper and kept in a freezer (-18^0 C), for the same period of time (10 days) as control. The samples and the control followed the same procedure as described in 7.1.1.d. and 7.1.1.e.

7.2.2.1.3. Statistical Methods

To compare differences between methods a statistical "evaluation of agreement" model was used (Lee *et al*, 1989). Two way ANOVA repeated measures on ranks was performed. If differences were found between the groups means, the Tukey method for all pairwise multiple comparisons was used to determine which groups of water treatment differed.

8. RESULTS

8.1. PHASE I

8.1.1. Testing Different Types of Filter Paper

The differences between different filter paper types is shown in Table 6 and 7. The concentration of analytes, from which the ratio has been calculated, is presented in the Appendix (Table 1). The difference in the value between filter paper types and the control (reference) is the difference for all ratio's. Filter paper number 6 (Whatman® brand), showed no significant difference for all ratios P/Cr (0.5125); Cl/Cr (0.7475); Mg/Cr (0.6213), Ca/Cr (0.5917), Na/Cr (0.7561), and K/Cr (0.5984). Although the difference in the *p* values between the filter paper types and control were different, some filter paper types also gave good results, like Whatman® no 42. However, the differences for Whatman® number 6 were smaller.

8.1.2. Testing the Diluent

Each value shown in Table 7 is an average of five measurements. The overall measured concentration of ~~the~~ each analyte did not differ significantly when compared with the expected concentration. Mg results were slightly higher than both expected and calculated results. The other analyte results were slightly lower than the expected values. Agreement between the two methods (the expected value and the calculated value), showed a significant interclass correlation (R_I >0.75) and 95% confidence interval, Mg (0.9912); Ca (0.9941); PO^{-}_4 (0.9895); Na (0.9762); Cl (0.9949); K (0.9924); Cr. (0.9983). The regression calculated values for each analyte showed a discrepancy in slope, but there were no significant outliers when plotting the regressions. However, the values for creatinine, although of acceptable linearity (Figure 1), showed a marked slope discrepancy (-18%). The exact cause of this was not determined. However, a reasonable assumption was that the creatinine analytical reagent had deteriorated with storage time. The reagent, although of acceptable "label" purity, had exceeded its specified shelf life which the

investigator had assumed to be of no significance. The sodium values show large differences, mainly at low values (Figure 6). Figure 2, 3, 4, 5, and 7, show the regression lines between the expected and calculated concentration in the diluent for phosphate, chloride, magnesium, calcium and potassium respectively. A slight variation of 9-13% between ideal regression (expected concentration) line and "best fit" regression line (calculated concentration) does not affect the interclass correlation significantly.

8.1.3. Determination of the Reproducibility of the Method

The reproducibility of the dry filter paper method shows a fair variability with a coefficient of variation (C.V.%) between 0~15 % (Tables 9 and 10). Sodium and calcium concentration as well as the Ca/Cr and Na/Cr ratio show the greatest variability between 12~15%, when compared to other analytes. To simulate urine with different concentrations of analytes and to determine the reproducibility of the method, artificial urine was diluted 1:5 with DDDW. The results are presented in Tables 9 and 10. The coefficient of variation of creatinine concentration in artificial urine (2.844%) was lower in 1:5 dilution. For other minerals and electrolytes as well as for the mineral/elctrolyte to creatinine ratio, the coefficient of variation was always higher in the 1:5 dilution than in the artificial urine, except for K/Cr, which presented with a C.V.% of 2.1899% for the 1:5 dilution against 2.406% in artificial urine.

8.1.4. Testing the Effect of Temperature and the Storage Time on the Method

The mean and standard deviation for the mineral/electrolyte to creatinine ratio of artificial urine and goat urine are presented in Tables 11 and 12 respectively. The concentrations from which the ratios were calculated is presented in Appendix (Tables 1 and 2). Comparison of means between a temperature of 22^0C, 30^0C and the controls are presented. Comparisons of the variation of mineral/electrolyte to creatinine ratio of artificial and goat urine over time (10 days) is presented graphically in Figure 8-13. There were no significant differences between different temperatures and control, except for potassium/creatinine (K/Cr) for the temperature of 30^0C and control for artificial urine

($P<0.001$) as well as between 22^{0}C and control for both artificial ($P<0.001$) and goat urine ($P=0.02$). A different pattern was shown by the mineral/electrolyte to creatinine ratios of artificial urine and goat urine relating to variation over time. Cl/Cr ratio (Figure 9), Ca/Cr ratio (Figure 11), and Na/Cr ratio (Figure 12) show irregular peaks, whereas P/Cr (Figure 1), Mg/Cr ratio (Figure 10), and K/Cr ratio (Figure 13) present more regular partterns for both artificial and goat urine, over time. For the Mg/Cr ratio and the P/Cr ratio there was a significant decrease on day 2 from the corresponding value using the filter paper method for the goat urine. The P/Cr ratio control for goat urine showed a sharp rise after 2 days from a mean 6.43 to a mean 15.81 (Figure 8). The results obtained on each day of the trial, showed a large difference between artificial urine and goat urine for P/Cr (Figure 8), Mg/Cr (Figures 10), and K/Cr (Figure 13), whereas for Cl/Cr ratio (Figure 9), Ca/Cr ratio (Figure 11), and Na/Cr ratio (Figure 12), the differences were small.

8.1.5. Testing the Effect of Different Volumes of Impregnation and Different Urine Dilutions on the Method

Comparisons between the control and the method for mineral/electrolyte to creatinine ratio are presented in Table 13-15. The concentrations of analytes from which the ratios were calculated are presented in Tables 4-7 in the Appendix. There was no statistical difference between the control and the method for all volumes and dilutions except volume 0.525 ml in dilution 1:10, ($P=0.002$), for P/Cr ratio (Table 13). A comparison of the effect of different impregnation volumes and the effect of different urine concentrations (dilutions) on P/Cr ratio, Cl/Cr ratio, Mg/Cr ratio, Ca/Cr ratio, Na/Cr ratio, and K/Cr ratio values, are presented in Tables 16, 17, 18, 19, 20, and 21 respectively. The tables present the mean values of each treatment and the significant difference between the treatment groups. The *p* values are presented in Table 8, in the Appendix. The volumes and dilutions did not affect the P/Cr ratio values significantly (Table 16), and the values were highly interchangeable. In comparisons between volumes, the Cl/Cr ratio (Table 17) values were not affected significanlty by the diluted (1:5) urine, but there was a significant difference between volumes with the 1:10 dilution. The Cl/Cr

ratio values for volumes of 0.175 ml, 0.35 ml and 0.7 ml were significantly affected by dilution. The means between control and 1:10 dilution and between 1:5 dilution and 1:10 dilution differed significantly. The Mg/Cr ratio (Table 18) value for undiluted urine and the 1:5 dilution were not affected significantly, when comparing between volumes, but the values were significantly different between 0.175 ml and 0.35 ml; between 0.175 ml and 0.7 ml; and between 0.35 ml and 0.525 ml for the 1:10 dilution. When comparing the Mg/Cr ratio values of each dilution for each volume of impregnation, there was a significant difference between normal urine and the 1:10 dilution and between the 1:5 and 1:10 dilutions. The Ca/Cr ratio (Table 19) values for undiluted and the 1:5 dilution were not affected significantly, when comparing between volumes, but were significantly different between 0.175 ml and 0.35 ml; between 0.175 ml and 0.7 ml; between 0.175 ml and 0.525 ml; and between 0.35 ml and 0.525 ml for the 1:10 dilution. Comparing the Ca/Cr ratio values of each dilution for volume of impregnation, there was a significant difference between normal urine and the 1:10 dilution and between the 1:5 and 1:10 dilutions. The Na/Cr ratio (Table 20) value was the most significantly affected by the dilutions. All the ratio values of each volume of impregnation were significantly affected by different dilutions. The K/Cr ratio (Table 21) was not affected significantly by application volume for undiluted urine and the 1:5 dilution, but there were significant difference between volumes for the 1:10 dilution. The K/Cr ratio values of all volumes were affected significantly by the dilutions. The values between undiluted urine and the 1:10 dilution and the values between the 1:5 and 1:10 dilution differed significantly.

8.2. TABLES – PHASE I (*in-vitro*)

Table 6: Mineral/electrolyte to creatinine ratio of artificial urine on different filter paper types Whatman®[a] (**), identified by numbers, expressed as mean, standard deviation (Std. Dev.) of three samples. Comparison between control (Ref) and filter paper types (FPT).

	FP no	2**		3**		5**		6**		42**		43**		44**	
Ratio		**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**
P/Cr	**Mean**	0.027	0.217	0.027	0.026	0.027	0.031	0.027	0.029	0.027	0.026	0.027	0.031	0.027	0.033
	Std dev	0.027	0.0095	0.265	0.013	0.027	0.007	0.027	0.004	0.027	0.003	0.027	0.006	0.027	0.009
	P	**0.4448**		**0.8964**		**0.3809**		**0.5125**		**0.775**		**0.502**		**0.3725**	
Cl/Cr	**Mean**	1.299	1.108	1.299	1.632	1.299	1.993	1.299	1.237	1.299	1.989	1.299	2.555	1.299	3.136
	Std dev	0.076	0.26	0.076	0.323	0.076	0.358	0.076	0.282	0.076	0.487	0.076	0.316	0.076	0.041
	P	**0.3319**		**0.2116**		**0.0723**		**0.7465**		**0.1302**		**0.3266**		**0.00005***	
Mg/Cr	**Mean**	1.117	0.103	1.117	0.112	1.117	0.121	1.117	0.112	1.117	0.121	1.117	0.137	1.117	0.127
	Std dev	0.007	0.007	0.007	0.003	0.007	0.011	0.007	0.006	0.007	0.008	0.007	0.001	0.007	0.008
	P	**0.0669**		**0.3337**		**0.3998**		**0.6213**		**0.6096**		**0.2404**		**0.1908**	
Ca/Cr	**Mean**	0.035	0.024	0.352	0.029	0.352	0.042	0.352	0.032	0.352	0.036	0.352	0.04	0.352	0.042
	Std dev	0.004	0.0052	0.435	0.002	0.435	0.003	0.435	0.007	0.435	0.000	0.435	0.000	0.435	0.004
	P	**0.0428***		**0.1208**		**0.1098**		**0.5917**		**0.6971**		**0.9694**		**0.124**	
Na/Cr	**Mean**	0.794	1.712	0.794	1.768	0.794	1.971	0.794	0.839	0.794	1.138	0.794	2.173	0.794	2.481
	Std dev	0.046	0.449	0.046	0.485	0.046	0.398	0.046	0.08	0.046	0.27	0.046	0.342	0.046	0.959
	P	**0.0697**		**0.0723**		**0.0344**		**0.7561**		**0.1541**		**0.0346***		**0.0924**	
K/Cr	**Mean**	3.164	2.812	3.164	2.878	3.164	3.366	3.164	2.962	3.164	2.902	3.164	3.251	3.164	3.226
	Std dev	0.203	0.017	0.203	0.855	0.203	0.169	0.203	0.131	0.203	0.091	0.203	0.048	0.203	0.084
	P	**0.0943**		**0.1211**		**0.2585**		**0.5984**		**0.142**		**0.4312**		**0.6649**	

*** Statistically difference between control and Filter paper type. *P* values for comparison of two groups (T-test)**

[a] Merck™, NT Laboratory supplies (Pty) Ltd, Midrand, Gauteng, South Africa

Table 7: Mineral/electrolyte to creatinine ratio of artificial urine on different filter paper types Whatman®[a] (**) and Schleicher & Schuell[b] (*), identified by numbers, expressed as mean, standard deviation (Std. Dev.) of three samples. Comparison between control (Ref) and filter paper types (FPT).

	FP no	542**		860*		593*		595*		597*		598*	
Ratio		**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**	**Refer**	**FPT**
P/Cr	**Mean**	0.027	0.030	0.027	0.024	0.027	0.026	0.027	0.039	0.027	0.02	0.027	0.018
	Std dev	0.027	0.008	0.027	0.131	0.027	0.008	0.027	0.008	0.027	0.006	0.027	0.008
	P	**0.5486**		**0.6926**		**0.8241**		**0.1217**		**0.1579**		**0.1956**	
Cl/Cr	**Mean**	1.299	2.163	1.299	2.911	1.299	2.167	1.299	2.303	1.299	2.363	1.299	2.605
	Std dev	0.076	0.502	0.076	1.245	0.076	0.26	0.076	0.313	0.076	0.286	0.076	0.297
	P	**0.0935**		**0.1537**		**0.008***		**0.0254***		**0.0179**		**0.0125***	
Mg/Cr	**Mean**	0.117	0.132	0.117	-0.005	0.117	0.137	0.117	0.127	0.117	0.118	0.117	0.117
	Std dev	0.007	0.000	0.007	0.011	0.007	0.003	0.007	0.006	0.007	0.002	0.007	0.002
	P	**0.0792**		**0.0002**		**0.034**		**0.1483**		**0.8859**		**0.9815**	
Ca/Cr	**Mean**	0.035	0.036	0.035	0.033	0.035	0.069	0.035	0.039	0.035	0.036	0.035	0.026
	Std dev	0.004	0.002	0.004	0.004	0.004	0.033	0.004	0.00	0.004	0.008	0.004	0.003
	P	**0.8063**		**0.4897**		**0.2208**		**0.2855**		**0.8579**		**0.0453***	
Na/Cr	**Mean**	0.794	3.05	0.794	1.796	0.794	1.852	0.794	1.75	0.794	1.794	0.794	1.469
	Std dev	0.046	0.586	0.046	0.289	0.046	0.341	0.046	0.617	0.046	0.293	0.046	0.026
	P	**0.0212***		**0.0242***		**0.031***		**0.1145**		**0.0252***		**0.0001***	
K/Cr	**Mean**	3.164	3.253	3.164	3.301	3.164	3.338	3.164	3.242	3.164	3.258	3.164	3.319
	Std dev	0.203	0.091	0.203	0.1	0.203	0.89	0.203	0.05	0.203	0.027	0.203	0.107
	P	**0.5427**		**0.3738**		**0.277**		**0.5795**		**0.5104**		**0.3279**	

*** Statistically difference between control and Filter paper type. *P* values for comparison of two groups (T-test)**

[a] Merck™, NT Laboratory supplies (Pty) Ltd, Midrand, Gauteng, South Africa
[b] Schleicher & Schuell filter papers, Lasec, Laboratory & Scientific Equipment, Pty, Ltd, Cape Town, South Africa

Table 8: Reproducibility of creatinine concentration and minerals/electrolytes to creatinine ratio of artificial urine by the dry filter paper method (DFPM), expressed as mean, standard error, and coefficient of variation (c.v.%) between samples. Ten replicates were read at the artificial urine concentration (AU) and ten at the one fifth of that concentration (1:5).

Ratios	Creatinine (µmol/l)		P/Cr		Cl/Cr		Mg/Cr		Ca/Cr		Na/Cr		K/Cr	
	A.U.	1:5	A.U.	1:5	A.U.	1:5	A.U.	1:5	A.U	1:5	A.U.	1:5	A.U	1:5
1	9612.167	2062.167	0.0245	0.0777	0.04883	0.20335	1.4444	1.3512	2.73178	2.5499	4.1545	10.8785	0.12261	0.2442
2	9662.167	2062.167	0.0259	0.0666	0.04340	0.17910	1.4369	1.3982	2.76939	2.5499	4.3917	15.7278	0.12197	0.2272
3	9612.167	2062.167	0.0297	0.0777	0.04363	0.13061	1.3403	1.3512	2.73178	2.5499	4.3935	13.3032	0.12261	0.2223
4	9912.167	2062.167	0.0263	0.0777	0.03726	0.20335	1.2996	1.3982	2.69954	2.5499	4.2809	13.3032	0.12898	0.2199
5	9362.167	1968.167	0.0268	0.0814	0.04318	0.20290	1.3265	1.3670	2.70365	2.4969	4.3691	11.3981	0.12649	0.2431
6	9412.167	2162.167	0.0278	0.0741	0.04479	0.17082	1.3761	1.3335	2.85813	2.432	4.5324	15.0000	0.13122	0.219
7	9662.167	1999.167	0.0276	0.0801	0.04455	0.20975	1.3688	1.3422	2.84295	2.452	3.9771	11.2213	0.12521	0.2519
8	9862.167	1969.167	0.0264	0.0686	0.05375	0.17486	1.3334	1.4642	2.71764	2.589	3.6155	11.3923	0.13232	0.2430
9	9712.167	2012.167	0.0239	0.0672	0.04759	0.20840	1.4077	1.433	2.66253	2.568	4.8096	13.6337	0.12964	0.2353
10	9812.167	2012.167	0.0265	0.0796	0.05802	0.19597	1.3130	1.3335	2.67610	2.4580	5.3437	13.6337	0.12520	0.2378
Mean	**9662.167**	**2037.167**	**0.0265**	**0.075**	**0.0465**	**0.18791**	**1.3647**	**1.377**	**2.73935**	**2.5195**	**4.4409**	**12.9492**	**0.12662**	**0.2344**
Std.Erro	**56.27314**	**18.319**	**0.0001**	**0.0018**	**0.00186**	**0.00781**	**0.0162**	**0.01419**	**0.02084**	**0.01745**	**0.1569**	**0.5294**	**0.00118**	**0.0037**
C. V.(%)	**1.842**	**2.844**	**6.246**	**7.719**	**12.681**	**13.1398**	**3.743**	**3.258**	**2.406**	**2.1899**	**11.171**	**12.927**	**2.957**	**4.941**

C.V.(%) calculated as follows: C.V(%)=100 x standard deviation/mean

Table 9: Descriptive statistics of analyte concentrations (expected and calculated) for evaluating the diluent, expressed as overall mean, each concentration value is a mean of five replicates.

	Creatinine		Phosphate		Chloride		Magnesium		Calcium		Sodium		Potassium	
	µmol/l		mmol/l/		mmol/l		mmol/l		mmol/l		mmol/l		mmol/l	
Equation	(Read*60)-(49*73)		(Read*60)-(49*0.866)		(Read*60)-(49*110)		(Reade*60)-(49*0.56)		(Read*60)-(49*1.68)		(Read*60)-(49*140)		(Read*60)-(49*3.6)	
Dilution	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc
1	9570	9218.5	2.955	2.865	141	144	12.3	12.56	4.99	4.94	63.5	63	293	293.6
1:1.5	6380	6020.5	1.97	1.781	94	88	8.2	8.28	3.33	3.144	42.3	36	195.3	196
1:2.5	3828	3630.5	1.184	1.193	56.4	50	4.92	5.68	1.996	2.08	25.4	24.8	117.2	118.5
1:5	1914	1834.5	0.591	0.525	28.2	30	2.46	2.96	0.998	1.08	12.7	9	58.6	54.5
1:10	957	869.5	0.2955	0.261	14.1	10	1.23	1.48	0.499	0.2	6.35	5	29.3	25.5
1:15	638	578.5	0.197	0.137	9.4	6	0.82	0.788	0.333	0.28	4.23	2.5	19.53	19.5
1:30	319	260.5	0.0985	0.069	4.7	8	0.41	0.484	0.166	0.2	2.12	4	9.767	9.5
1:50	191.4	80.9	0.0591	0.105	2.82	6.4	0.246	0.22	0.0998	0.34	1.27	1.2	5.86	4.5
1:100	95.7	98.5	0.0296	0.025	1.41	0.4	0.123	0.22	0.0499	0.14	0.635	0.7	2.93	0
Mean	**2644.2**	**2499.3**	**0.725**	**0.651**	**38.958**	**38.044**	**2.957**	**3.164**	**1.200**	**1.180**	**17.541**	**16.1667**	**80.953**	**80.178**
Std E.	**78.9398**		**0.1172**		**25.4304**		**0.2865**		**0.2053**		**4.1678**		**4.7344**	
Intercept	**-46.498**		**0.0011**		**-0.7089**		**0.1612**		**0.03219**		**-0.6643**		**-1.9799**	
Slope	**0.9631**		**0.9523**		**0.9919**		**1.0167**		**0.9721**		**0.9601**		**1.0108**	
R_I	**0.9983**		**0.9895**		**0.9949**		**0.9912**		**0.9941**		**0.9762**		**0.9924**	
p	**0.8264**		**0.8513**		**0.92249**		**0.8022**		**0.9852**		**0.7564**		**0.9575**	
R	**0.9997**		**0.9924**		**0.8792**		**0.9977**		**0.9921**		**0.9799**		**0.9989**	
R^2	**0.9994**		**0.9848**		**0.7729**		**0.9954**		**0.9843**		**0.9601**		**0.9977**	

The results presented in the table are a comparison between the expected (Exp.) concentration and calculated (Calc.) concentrations. The calculated concentration were calculated by the formula presented in column for each analyte. The table shows the Overall mean (mean.); standard error of calculated concentration for each know concentration in a regression (Std E.); Intercept (intercept.); Slope (Slope); Interclass correlation between two groups (R_I); and the probability (*p*); R, and R Square (R^2).

8. 3. FIGURES – PHASE I

Figure 2: Linear regression between creatinine expected concentration and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 0.9631x – 46.498) and R_I as Interclass correlation =0.9983

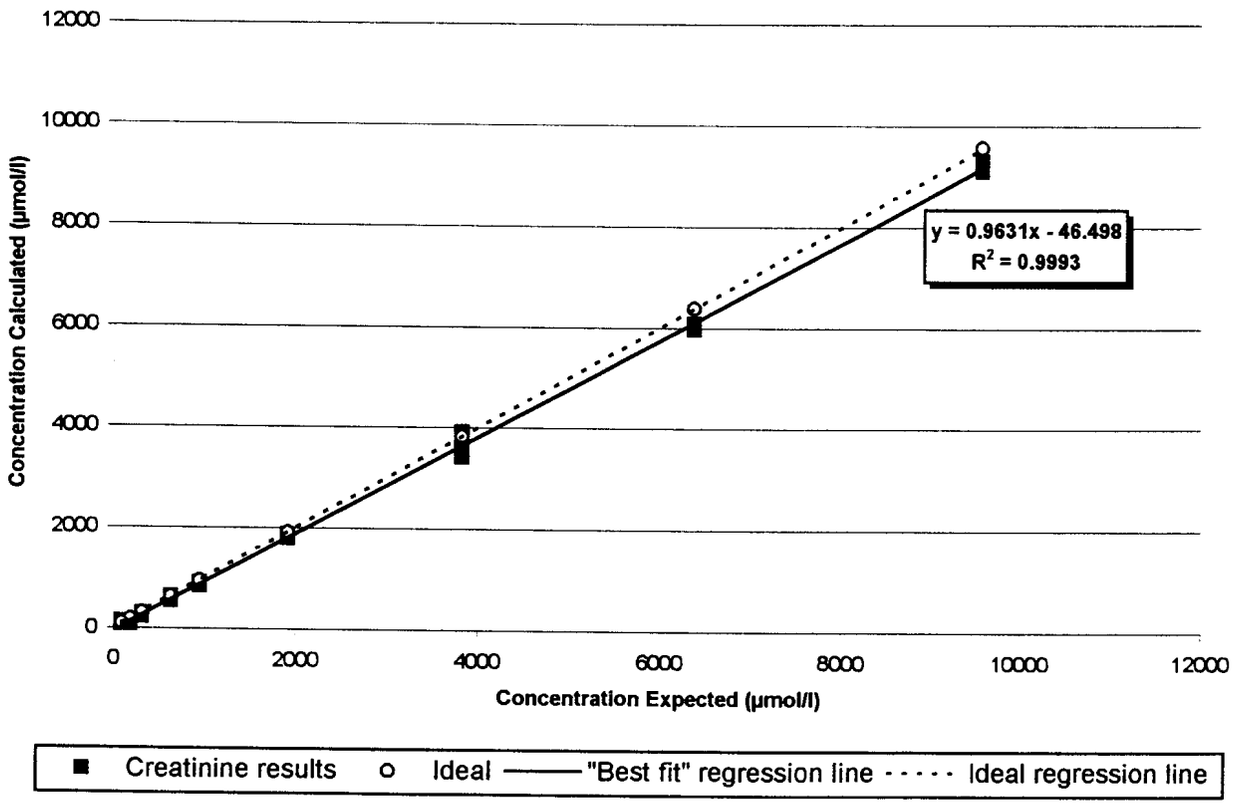


Figure 3: Linear regression between <u>phosphate</u> expected and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 0.9523x + 0.011) and R_I as interclass correlation=0.9895.

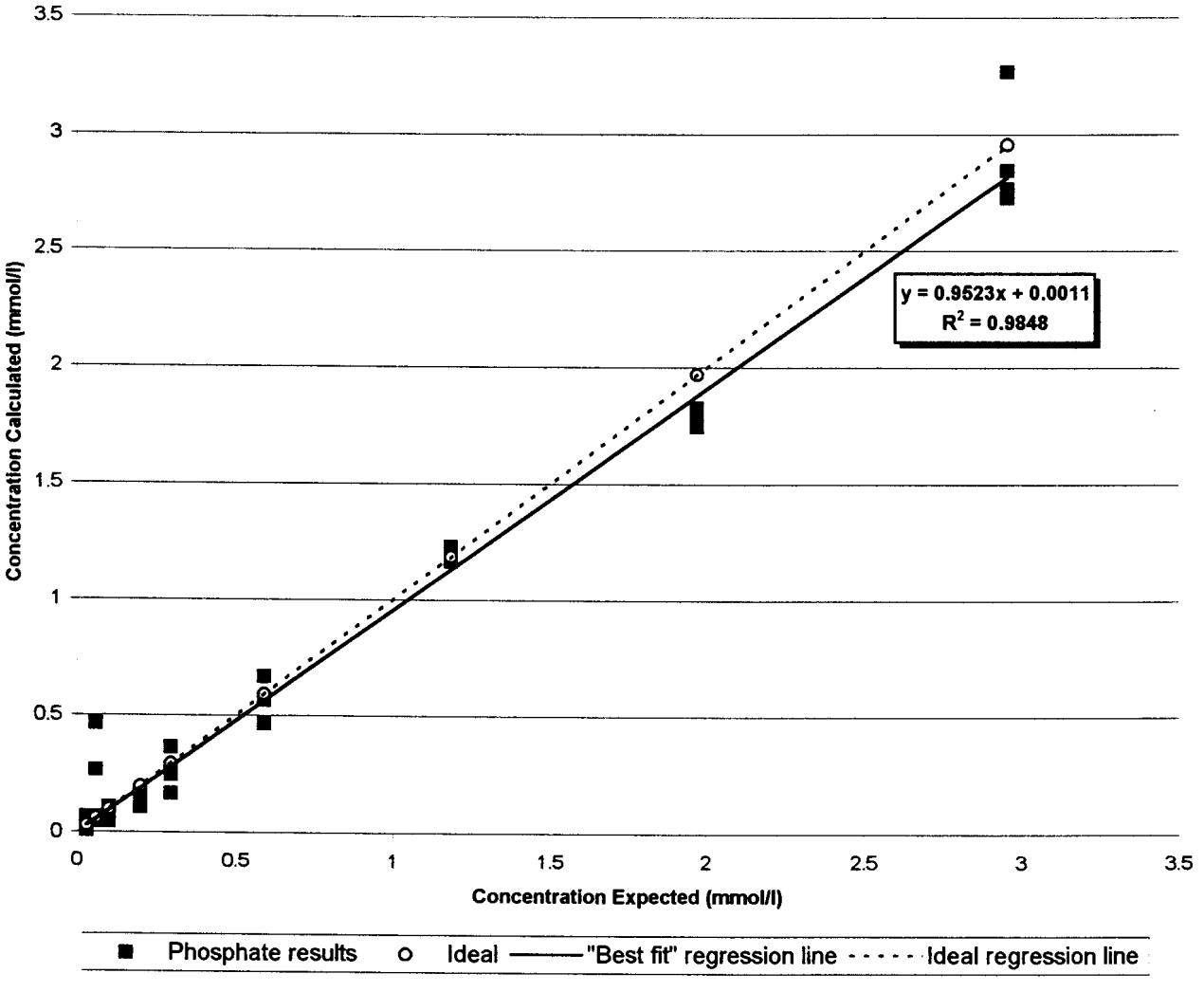


Figure 4: Linear regression between chloride expected concentration and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 0.9919x - 0.7089) and R_I interclass correlation =0.9949.

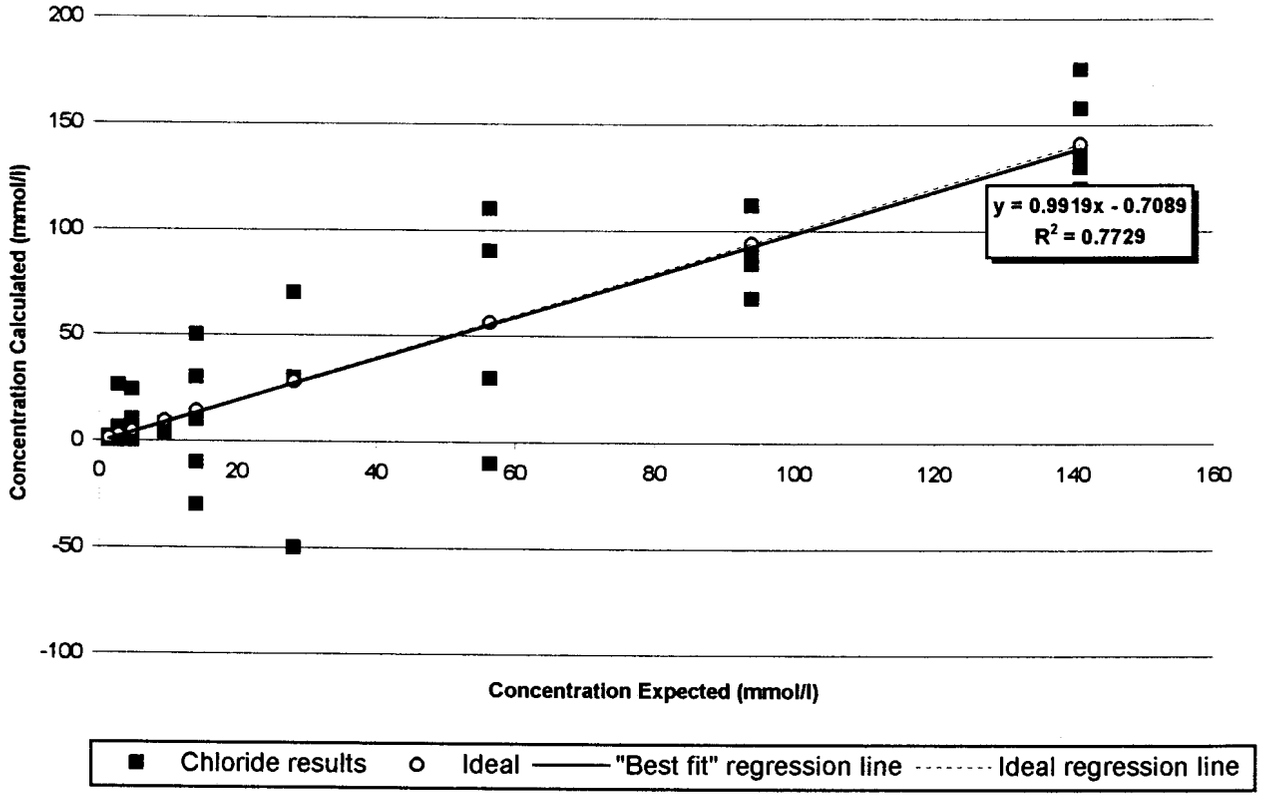


Figure 5: Linear refression between magnesium expected concentration and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 1.0167x + 0.1612) and R_I interclass correlation =0.9912.

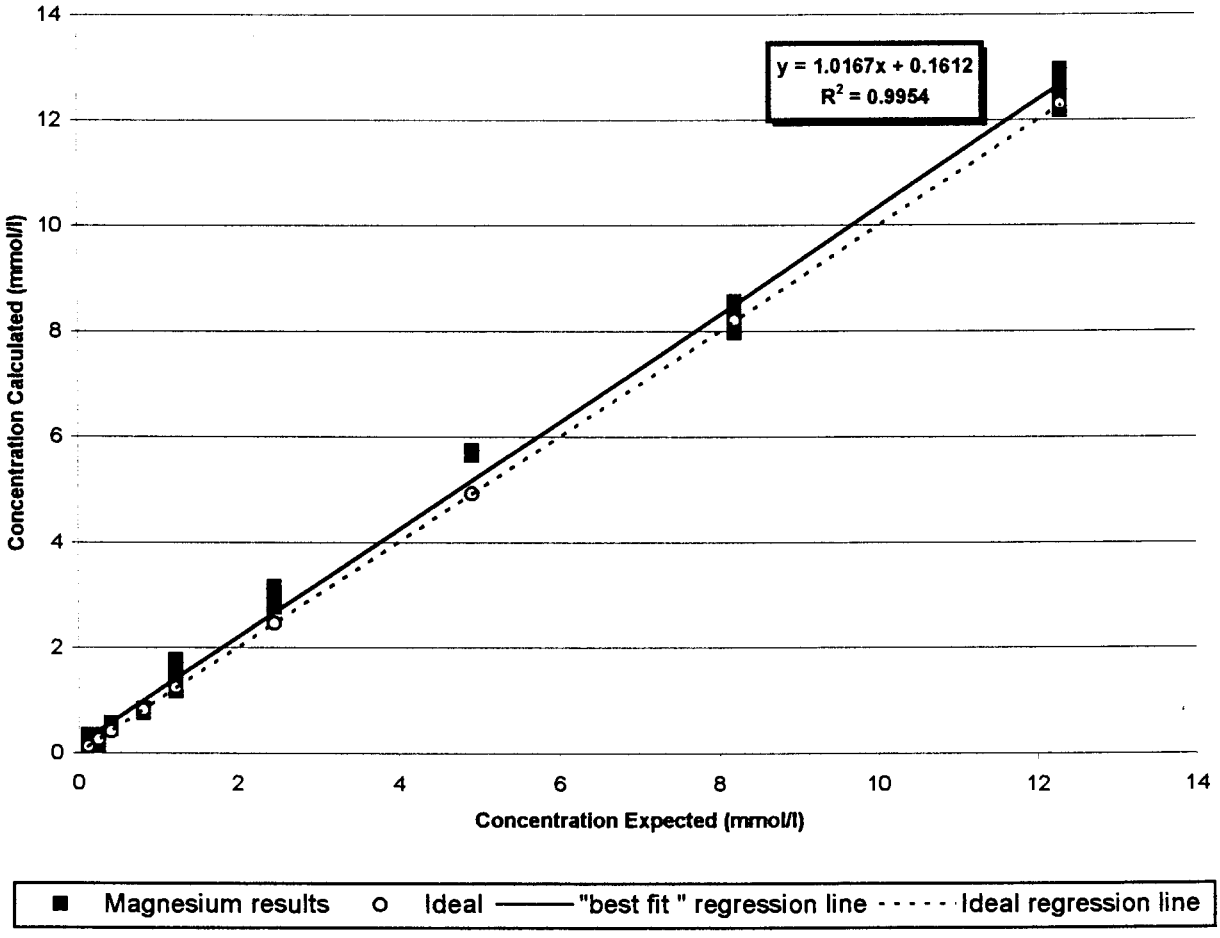


Figure 6: Linear regression between calcium expected concentration and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 0.9721x + 0.0322) and R_I interclass correlation =0.9941.

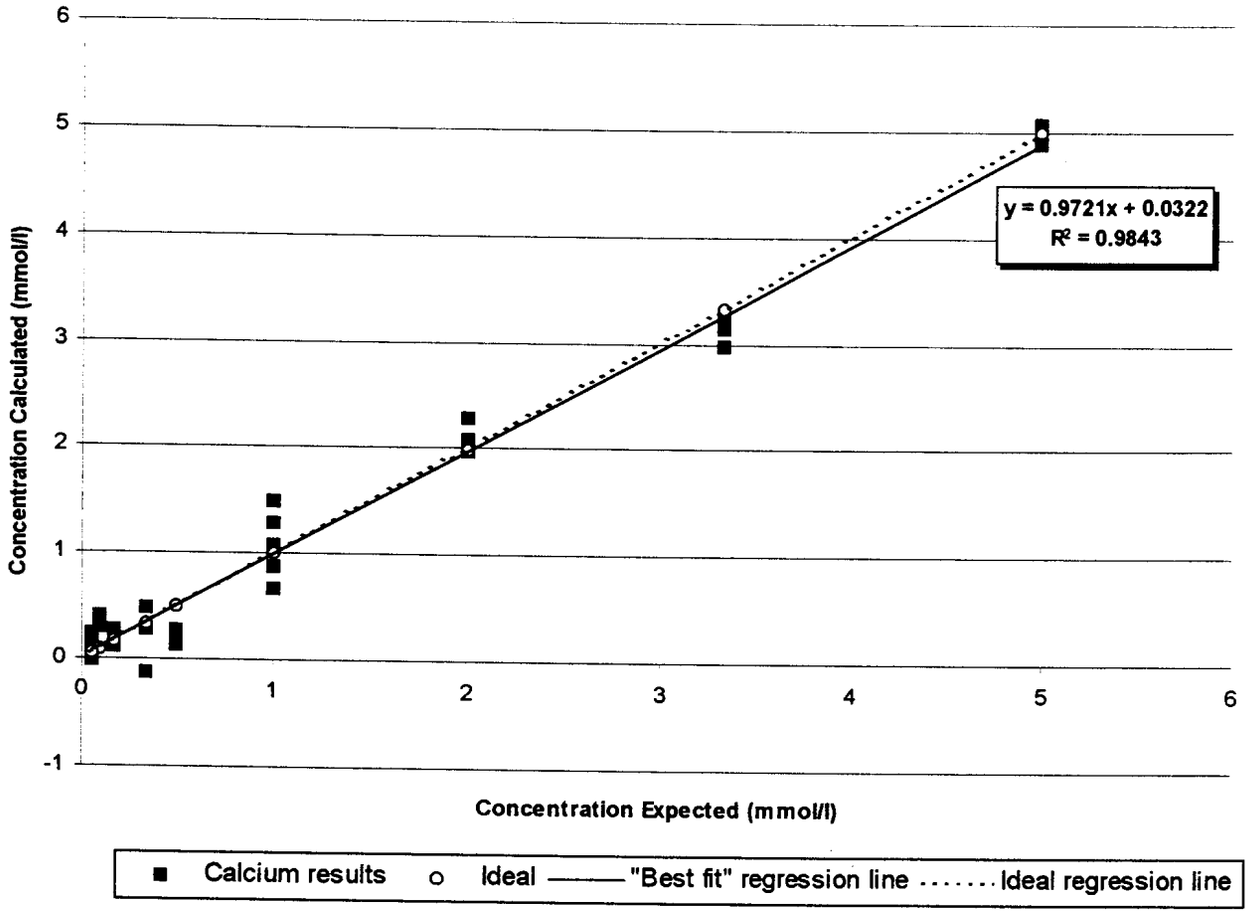


Figure 7: Linear correlation between <u>sodium</u> known concentration (ideal regression line) and calculated concentration ("best fit" regression line) for the diluent, regression equation (y= 0.9601x - 0.6643) and R_I interclass correlation =0.9762.

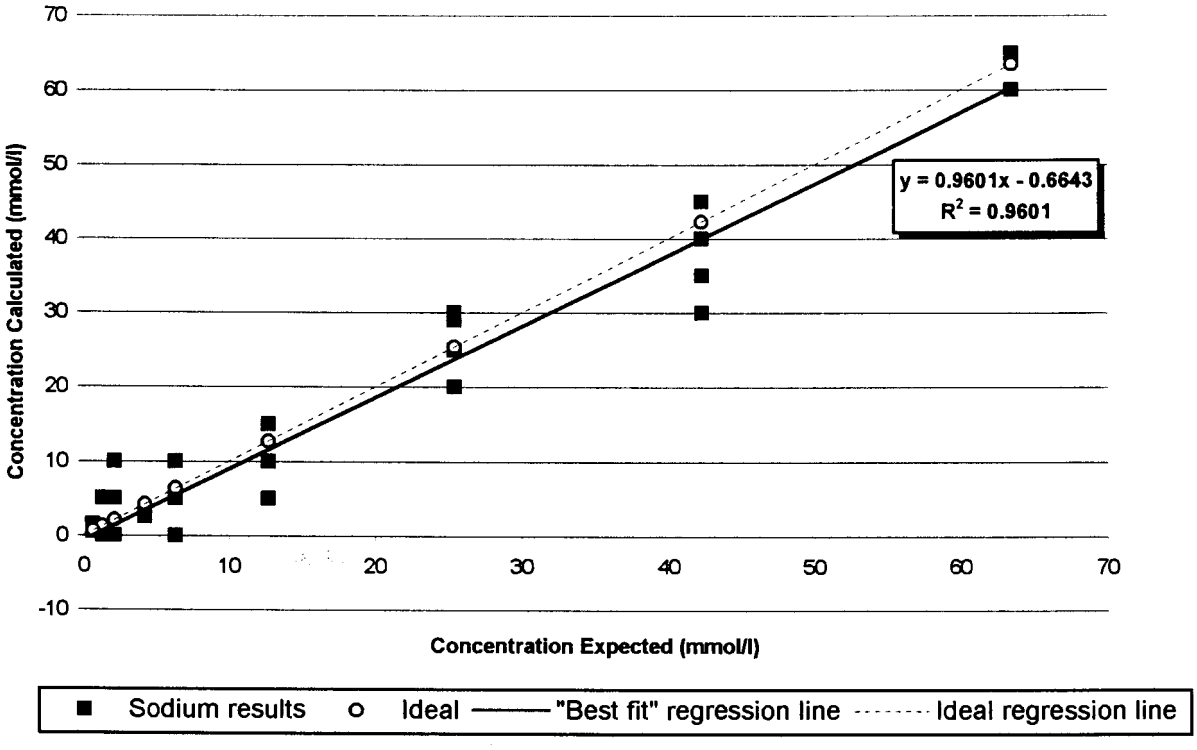
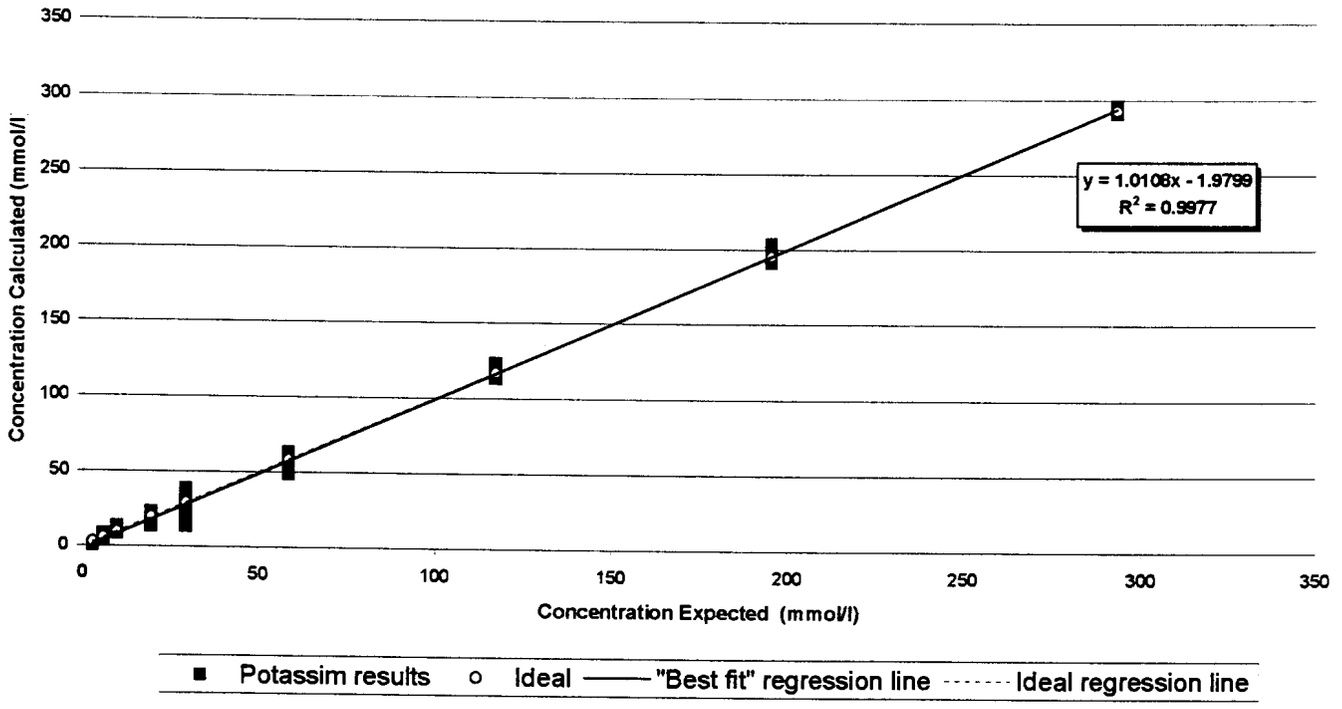


Figure 8: Linear regression between potassium expected and calculated concentration ("best fit" regression line) in the diluent, regression equation (y= 1.0108x – 1.9799) and R_I interclass correlation =0.9824.



8.4. TABLES

Table 10: Descriptive statistics comparing mineral/electrolyte to creatinine ratio of artificial urine with control (Ref) at two different temperatures (22^{0}C and 30^{0}C), over a period of ten days.

Ratio	P/Cr			Cl/CR			Mg/Cr			Ca/Cr			Na/Cr			K/Cr		
	Temp. (^{0}C)			Temp. (^{0}C)			Temp. (^{0}C)			Temp. (^{0}C)			Temp. (^{0}C)			Temp. (^{0}C)		
Days	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**
1	2.79	2.41	3.85	132.1	104.35	432.26	10.64	10.69	11.51	5.52	5.05	6.25	193.47	192.77	-58.45	281.91	280.89	262.44
2	3.72	3.86	2.38	362.44	458.72	246.49	11.35	11.31	8.00	3.82	4.42	2.91	-72.02	-92.27	146.03	290.55	286.03	279.89
3	3.28	3.55	4.07	512.57	396.5	256.16	11.88	12.17	12.43	6.36	5.47	4.89	34.14	34.2	-141.48	280.34	279.94	234.56
4	3.05	3.43	3.33	248.52	277.34	289.48	12.24	12.8	10.88	7.51	7.40	3.54	100.08	118.72	-45.19	286.67	288.33	268.67
5	3.55	3.6	3.3	352.29	418.65	147.00	11.65	10.98	12.23	7.79	7.96	5.09	165.94	190.41	-6.19	308.04	300.96	268.14
6	3.23	3.37	3.18	599.2	664.19	314.35	11.32	12.08	11.59	6.44	5.65	1.63	104.00	111.78	106.17	286.81	287.96	274.44
7	3.26	3.61	2.85	589.91	291.75	88.48	13.1	13.55	11.34	4.54	4.28	7.20	75.51	82.76	-149.07	279.25	280	263.86
8	3.29	3.82	3.5	476.34	356.92	308.29	11.68	12.23	11.53	4.48	4.26	5.40	-215.0	-208.92	39.76	285.40	284.4	270.18
9	3.2	3.3	3.58	322.63	260.12	387.48	10.16	9.72	10.85	4.19	3.09	4.60	70.99	69.19	202.36	284.42	276.31	277.91
10	3.34	3.62	4.05	271.11	297.54	455.64	11.41	12.29	10.70	4.89	4.7	6.12	157.75	130.56	46.49	283.95	281.65	276.29
Mean	**3.271**	**3.356**	**3.406**	**386.71**	**352.61**	**292.56**	**11.54**	**11.70**	**11.11**	**5.55**	**5.23**	**7.76**	**61.482**	**63.019**	**14.053**	**286.70**	**284.64**	**267.64**
Std Dev.	**0.253**	**0.479**	**0.530**	**154.44**	**148.04**	**116.49**	**0.81**	**1.114**	**1.23**	**1.405**	**1.48**	**1.68**	**123.01**	**125.66**	**116.92**	**8.201**	**6.904**	**12..996**
P (22/30)	**0.162**			**0.620**			**0.599**			**0.620**			**0.910**			**0.521**		
P (30/Ref)		**0.816**			**0.327**			**0.213**			**0.519**			**0.379**			**<0.001***	
P (22/Ref)	**0.467**			**0.141**			**0.521**			**0.267**			**0.388**			**<0.001***		

***P* (22/30) = The "*P*" value for the test of the hypothesis that mean$_{T22}$=mean$_{T30}$**

***Statistically different between temperature and control (Ref)**

Two way ANOVA. If the test for equal mean and equal variance failed, non-parametric pairwise multiple comparison tests were performed (Tukey)

Table 11: Descriptive statistics comparing mineral/electrolyte to creatinine ratio of goat urine with control (Ref) at two different temperatures (22^0C and 30^0C), over a period of ten days.

Ratio	P/Cr			Cl/CR			Mg/Cr			Ca/Cr			Na/Cr			K/Cr		
	Temp. (^{0} C)			Temp. (^{0} C)			Temp. (^{0} C)			Temp. (^{0} C)			Temp. (^{0} C)			Temp. (^{0} C)		
Days	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**	**22**	**30**	**Ref**
1	15.4	16.84	6.43	225.95	487.37	228.19	7.44	8.41	8.03	8.99	8.35	7.61	117.78	91.35	22.22	102.25	95.56	98.61
2	14.95	15.52	6.15	220.58	237.95	126.37	6.71	5.11	4.81	5.51	5.75	3.42	171.53	267.71	126.95	101.66	106.68	102.67
3	14.71	16.55	15.81	255.66	312.13	298.01	7.2	7.96	7.85	7.27	6.69	6.06	29.31	28.76	-76.46	99.46	97.02	99.14
4	15.05	16.86	15.51	250.53	241.67	281.35	7.23	8.28	7.37	6.68	6.94	4.29	110.5	114.82	51.31	103.62	101.18	100.11
5	14.56	15.22	15.43	182.48	136.45	155.91	7.35	8.11	7.67	5.53	4.8	5.64	156.68	155.01	-94.16	100.86	99.43	93.43
6	14.72	15.45	16.34	219.89	196.09	203.24	6.82	7.37	7.37	6.35	5.69	3.74	170.66	191.77	186.99	104.22	104.52	100.99
7	15.34	15.55	15.82	121.34	182.86	96.51	6.31	7.39	7.01	3.85	5.12	3.74	145.06	149.5	102.45	107.93	106.52	104.1
8	14.45	15.13	15.59	293.40	221.17	183.24	7.26	8.23	6.78	5.91	7.04	2.6	170.62	132.91	91.79	105.11	91.25	98.83
9	15.25	15.86	15.67	300.64	306.58	446.42	7.31	7.61	7.22	5.52	6.84	7.01	283.81	158.06	228.69	109.14	103.83	101.49
10	15.29	15.75	15.02	341.61	275.75	363.00	7.33	6.97	6.75	5.52	6.58	5.53	131.83	138.25	80.69	105.78	104.93	104.59
Mean	**15.07**	**15.87**	**13.78**	**241.21**	**259.80**	**238.22**	**7.093**	**7.544**	**7.082**	**6.113**	**6.38**	**4.05**	**148.78**	**152.82**	**72.05**	**104.00**	**101.23**	**100.4**
Std Dev.	**0.35**	**0.647**	**3.961**	**62.860**	**96.874**	**109.58**	**0.363**	**0.976**	**0.906**	**1.357**	**1.051**	**4.988**	**63.917**	**72.288**	**102.6**	**3.082**	**5.102**	**3.229**
P (22/30)	**0.003***			**0.617**			**0.187**			**0.629**		**1.636**	**0.896**			**0.159**		
P (30/Ref)		**0.241**			**0.646**			**0.089**			**0.036***			**0.0057**			**0.666**	
P (22/Ref)	**0.104**			**0.941**			**0.521**			**0.111**			**0.060**			**0.020***		

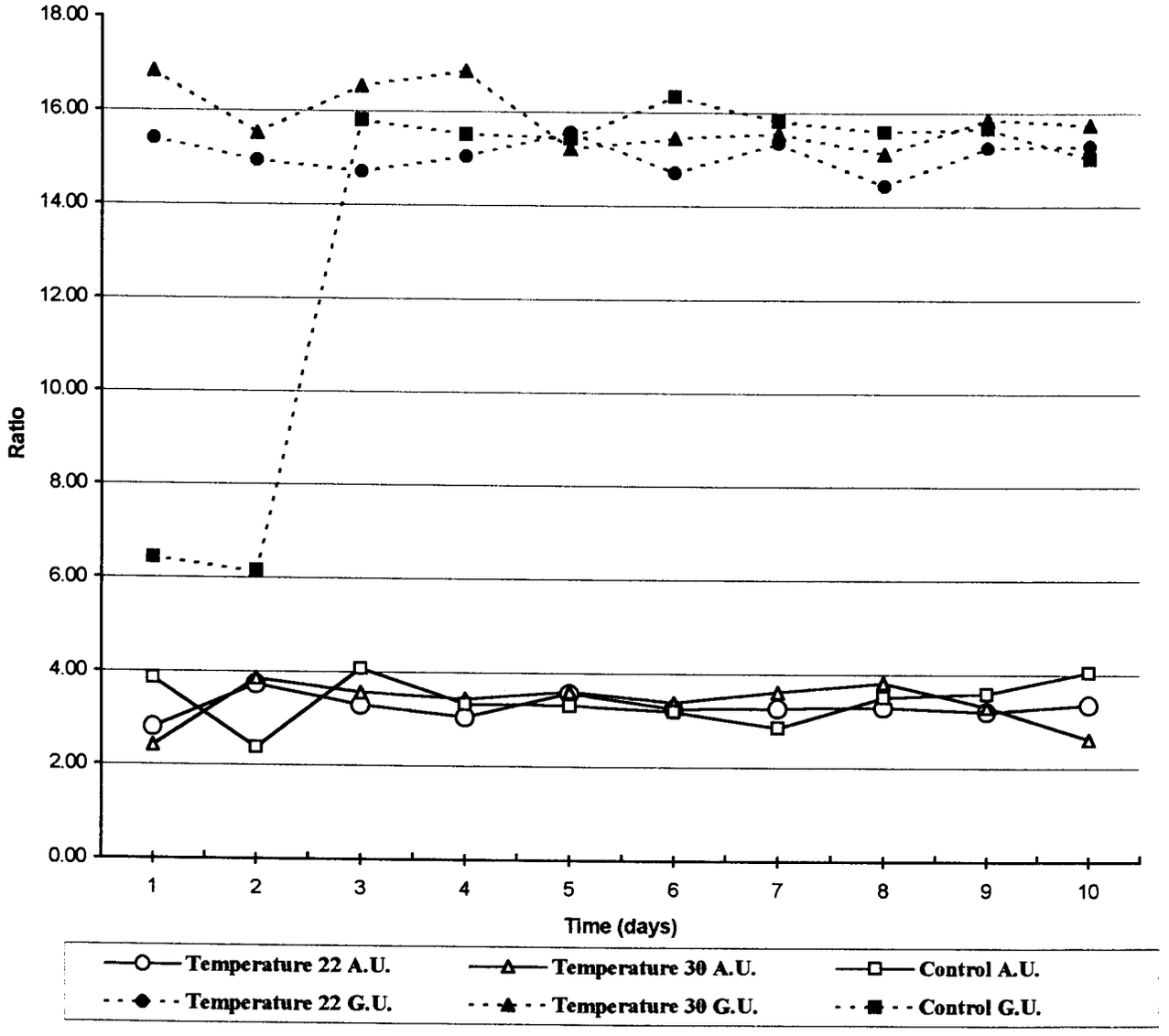
***P* (22/30) = The "*P*" value for the test of the hypothesis that $mean_{T22}=mean_{T30}$**

***Statistically different between temperature and control (Ref)**

Two way ANOVA. If the test for equal mean and equal variance failed, non-parametric pairwise multiple comparison tests were performed (Tukey)

8.5. FIGURES

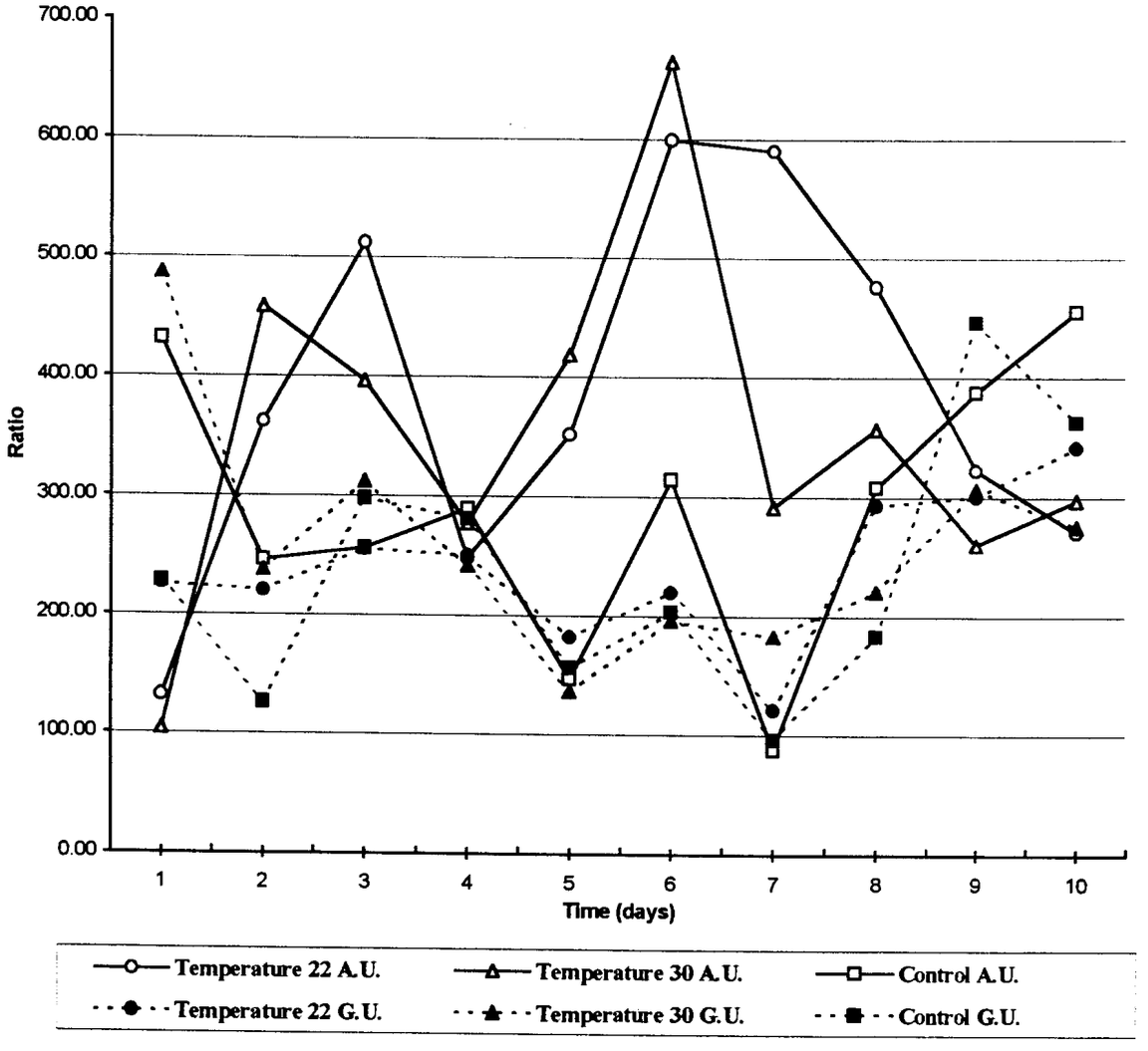
Figure 9: Variation of Phosphate/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

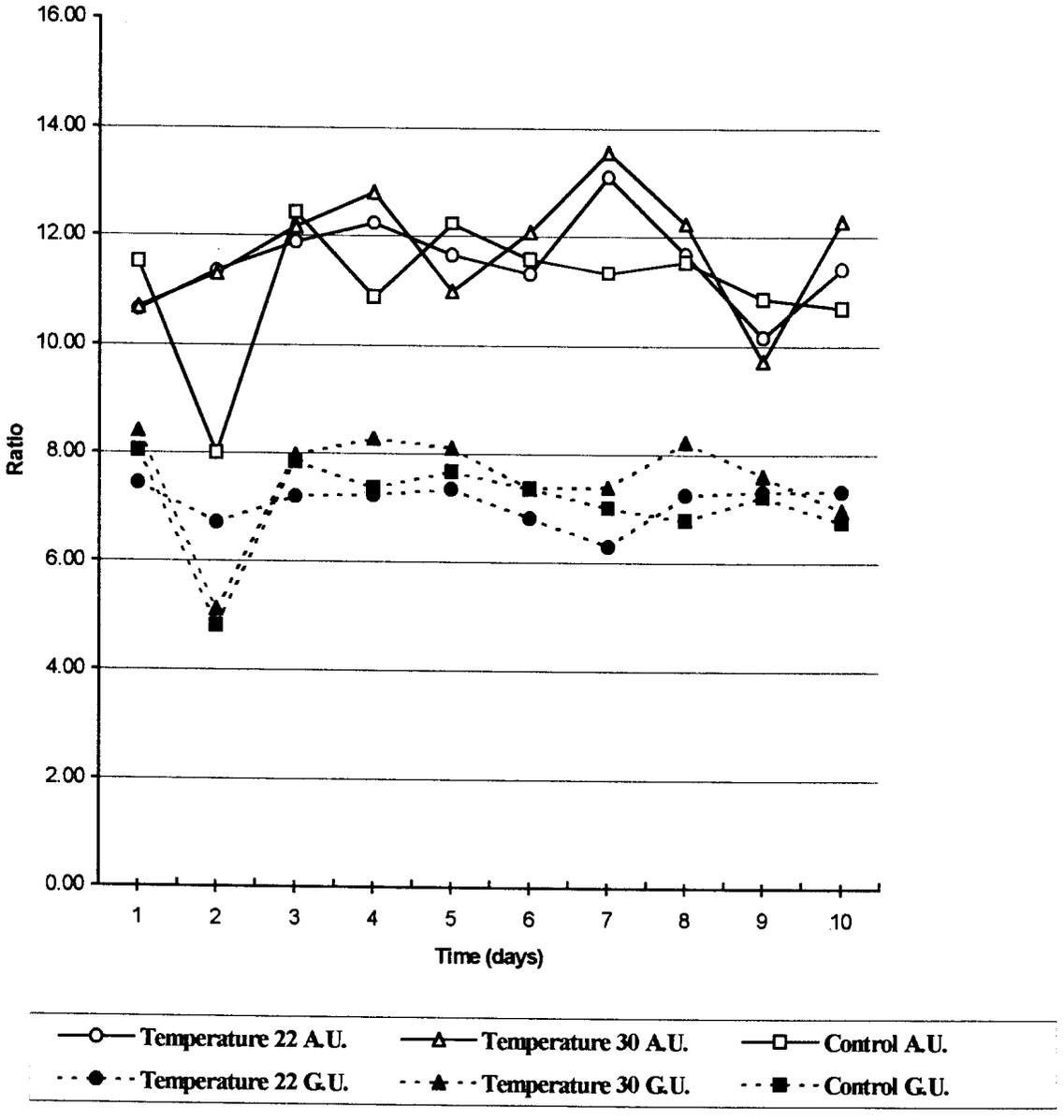
Figure 10: Variation of Chloride/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

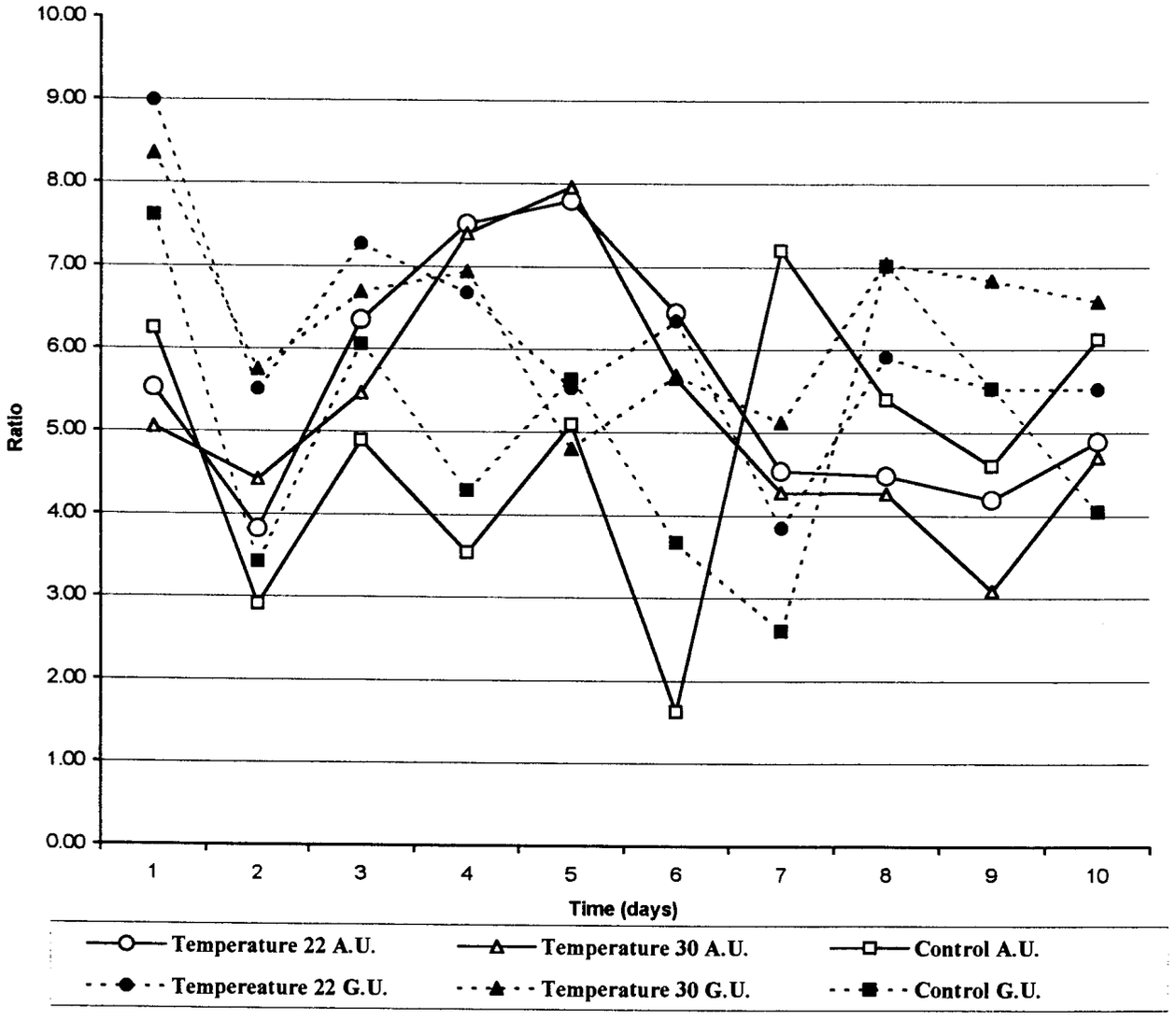
Figure 11: Variation of Magnesium/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

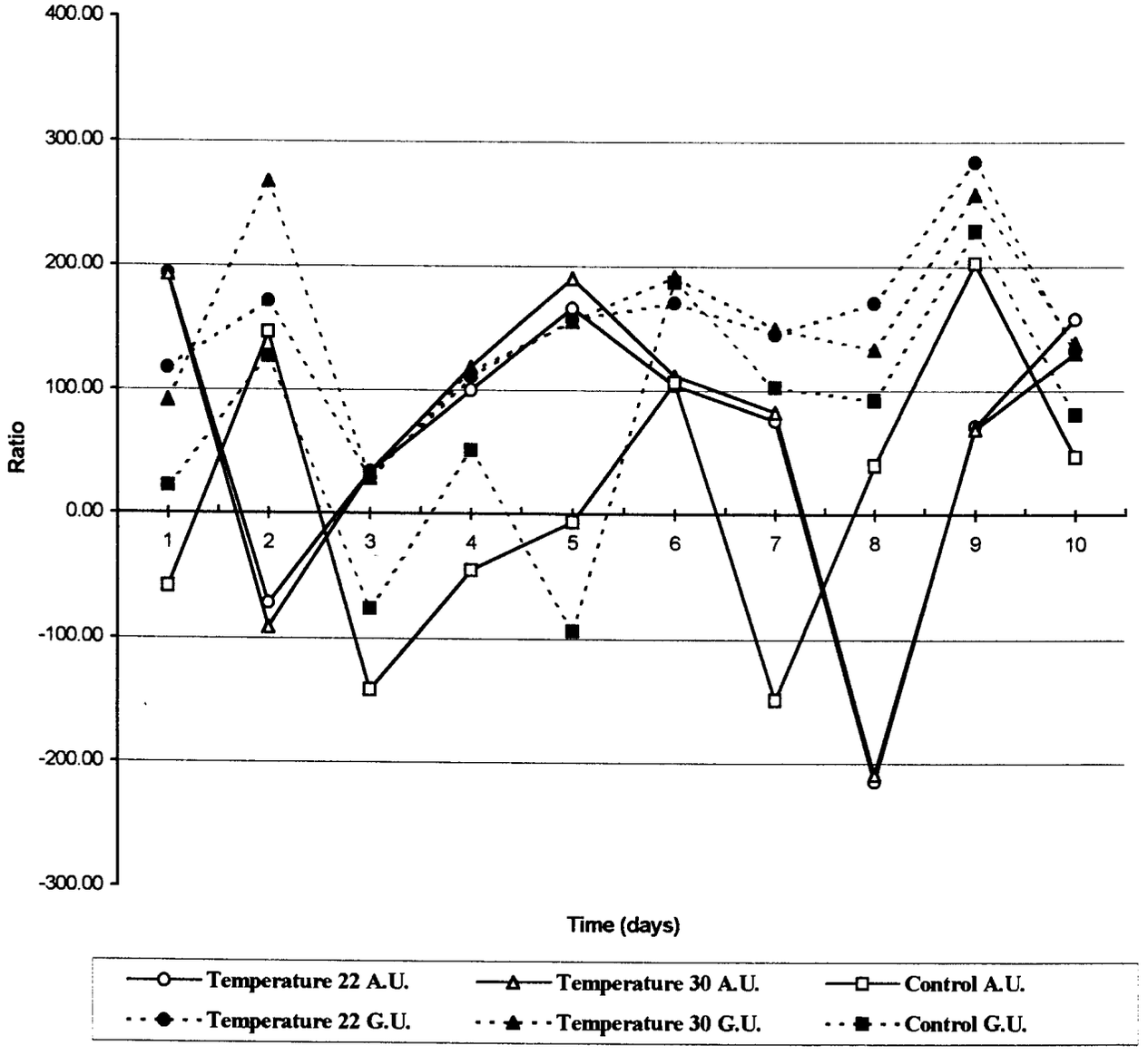
Figure 12: Variation of Calcium/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

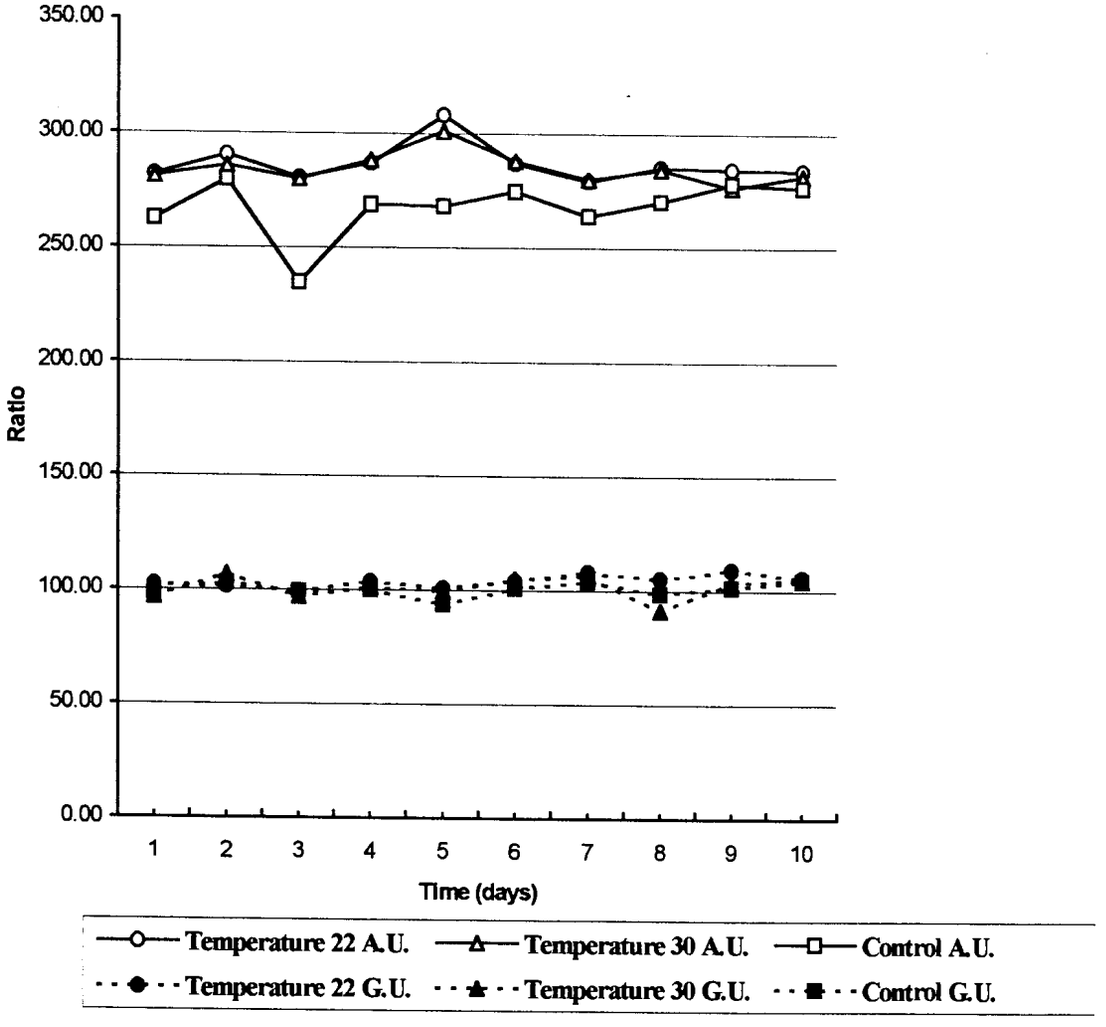
Figure 13: Variation of Sodium/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

Figure 14: Variation of Potassium/Creatinine ratio of artificial urine and goat urine at temperatures of 22^{0}C and 30^{0}C for a period of 10 days.



A.U.=Artificila Urine

G.U.=Goat Urine

8.6. TABLES

Table 12 :Mineral/electrolyte:Creatinine ratios (P/Cr and Cl/Cr) of goat urine by DFPM. The filter paper was impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), and eluted to give three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10). Comparison between control (refrigerated goat urine) and the DFPM, expressed as mean and standard deviation.

	Ratios											
	P/Cr						Cl/Cr					
Goat Urine	a		b		c		a		b		c	
Volume	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
V1	0.3128	0.3436	0.2686	0.2306	0.0209	0.0337	36.1299	66.7434	33.8487	47.9902	2.4559	2.3040
	0.3409	0.5201	0.1985	0.2509	0.0086	0.0000	31.9207	79.9208	18.9606	35.9857	3.3448	1.3105
	0.5434	0.2760	0.0942	0.1391	0.0212	0.0000	31.9207	20.4167	101.0853	40.5118	2.4847	4.7772
	0.4153	0.1776	0.1544	0.2131	0.0205	0.0345	33.6784	23.6778	44.9345	40.5118	3.5345	3.8393
	0.2760	0.4153	0.1735	0.0853	0.0000	0.0000	37.8465	14.7259	53.3071	43.8428	4.6688	4.3713
Mean	**0.3777**	**0.3465**	**0.1779**	**0.1838**	**0.0142**	**0.0137**	**34.2995**	**41.0969**	**50.4272**	**41.7685**	**3.2977**	**3.3204**
Std dev	**0.1058**	**0.1307**	**0.0637**	**0.0694**	**0.0096**	**0.0187**	**2.6285**	**29.9649**	**31.1035**	**4.4613**	**0.9094**	**1.4636**
P	**1.000**		**0.891**		**0.841**[+]		**0.627**		**0.841**[+]		**0.997**	
V2	0.2354	0.1735	0.0485	0.0461	0.0046	0.0103	32.2772	31.6584	16.4586	13.0644	2.3770	1.7789
	0.2509	0.3169	0.0821	0.0872	0.0103	0.0222	33.7467	33.7467	9.1218	19.0174	2.9208	2.4048
	0.5448	0.5484	0.0755	0.1082	0.0104	0.0156	31.6584	31.6484	13.7675	11.7065	2.9375	2.2291
	0.5131	0.4488	0.0846	0.0461	0.0160	0.0100	29.8135	33.0871	15.4282	10.0000	2.3499	2.1616
	0.4099	0.3382	0.0544	0.0797	0.0046	0.0161	36.1299	29.7135	15.4282	14.5506	1.2012	2.9375
Mean	**0.3908**	**0.3644**	**0.0690**	**0.0735**	**0.0092**	**0.0148**	**32.7552**	**31.9928**	**14.0409**	**13.6678**	**2.3573**	**2.3024**
Std dev	**0.1438**	**0.1406**	**0.0165**	**0.0271**	**0.0048**	**0.0050**	**2.3692**	**1.5204**	**2.9141**	**3.4301**	**0.7056**	**0.4222**
P	**0.777**		**0.762**		**0.106**		**0.577**		**0.858**		**0.885**	
V3	0.0399	0.1057	0.0729	0.0595	0.0143	0.0103	9.1143	10.1994	7.8911	6.6073	1.9222	1.1491
	0.0681	0.0861	0.0595	0.0595	0.0031	0.0030	12.4295	9.3207	10.8482	8.7277	2.3419	1.9367
	0.0579	0.0872	0.0570	0.0360	0.0000	0.0068	13.2092	15.9080	10.4068	8.2057	2.6340	1.9222
	0.0579	0.1072	0.0327	0.0353	0.0068	0.0066	19.6277	19.5566	7.4619	10.0000	2.3153	1.8729
	0.0267	0.1351	0.0391	0.0570	0.0069	0.0029	10.3316	18.1676	6.7504	8.3727	2.3329	3.7033
Mean	**0.0501**	**0.1043**	**0.0522**	**0.0494**	**0.0062**	**0.0059**	**12.9425**	**14.6304**	**8.6717**	**8.3827**	**2.3092**	**2.1168**
Std dev	**0.1438**	**0.0199**	**0.0163**	**0.0127**	**0.0054**	**0.0031**	**4.0773**	**4.6432**	**1.8379**	**1.2164**	**0.2535**	**0.9465**
P	**0.002***		**0.769**		**0.923**		**0.558**		**0.777**		**0.222**[+]	
V4 (Dipping)	0.1052	0.0872	0.0575	0.0612	0.0000	0.0000	19.6792	19.0174	4.7068	6.6216	1.4527	1.5496
	0.0735	0.0637	0.0261	0.0749	0.0052	0.0000	10.7862	16.9137	7.3973	6.4152	1.7466	2.0150
	0.0755	0.0698	0.0310	0.0300	0.0000	0.0023	16.4586	15.2291	7.0770	6.8418	2.0498	1.7269
	0.01082	0.0947	0.0508	0.0281	0.0023	0.0051	17.3948	12.7390	11.0877	7.9738	1.7367	1.7417
	0.0797	0.1494	0.0430	0.0277	0.0000	0.0025	17.3948	19.0174	6.3168	6.3168	2.0093	1.6030
Mean	**0.0884**	**0.0930**	**0.0417**	**0.0444**	**0.0015**	**0.0020**	**16.3427**	**16.5833**	**7.3177**	**6.8338**	**1.7990**	**1.7272**
Std dev	**0.0169**	**0.0339**	**0.0132**	**0.0222**	**0.0023**	**0.0021**	**3.3254**	**2.6726**	**2.3495**	**0.6684**	**0.2417**	**0.1803**
P	**0.795**		**0.822**		**0.730**		**0.903**		**0.730**		**0.609**	

***Statiscally significant difference between control and the DFPM for the mineral/electrolyte to creatinine ratio. *P*<0.05; [+]statiscally significant difference by the Mann-Whitney rank sum test;.(0.000) reading below detection limit of analyser; undiluted=the normal elution rate applied in the other studies.**

Table 13 : Mineral/electrolyte:Creatinine ratios (Mg/Cr and Ca/Cr) of goat urine by DFPM. The filter paper was impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), and eluted to give three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10). Comparison between control (refrigerated goat urine) and the DFPM, expressed as mean and standard deviation.

	Ratios											
	Mg/Cr						Ca/Cr					
Goat Urine	a		b		c		a		b		c	
Volume	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
V1+	0.5861	0.4005	0.3305	0.2943	0.2583	0.3705	0.8388	1.1275	0.1088	0.1319	0.0233	0.0524
	0.4929	0.4929	0.3056	0.3109	0.3281	0.2645	0.7348	1.0932	0.1041	0.1278	0.0276	0.0000
	0.5861	0.4624	0.2874	0.2840	0.3351	0.2686	1.9767	1.0039	0.0894	0.1529	0.0341	0.0834
	0.3189	0.4929	0.3230	0.3385	0.2986	0.2438	1.7686	1.5945	0.1487	0.0810	0.0310	0.0447
	0.1040	0.3189	0.3230	0.2811	0.3568	0.2740	0.9055	1.1135	0.1258	0.0491	0.0465	0.0447
Mean	**0.4176**	**0.4335**	**0.3139**	**0.3018**	**0.3154**	**0.2843**	**1.2449**	**1.1865**	**0.1154**	**0.1086**	**0.0325**	**0.0450**
Std dev	**0.2065**	**0.0743**	**0.0174**	**0.0236**	**0.0381**	**0.0495**	**0.5810**	**0.2331**	**0.0227**	**0.0424**	**0.0088**	**0.0298**
P	**0.875**		**0.383**		**0.383**		**0.840**		**0.760**		**0.394**	
V2	0.2896	0.4189	0.3146	0.2824	0.3245	0.2811	1.3119	1.0644	0.0997	0.1951	0.0403	0.0521
	0.3006	0.4279	0.2939	0.3124	0.2960	0.3035	0.8047	0.6728	0.0891	0.2375	0.0605	0.0515
	0.4164	0.3853	0.3128	0.3079	0.2660	0.2738	0.6312	0.6312	0.2889	0.0837	0.0582	0.0429
	0.3832	0.3355	0.3110	0.3007	0.3711	0.2723	0.5944	0.8707	0.1124	0.0430	0.0375	0.0404
	0.2694	0.4500	0.2865	0.3070	0.3295	0.2528	0.5791	0.7110	0.1560	0.1465	0.0429	0.0570
Mean	**0.3318**	**0.4035**	**0.3037**	**0.3021**	**0.3174**	**0.2767**	**0.7843**	**0.7900**	**0.1492**	**0.1412**	**0.0479**	**0.0488**
Std dev	**0.0641**	**0.0446**	**0.0127**	**0.0118**	**0.0393**	**0.0183**	**0.3084**	**0.1782**	**0.0821**	**0.0793**	**0.0107**	**0.0069**
P	**0.074**		**0.837**		**0.069**		**0.972**		**0.879**		**0.877**	
V3	0.2319	0.2486	0.3023	0.2820	0.2280	0.1397	0.4473	0.2432	0.1399	0.1073	0.0531	0.0525
	0.3030	0.2598	0.3081	03046	0.1895	0.2012	0.3936	0.2763	0.0000	0.0062	0.0411	0.1005
	0.2719	0.3256	0.3053	0.3099	0.2011	0.2125	0.2953	0.4104	0.0323	0.0000	0.0528	0.0622
	0.2719	0.4768	0.3008	0.2836	0.2253	0.1908	0.2953	0.5046	0.2473	0.2349	0.0582	0.0563
	0.3141	0.4074	0.3108	0.3019	0.3460	0.3263	0.3050	0.4332	0.4282	0.4323	0.0409	0.0528
Mean	**0.2785**	**0.3436**	**0.3055**	**0.2964**	**0.2360**	**0.2141**	**0.3473**	**0.3736**	**0.1695**	**0.1561**	**0.0492**	**0.0649**
Std dev	**0.0321**	**0.977**	**0.0041**	**0.0127**	**0.0625**	**0.0686**	**0.0695**	**0.1102**	**0.1742**	**0.1851**	**0.0078**	**0.0203**
P	**0.195**		**0.648**		**0.548⁺**		**0.664**		**0.879**		**0.147**	
V4 (Dipping)	0.4500	0.0000	0.3023	0.3124	0.1746	0.0338	0.5863	0.4104	0.0000	0.0987	0.0255	0.1106
	0.4007	0.3111	0.3041	0.2902	0.1290	0.2290	0.4507	0.3374	0.0104	0.2770	0.0988	0.1080
	1.0605	0.4402	0.3009	0.2893	0.1812	0.1226	0.3821	0.4532	0.3758	0.2083	0.0845	0.0836
	0.4882	0.4402	0.2995	0.2861	0.1912	0.1878	0.3754	0.4532	0.3965	0.3951	0.1044	0.1134
	0.0000	0.3790	0.2922	0.2897	0.2149	0.0696	0.3470	0.3794	0.1361	0.1040	0.0703	0.0552
Avarege	**0.4799**	**0.3141**	**0.2998**	**0.2937**	**0.1782**	**0.1296**	**0.4283**	**0.4067**	**0.1838**	**0.2166**	**0.0767**	**0.0942**
Std dev	**0.3790**	**0.1835**	**0.0046**	**0.0111**	**0.0314**	**0.0793**	**0.0962**	**0.0497**	**0.1925**	**0.1247**	**0.0315**	**0.0248**
P	**0.404**		**0.293**		**0.421⁺**		**0.667**		**0.757**		**0.359**	

***Statiscally significant difference between control and the DFPM for the mineral/electrolyte to creatinine ratio. *P*<0.05; ⁺statiscally significant difference by the Mann-Whitney rank sum test;.(0.000) reading below detection limit of analyser; undiluted=the normal elution rate applied in the other studies.**

Table 14 : Mineral/electrolyte:Creatinine ratios (Na/Cr and K/Cr) of goat urine by DFPM. The filter paper was impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), and eluted to give three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10). Comparison between control (refrigerated goat urine) and the DFPM, expressed as mean and standard deviation.

	Ratios											
	Na/Cr						K/Cr					
Goat Urine	a		b		c		a		b		c	
Volume	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
V1	4.1344	4.6486	0.4245	0.0000	0.0000	0.0000	2.7121	2.4418	4.0568	4.0676	3.8342	3.6384
	6.5383	1.3689	0.1414	0.4233	0.0000	0.0000	2.9757	2.7736	3.9534	4.1042	4.1710	4.4303
	0.0000	1.2326	0.1426	0.1398	0.0000	0.0000	3.9307	3.8085	3.9761	3.9547	3.8342	4.0523
	4.2520	1.2326	0.1406	0.4257	0.0000	0.0000	3.9307	4.6820	3.9534	3.8777	4.2967	3.3830
	0.0000	1.5392	0.1308	0.1551	0.0000	0.0000	4.0610	4.3494	3.9877	4.0910	4.2967	3.7679
Mean	**2.9849**	**2.0044**	**0.1978**	**0.2288**	**0.0000**	**0.0000**	**3.5220**	**3.6110**	**3.9855**	**4.0190**	**4.0866**	**3.8544**
Std dev	**2.8884**	**1.4835**	**0.1268**	**0.1886**	**0.0000**	**0.0000**	**0.6283**	**0.9746**	**0.0425**	**0.0986**	**0.2360**	**0.4022**
P	**0.519**		**0.841⁺**		-		**0.868**		**0.505**		**0.298**	
V2	3.0631	0.0000	0.9374	0.9201	0.0000	0.0000	4.1358	4.9608	4.0393	4.1873	4.1185	4.0618
	3.2652	3.2652	0.5724	0.1874	0.0000	0.0000	4.7025	3.8961	4.0909	4.0845	3.9398	4.4027
	0.0000	3.0631	0.1832	0.5617	0.0000	0.0000	8.2326	4.1811	3.9823	4.0730	4.8569	4.1968
	0.000	3.2652	0.5659	0.5473	0.0000	0.0000	3.5459	5.1654	3.9541	3.8799	4.9398	4.0910
	7.7331	2.8846	0.5703	0.1812	0.0000	0.0000	5.1654	4.6376	3.8471	4.3943	4.1817	3.7213
Mean	**2.8123**	**2.4956**	**0.5659**	**0.4795**	**0.0000**	**0.0000**	**5.1924**	**4.5682**	**3.9827**	**4.1238**	**4.4074**	**4.0947**
Std dev	**3.1741**	**1.4041**	**0.2667**	**0.3081**	**0.0000**	**0.0000**	**1.8002**	**0.5285**	**0.0924**	**0.1876**	**0.4579**	**0.2479**
P	**1.000⁺**		**0.648**		-		**0.478**		**0.170**		**0.216**	
V3	1.0961	0.9868	1.4409	0.8535	0.0000	0.0000	4.2168	5.3632	4.1422	4.0658	4.0736	4.2935
	6.0617	3.3854	0.8535	0.8735	0.0000	0.0000	4.2579	4.4934	4.2598	4.2114	4.5055	4.2935
	4.4769	4.6486	0.8584	0.8298	0.0000	0.0000	4.3724	4.0458	4.1919	4.2549	4.3222	4.1364
	4.7978	5.7148	0.8535	1.3772	0.0000	0.0000	4.3367	4.0458	4.2492	4.1825	3.6708	3.9578
	1.6412	5.3089	1.4163	0.8584	0.0000	0.0000	4.6212	5.0522	4.3579	4.1458	4.6031	4.7290
Mean	**3.6148**	**4.0089**	**1.0845**	**0.9585**	**0.0000**	**0.0000**	**4.3610**	**4.6001**	**4.2402**	**4.1721**	**4.2350**	**4.2820**
Std dev	**2.1429**	**1.9060**	**0.3142**	**0.2346**	**0.0000**	**0.0000**	**0.1580**	**0.5943**	**0.0810**	**0.0716**	**0.3744**	**0.2857**
P	**0.726**		**0.548⁺**		-		**0.841⁺**		**0.197**		**0.829**	
V4 (Dipping)	12.2944	6.0662	1.7351	1.7977	0.0000	0.0000	5.1040	5.1040	4.3231	4.4791	5.1186	5.1382
	4.4355	13.3961	2.6782	0.5813	0.0000	0.0000	5.4406	5.4406	3.8909	4.3739	3.7829	5.4406
	9.5930	0.0000	2.9298	4.0564	0.0000	0.0000	5.1040	5.1040	4.2565	4.4392	3.7829	3.5952
	16.3210	4.8720	2.8301	3.0742	0.0000	0.0000	4.8065	5.4406	4.1116	4.5895	3.4405	5.5076
	4.8720	16.3210	2.8301	1.5877	0.0000	0.0000	5.8249	4.8065	4.1116	3.8496	4.4851	3.4253
Mean	**9.5032**	**8.1310**	**2.6007**	**2.2195**	**0.0000**	**0.0000**	**5.2560**	**5.1791**	**4.1387**	**4.3462**	**4.1220**	**4.6214**
Std dev	**5.0351**	**6.6294**	**0.4921**	**1.3570**	**0.0000**	**0.0000**	**0.3892**	**0.2678**	**0.1664**	**0.2885**	**0.6745**	**1.0256**
P	**0.722**		**0.572**		-		**0.726**		**0.201**		**0.390**	

***Statiscally significant difference between control and the DFPM for the mineral/electrolyte to creatinine ratio. *P*<0.05; ⁺statiscally significant difference by the Mann-Whitney rank sum test;.(0.000) reading below detection limit of analyser; undiluted=the normal elution rate applied in the other studies.**

Table 15: Phosphate/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effect
P/Cr	V1	0.347	0.184	0.0137	$P<0.05^{a,b;\ a,c;\ b,c}$
	V2	0.364	0.0735	0.0148	$P<0.05^{a,c;\ b,c}$
	V3	0.104	0.0444	0.00594	$P<0.05^{a,c}$
	V4	0.0930	0.04947	0.00199	
	Volume effect	$P<0.05^{1,3;\ 1,4}$	$P<0.05^{1,2;\ 1,3;\ 1,4}$		

Table 16: Chloride/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effect
Cl/Cr	V1	41.768	41.097	3.320	$P< 0.05^{a,c;\ a,b}$
	V2	31.993	13.668	2.302	$P< 0.05^{a,c;\ b,c;}$
	V3	16.583	8.383	2.117	
	V4	14.630	6.834	1.727	$P< 0.05^{a,c}$
	Volume effect	$P<0.05^{1,2;\ 1,3;\ 1,4}$	$P<0.05^{1,2;\ 1,3;\ 1,4}$		

Table 17: Magnesium/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effect
Mg/Cr	V1	0.434	0.130	0.294	$P<0.05^{a,b;\ a,c}$
	V2	0.403	0.277	0.296	
	V3	0.344	0.214	0.302	
	V4	0.314	0.525	3.320	
	Volume effect			$P<0.05^{1,2;\ 1,3;\ 1,4}$	

^{1,2,3} Means with different superscript differ significantly (P< 0.05) Volume effect for each dilution

^{a,b,c} Means with different superscript differ significantly (P< 0.05) Dilution effect for each volume

Undiluted=as before in Table 12 – 14.
Two way ANOVA test. if equal mean and/or equal variance failed, all pairwise multiple comparison test (Tukey)

Table 18: Calcium/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effect
Ca/Cr	V1	1.187	0.109	0.0450	$P< 0.05^{a,c;\ b,c}$
	V2	0.790	0.141	0.0488	$P< 0.05^{a,c;\ b,c}$
	V3	0.374	0.156	0.0649	$P< 0.05^{a,c;\ b,c}$
	V4	0.407	0.217	0.0942	$P< 0.05^{a,c;\ b,c}$
	Volume effect	$P<0.05^{1,2;\ 1,3;\ 1,4}$			

Table 19: Sodium/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effct
Na/Cr	V1	2.004	0.229	0.000	$P< 0.05\ a^{c,\ b,c}$
	V2	2.496	0.480	0.000	
	V3	4.009	0.958	0.000	$P< 0.05^{a,c,\ b,c}$
	V4	8.131	2.219	0.000	
	Volume effect		$P<0.05^{1,2;\ 1,3;\ 1,4}$		

Table 20: Potassium/Creatinine ratio of goat urine from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), comparing the dilution effect and the volume effect, expressed as a mean.

		a	b	C	Dilution effect
K/Cr	V1	3.611	4.019	3.854	
	V2	4.568	4.124	4.095	$P< 0.05^{a,c;\ b,c}$
	V3	4.600	4.172	4.282	
	V4	5.179	4.346	4.621	
	Volume effect	$P< 0.05^{1,3}$			

^{1,2,3} Means with different superscript differ significantly (P< 0.05) Volume effect for each dilution

^{a,b,c} Means with different superscript differ significantly (P< 0.05) Dilution effect for each volume; Undiluted=as before in Table 12 - 14

Two way ANOVA test. If equal mean and/or equal variance failed, all pairwise multiple comparison test (Tukey)

8.7. PHASE II

8.7.1. Testing the Effect of Different Water Intakes on Mineral/Electrolyte to Creatinine Ratio in Goat Urine by Using the DFP Method

Results comparing the control and the method using goat's urine (group 1 water *ad libitum*) are presented in Table 21. Each result is the mean of five urine samples of each animal (n=5) over six days. Two urine samples were missing, because the goats did not produce enough urine. The concentration values used for ratio calculation are presented in Table 12 in the Appendix. Comparisons between the control (urine) and the method were conducted by using interclass correlation (R_I >0.75), coefficient of variation (%), and significant difference (P<0.05). For all ratios, except P/Cr ratio, there is no difference between the DFPM and control. Correlation coeficients (r^2) were: Cl/Cr-0.8593; Mg/Cr-0.9464; Ca/Cr-0.8291; Na/Cr-0.9293; K/Cr-0.9783. However, the coefficients of variation were very high, for all ratios.

Mineral/electrolyte to creatinine ratio values of each day by treatment groups are presented in Table 22 (Group 1), Table 23 (Group 2), and Table 24 (Group 3). The concentration at which the ratios were calculated are presented in Appendix, Tables 13, 14, and 15 for Group 1, Group 2, and Group 3 respectively. The results presented in Table 22, 23, and 24, show the means of 5 samples of goat urine for each day, and a comparison between control (urine) and the DFPM. There were no significant difference between control and the DFPM, except in Group 2, for Cl/Cr ratio on day 1.

Data for the effect of water intake and the effect of day on mineral/electrolyte to creatinine ratio, is presented using sample means for each ratio in Table 25 (P/Cr), Table 26 (Cl/Cr), Table 27 (Mg/Cr), Table 28 (Ca/Cr), Table 29 (Na/Cr), and Table 30 (K/Cr). The *p* values are presented in Tables 16 and 17 in the Appendix. All mineral/electrolyte to creatinine ratios were unaffected by water intake and by the duration of the treatment. However, Cl/Cr ratio values were significantly different (P<0.05) between Groups 1 and 2 on Day 2, between all Groups on Day 3, and between Group 1 and 2, and 1 and 3 on Day

6. There was also a significant difference between Day 1 and the rest of the period of time.

8.8. Tables – Phase II (*in vitro and in-vivo*)

Table 21: Mineral/electrolyte to creatinine ratios of goat urine. Comparison of the Dry Filter Paper Method (DFPM) and direct urine dilution (control). Each value shows the mean of a duplicate of a pool of goat urine samples. Coefficient of variation (C.V%.), *p* value, and interclass correlation (R_I) between control and DFPM.

	Ratios											
	P/Cr		Cl/Cr		Mg/Cr		Ca/Cr		Na/Cr		K/Cr	
	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM
1	0.00935	0.0185	0.0130	0.01289	0.2127	0.2386	0.114	0.1315	1.1345	0.197	3.5085	3.5687
2	0.0059	0.0058	0.0128	0.0127	0.3414	0.353	0.1058	0.1049	1.2322	1.5114	2.6178	2.8270
3	0.0212	0.0083	0.0194	0.01994	0.3371	0.3231	0.1967	0.1850	2.7254	2.6799	4.7082	4.7130
4	-0.0012	0.0095	-0.0017	0.0069	0.2591	0.2462	0.4291	0.0635	1.3185	1.6696	3.8102	3.8874
5	-0.0034	-0.0005	0.0105	0.0076	0.2029	0.1884	0.0454	0.0336	1.1799	0.8832	3.4154	3.4141
6	-0.0005	0.0044	0.0170	0.01410	0.2557	0.2340	0.0611	0.0496	1.3136	1.2780	4.7913	4.6614
7	-0.0043	0.0058	0.0093	0.0097	0.1870	0.2033	0.0219	0.0269	1.0214	1.0654	3.6466	3.7628
8	-0.0021	0.0278	0.0498	0.0468	0.5898	0.5449	0.1171	0.0408	4.4110	5.1394	3.9921	4.0657
9	0.0002	0.0002	0.0075	0.0308	0.2179	0.2609	0.1783	0.1889	1.8335	2.0345	3.0826	2.2745
10	0.0001	-0.0057	0.0195	0.0141	0.2032	0.1952	0.0516	0.0668	0.6175	0.9152	4.4193	4.4049
11	-0.002	-0.0020	0.02	0.0204	0.1914	0.1912	0.061	0.0725	1.0453	0.8611	3.4352	4.8573
12	-0.0021	0.0023	0.0126	0.0153	0.2216	0.2326	0.0517	0.0516	0.6793	0.9279	2.9543	2.9761
13	0.0326	0.0005	0.0926	0.0429	1.1488	0.9473	0.2641	0.2092	3.7912	4.9350	2.8561	2.5334
14	-0.0105	0.0108	0.0352	0.0210	0.1681	0.1929	0.0623	0.0829	2.7851	2.7556	2.9715	2.9815
15	-0.0091	-0.0022	0.0160	0.0147	0.1619	0.181	0.0544	0.0584	0.9644	1.4119	3.3721	3.3802
16	0.1030	-0.0141	0.0584	0.0572	0.5086	0.5264	0.0705	0.0905	4.5408	5.879	3.6826	3.4835
17	-0.0039	-0.0019	0.0107	0.0155	0.1993	0.1818	0.0642	0.0499	1.0207	0.6211	4.5598	4.6722
18	0.0412	-0.1017	0.1962	0.3092	1.3179	1.7800	0.1429	0.2393	0.9487	0.9692	2.8931	3.0616
19	-0.0114	-0.0062	0.0122	0.0287	0.1648	0.2331	0.0532	0.0598	1.0867	0.8074	4.3415	4.1044
20	0.0033	-0.0062	0.0123	0.0096	0.2353	0.2633	0.0346	0.0378	1.1717	1.1632	4.1533	4.2039
21	-0.0248	-0.0004	0.0154	0.0129	0.2081	0.2139	0.0564	0.0584	0.5672	0.5638	5.2958	5.2972
22	-0.0047	-0.0017	0.0094	0.0079	0.1990	0.2127	0.042	0.04022	1.1804	0.3340	2.6843	2.6383
23	-0.0048	-0.0047	0.8357	0.0409	0.2018	0.2398	0.036	0.1263	1.2654	1.1898	11.78	11.2742
Mean	**0.0057**	**-0.0023**	**0.0318**	**0.0335**	**0.3362**	**0.3558**	**0.0838**	**0.0899**	**1.6445**	**1.7303**	**4.0421**	**4.0454**
Std.Dev	**0.0254**	**0.0234**	**0.0432**	**0.617**	**0.3032**	**0.3554**	**0.0604**	**0.0613**	**1.1705**	**1.5619**	**1.820**	**1.7771**
C.V.%	**443.333**	**-1006.92**	**135.814**	**183.843**	**90.162**	**99.877**	**72.069**	**68.124**	**71.156**	**90.268**	**45.571**	**43.929**
P	**0.3517**		**0.7769**		**0.3977**		**0.4221**		**0.4425**		**0.9685**	
R_I	**-0.3841**		**0.8593**		**0.9464**		**0.8291**		**0.9293**		**0.9783**	

***P* values for comparing two groups (T-test), P<0.05; R_I interclass correlation**

Table 22: Mineral/electrolyte to creatinine ratios in Group 1 (*ad libitum* water intake) of goat urine over a period of six days. Comparison between control (urine) and dry filter paper method (DFPM).

		Group 1											
		P/Cr		Cl/Cr		Mg/Cr		Ca/Cr		Na/Cr		K/Cr	
Day		Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	Mean	0.0121	0.0109	0.0151	0.0152	0.2970	0.3050	0.1390	0.1400	1.697	1.463	3.6120	3.7030
	St. d	0.0081	0.00674	0.00376	0.0737	0.0731	0.00593	0.0503	0.0408	0.892	1.242	1.049	0.950
	P	**0.847***		**1.000***		**0.893**		**0.967**		**0.804**		**0.916**	
2	Mean	-0.0023	0.0093	0.0170	0.0170	0.2990	0.2830	0.0577	0.0429	1.849	2.007	5.7290	5.5470
	St. d	0.001587	0.00109	0.0195	0.0169	0.166	0.148	0.0360	0.0143	1.437	1.775	4.087	3.641
	P	**0.016**		**0.841***		**0.841***		**0.918**		**1.000***		**1.000***	
3	Mean	0.0057	-0.001	0.0304	0.0247	0.3970	0.3650	0.1210	0.1180	1.5930	1.9360	7.2120	6.1080
	St. d	0.00150	0.0305	0.0351	0.0121	0.421	0.327	0.0961	0.0749	1.321	1.748	7.800	5.032
	P	**1.000***		**0.548***		**0.841***		**0.841***		**0.736**		**0.841***	
4	Mean	0.0241	-0.0218	0.0633	0.0835	0.4710	0.5720	0.0789	0.1040	4.3850	7.5160	13.3700	16.7860
	St. d	0.0490	0.0455	0.0766	0.127	0.495	0.691	0.0363	0.0773	4.830	11.025	16.818	23.273
	P	**0.163**		**1.000***		**1.000***		**0.841***		**1.000***		**0.690***	
5	Mean	-0.0111	-0.0036	0.0124	0.0148	0.2020	0.2310	0.0465	0.0490	0.9440	0.8760	3.6270	3.649
	St. d	0.00982	0.00306	0.00245	0.00951	0.00951	0.0236	0.0101	0.0117	0.267	0.254	0.702	0.705
	P	**0.199**		**0.642**		**0.690**		**0.757**		**0.726**		**0.965**	
6	Mean	-0.0069	-0.0122	0.0352	0.0283	0.3250	0.3110	0.0625	0.0653	1.6470	1.4160	5.6510	5.5040
	St. d	0.00919	0.0170	0.0287	0.0126	0.0126	0.178	0.0474	0.0405	1.411	1.378	3.551	3.362
	P	**0.690***		**0.639**		**0.174**		**0.841***		**0.548***		**0.841***	

*** Mann-Whitney rank Sum Test, P<0.05**

+ Statistical Difference between control and the DFPM

Table 23: Mineral/electrolyte to creatinine ratios in Group 2 (50 % water intake) of goat urine over a period of six days. Comparison between control (urine) and dry filter paper method (DFPM).

		Group 2											
		P/Cr		Cl/Cr		Mg/Cr		Ca/ Cr		Na/Cr		K/Cr	
Day		Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	*Mean*	0.0043	0.0042	1.9020	1.7180	0.2410	0.2360	0.1100	0.1070	1.1380	1.2140	3.9400	3.6830
	Std. dev	0.00594	0.00517	2.011	1.530	0.169	0.154	0.0688	0.0522	0.13	0.253	1.173	1.454
	P	**0.841***		**0.036+**		**0.690***		**0.939**		**0.570**		**0.808**	
2	*Mean*	0.0001	0.0001	1.6110	1.3780	0.2280	0.2200	0.0550	0.4992	1.4700	1.3090	4.6490	3.9800
	Std. dev	0.00165	0.00371	1.289	1.320	0.123	0.124	0.0296	0.0258	0.189	0.257	1.255	0.851
	P	**0.548***		**0.548***		**0.968**		**0.736**		**0.293**		**0.348**	
3	*Mean*	0.0035	0.0033	1.7770	1.8270	0.2350	0.2390	0.4930	0.0499	1.2020	1.2330	3.9550	4.0430
	Std. dev	0.00762	0.00256	0.482	0.312	0.130	0.139	0.0222	0.0230	0.359	0.240	0.902	1.067
	P	**0.222***		**0.850**		**0.963**		**0.970**		**0.877**		**0.892**	
4	*Mean*	-0.0057	-0.0046	2.0280	1.9680	0.2190	0.2330	0.0424	0.0426	1.7260	1.7150	3.8690	3.8950
	Std. dev	0.00306	0.0011	0.721	0.908	0.168	0.166	0.0396	0.0301	0.407	0.617	1.603	2.183
	P	**0.548***		**0.911**		**0.690**		**0.841***		**0.998**		**0.983**	
5	*Mean*	-0.0047	-0.0041	1.3720	1.5070	0.2480	0.2540	0.0452	0.0400	1.4710	1.2300	4.1710	3.9640
	Std. dev	000423	0.00389	0.680	0.137	0.119	0.124	0.0252	0.0286	1.164	1.032	2.152	1.856
	P	**0.824**		**0.848**		**0.945**		**0.767**		**0.548***		**0.867**	
6	*Mean*	-0.0009	-0.0011	1.2560	1.1550	0.2450	0.2400	0.04280	0.0346	1.3230	1.1570	4.1660	4.0001
	Std. dev	0.00137	0.00239	0.580	0.744	0.119	0.110	0.0217	0.0205	0.476	0.280	1.256	1.404
	P	**0.862**		**0.817**		**0.841***		**0.556**		**0520**		**0.863**	

*** Mann-Whitney rank Sum Test, P<0.05**

+ Statistical Difference between control and the DFPM

Table 24: Mineral/electrolyte to creatinine ratios in Group 3 (30% water intake) of goat urine over period of six days. Comparison between control (urine) and dry filter paper method (DFPM).

		Group 3											
		P/Cr		Cl/Cr		Mg/Cr		Ca/Cr		Na/Cr		K/Cr	
Day		Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	*Mean*	0.0046	0.0041	1.4670	1.4080	0.2110	0.2120	0.0962	0.0961	1.1030	1.2090	3.330	3.312
	Std. dev	0.00436	000041	0.617	0.622	0.159	0.153	0.0631	0.0633	0.649	0.589	1.738	1.640
	P	**0.548***		**0.884**		**0.988**		**0.999**		**0.793**		**1.000***	
2	*Mean*	0.0007	0.0011	0.8190	0.7920	0.2180	0.2070	0.0336	0.0299	1.3190	1.3130	3.377	3.179
	Std. dev	0.00352	0.00462	0910	0.730	0.140	0.134	0.0153	0.0124	0.539	0.647	1.886	1.702
	P	**1.000***		**0.960**		**0.548***		**0.683**		**0.988**		**0.867**	
3	*Mean*	0.0001	0.0002	1.1160	1.1040	0.1580	0.1650	0.0354	0.0372	1.2240	1.1630	3.066	3.119
	Std. dev	0.000	0.0018	0.669	0.581	0.0964	0.101	0.0197	0.0192	0.603	0.227	0.665	0.814
	P	**0.841***		**0.977**		**0.919**		**0.690**		**0.838**		**0.913**	
4	*Mean*	0.0063	0.0053	1.3840	1.2260	0.1360	0.1390	0.0225	0.0228	1.2890	1.2770	2.542	2.323
	Std. dev	0.00122	0.0092	0.509	0.189	0.105	0.101	0.0114	0.0125	0.0847	0.236	0.423	0.191
	P	**0.916**		**0.634**		**1.000***		**0.977**		**0.941**		**0.460**	
5	*Mean*	0.0062	0.0053	0.8950	0.8340	0.2290	0.2060	0.0367	0.0363	0.9570	0.8270	3.3873	3.087
	Std. dev	0.0195	0..0175	0.582	0.502	0.0715	0.0932	0.0112	0.0160	0.261	0.155	0.971	0.978
	P	**1.000***		**0.864**		**0.674**		**0.971**		**0.366**		**0.421***	
6	*Mean*	-0.0020	-0.0022	1.9490	1.8840	0.2070	0.2070	0.0294	0.0292	1.2880	1.1390	2.948	3.357
	Std. dev	0.00334	0.004	0.471	0.528	0.0870	0.088	0.0116	0.0132	0.300	0.329	0.416	0.964
	P	**0.841***		**0.842**		**0.841***		**0.980**		**0.475**		**0.409**	

*** Mann-Whitney rank Sum Test, P<0.05**

+ Statistical Difference between control and the DFPM

Table 25: Phosphate/Creatinine ratio. Comparison of the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

P/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	0.0109	0.0093	-0.001	-0.0218	-0.0036	-0.0122
Group 2	0.0042	0.0001	0.0033	-0.0046	-0.0041	-0.0011
Group 3	0.0041	0.0011	0.0002	0.0053	0.0053	-0.0022

Table 26: Chloride/Creatinine ratio. Comparison of the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

Cl/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	0.0152	0.0170	0.0247	0.0835	0.0148	0.0283
Group 2	1.7180	1.37780	1.8270	1.9680	1.5070	1.1550
Group 3	1.4080	0.7920	1.1040	1.2260	0.8340	1.8840
		$P< 0.05^{1,2}$	$P< 0.05^{1,2,3}$			$P< 0.05^{1,2;1,3}$

Water intake effect

$P< 0.05^{1,2;1,4;1,5;1,6}$ **Day Effect**

Table 27: Magnesium/Creatinine ratio. Comparison of the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

Mg/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	0.3050	0.2830	0.3650	0.5720	0.2310	0.3110
Group 2	0.2360	0.2200	0.2390	0.2330	0.2540	0.2400
Group 3	0.2120	0.2070	0.1650	0.1390	0.2060	0.2070

Table 28: Calcium/Creatinine ratio. Comparison of the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

Ca/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	0.1400	0.4029	0.1180	0.1040	0.0490	0.0653
Group 2	0.1070	0.4992	0.0499	0.0426	0.0400	0.0346
Group 3	0.0961	0.0299	0.0372	0.0228	0.0363	0.0292

Table 29: Sodium/Creatinine ratio. Comparison of the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

Na/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	1.463	2.007	1.9360	7.5160	0.8760	1.4160
Group 2	1.2140	1.3090	1.2330	1.7150	1.2300	1.1570
Group 3	1.2090	1.3130	1.1630	1.2770	0.8270	1.1390

Table 30: Potassium/Creatinine ratio. Comparison the effect *ad libitum* water intake (Group 1), 50 % water intake (Group 2), 30% water intake (Group 3), over a period of six days, expressed as a mean for each group.

K/Cr

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group 1	3.7030	5.5470	6.1080	16.7890	3.6490	5.5040
Group 2	3.6830	3.9800	4.0430	3.8950	3.9640	4.0001
Group 3	3.3120	3.1790	3.1190	2.3230	3.0870	3.3570

9. DISCUSSION

9.1. – Testing Different Types of the Filter Paper

Due to the variability of the filter paper range which could been used in the DFPM, it was decided evaluate filter papers from 2 different manufactures (Whatman® and Scheicher & Schuel filter papers). The Whatman® number 6 is considered, to have a slow flowrate, a high retention, and low wet strength, relatively thicker than other filter papers (0.18 mm) (Tietz, 1976; Cope, 1997) Others filter papers are either thicker, have a medium flowrate and medium retention, such as Whatman® number 3, (Cope, 1997), or the same charateristics but slightly thicker, such as Whatman® number 42. This could influence the amount of urine retained. Filter paper such as Whatman® number 5 present almost the same characteristic as Whatman® number 6 (Cope, 1997), but it is thinner, which probably has an influence on the speed of distribuition of the artificial urine throughout the filter paper. The Schleicher & Schuell filter papers used are considered to be medium fast, thick (597, 598), medium fast to slow, thin (593, 595), and medium fast, smooth (860) (Schleicher & Schuell, 1991), which substantially differ from Whatman® number 6.

Phosphates are best collected on a slower paper of medium texture and calcium is better collected using a very slow, fine filter paper, highlighting some of the urine retention qualities of the filter paper (Tietz, 1976). Results (Tables 6 and 7) show that the Whatman® number 6 filter paper, presents more consistency throughout. Although it does not have a particularity high retention capacity for some constituents. Nevertheless, the results given by other filter papers, did not differ significantly in most respects from the Whatman® number 6. Considering the constant and uniform p value presented by Whatman filter paper number 6, even when compared with very high quality filter papers, (such as 5, 42, 43, 44, and 542 for Whatman® and 860, 593, 595, 597, 598 for Schleicher & Schuell), it appears that this filter paper is generally better for the DFPM.

9.2. – Testing The Diluent

The very high variability of urine mineral/electrolyte concentrations in ruminants (Field, 1964), and the limited linearity range of routine analysis methods and apparatus, are constraints to the use of routine laboratory analysers in urine mineral and electrolyte assessment (Christopher *et al,* 1996; De Groot and Asfjes, 1960). One of the approaches is to use a diluent, with a predetermined mineral/electrolyte concentration, designed so that "mixing" artificial/goat urine with the diluent will bring a final mineral/electrolyte concentration to fall into the linear and controlled/standardised range of the particular analytical method and apparatus. Such a diluent was prepared and the result for **Creatinine** (Figure 2) shows a high interclass correlation (0.9983) between the expected and the calculated values. However, a slight difference in slope (from unity) and the –52 µmol/l intercept might be attributed to the possibility that the creatinine used could have had a lower activity and been unstable, due to the storage time. To compensate, creatinine values were quantitatively increased and gave improved results.

At high concentrations, **phosphate** may precipitate out of the alkaline solution and thus be unavailable for analysis. At low concentration the phosphate should be completely dissolved and should thus lead to measured values that are very close to the expected. To avoid this scenario, however, the solution was acidified. The results show a high interclass correlation (0.9895) between expected values and obtained results (Figure 3). In addition, the high interclass correlation probably shows that the diluent could be use for analysis of phosphate in urine, however 3.5% difference on the higher values between expected and calculated values should be taken in consideration.

Chloride (Figure 4) illustrated very good interclass correlation (0.9949) between the expected and calculated values. The slight variation and decreasing tendency could be explained by a reaction between the components in solution (Miller, 1984b), mainly with sodium ions. However, it does not explain that it influences the concentration of such a highly soluble compound. In addition, r^2 between expected values and calculated values is relatively low compared to the other analytes, due the great variability of the results which

probably could be explained by the poor precision of the method of analysis (ferric/mercuric) (Miller, 1984b).

The 10% slope difference in the **magnesium** results (Figure 5) could be caused by a higher magnesium concentration in the diluent than there should have been (inaccuracy during weighing) or by the concentration of magnesium not falling within the linearity range of the apparatus. When overall results are compared, this error is insignificant as is illustrated by an almost perfet interclass correlation (0.9941) of expected value and obtained results. Although, interclass correlation is high, the 10% variation of magnesium should be taken into consideration, and is probably attributable to coeficient of variation for the method for serum magnesium concentrations within normal reference limits is approximately 11% to 12% (Farrell, 1984a).

Calcium, at high concentrations, could become unavailable for analysis, due to the precipitation in the alkaline solution. During the preparation of low concentration solutions, the calcium should be completely dissolved. At higher concentrations (Figure 6) the solution was opaque due to the undissolved crystals. Direct spectrophotometric measurements of calcium in serum and urine are based on the formation of colour complexes between calcium and organic molecules (Farrell, 1984c), and the opacity could have an effect on increasing absorbance of the photometric readings. Excessive magnesium concentrations could reduce calcium and phosphorus availability (Payne, 1977). It is thought that magnesium not only competes with calcium but might also combine with phosphorus to form relatively insoluble salts, which can not be measured (Payne, 1977). Interference by magnesium ions should be eliminated by addition of 8-hydroxiquinolone to reagent (Walmasley *et al,* 1981). However, the relatively high magnesium concentration in these solutions would probably exceed the "quenching" effect of 8-hydroquinolone. Although, the interclass correlation of 0.9941, between expected values and the obtained results, these factors could have influenced the calcium results at higher concentrations leading to a 7% drop in the slope, shown by the slightly significant effect of the factors (Figure 7).

Sodium results show low interclass correlation (0.9762) compared to the other minerals. Because the I.S.E analyser does not give values in fractions of whole numbers, a slight error in calculated concentration could be expected in the results, due to the rounding error values being multiplied in the dilution equation, which should be more evident at low concentrations (Figure 8) as is the case.

It has been found that indirect ISE for **potassium** concentration in urine consistently underestimates samples from sheep and cattle, because of the presence of an anionic or zwitterionic Chimical that bind potassium and sequester it from interaction with ISE (Brooks *et al,* 1988). It is not known whether the artificial urine contained potassium chelators, although none were added. According to the results is does not seem that artificial urine contains any chelators and there is a good correlation between calculated and expected values (0.9924) (Figure 9). In addition, bias is variable, as shown by a standard error of 10.53, between calculated and expected values (Table 8). The variation obtained may be attributable, in part, to the higher potassium concentrations in artificial urine, which would be in agreement with work in llama's (Christopher *et al,* 1996).

During the trail it was found that the diluent showed instability of minerals/electrolytes, leading to variability. This variation could be attributable, in part, to the unexpected interaction between molecules, because the diluent is a mix of different Chimical compounds. Recommendations for further studies to develop a preservative and/or stabiliser compound, even a buffer, are advisable in order maintain the stability of concentrations.

9.3. – Determination of the Reproducibility Of The Method

Reproducibility of mineral/electrolyte to creatinine ratio of artificial urine using a dried filter paper method has been studied. The current study evaluated reproducibility of undiluted and diluted 1:5 artificial urine to simulated reproducibility of mineral/ electrolytes to creatinine ratio at higher and lower concentrations respectively. The values indicate relatively good reproducibility of the assay, because the variation, as measured by

standard deviation, is small relative to the mean (C.V.%), except for Cl/Cr ratio and Ca/Cr ratio. The latter two ratios presented relatively low reproducibility, for both undiluted and diluted 1:5 artificial urine. It is difficult to find any explanation why the C.V% values of those ratios, were higher.

The reproducibility data show a marked difference with studies using human urine (Takemori, 1980), where distilled water was used as diluent and flame photometer as analytical method for minerals/electrolytes. However, that study did not evaluate the other minerals/electrolytes, reporting only Na/Cr and K/Cr ratio. Comparing the results from this study and the Takemori study, for Na/Cr and K/Cr, there is not a large difference between coefficient of variations (C.V. %), which has a range of 0~3.7%.

9.4. - Testing the Effect of Temperature and the Storage Time On The Method

The stability of mineral/electrolyte to creatinine ratio (E/Cr) in artificial and goats urine specimens on dried filter paper stored at two different temperatures over ten days was evaluated in this study. While it was found that the P/Cr, Mg/Cr, and K/Cr ratios were stable over the time see Figures 9, 11, 14, however the Cl/Cr (Figure 10), Ca/Cr (Figure 12), and Na/Cr ratios (Figure 13) were found to be much less stable over the same time period. However, the average result do not differ significantly from the control in both artificial urine as well as goat urine. The instability of those specific ratios, could be explained by the influence of the diluent, because those minerals/electrolytes specifically presented high coefficients of variation, between 12~13%. Another reason would be the variation of pH, because the diluent has not been submitted to any stabiliser, buffer or preservative. The diluent used was acidified, so precipatation would be an unlikely explanation.

The instability of ratios could not be attributed to the creatinine, because its was found to be fairly stable over the period of time. (Figure 9). In this study creatinine seems to confirm the robustness of the method, alluded to in other studies (Fuller and Ellia, 1988;

Soliman *et al,* 1986; Spierto *et al,* 1997), where the results of creatinine were stable in different preservation conditions, such as freezing or freezing with thymol (Soliman *et al,* 1986), storage at laboratory conditions of a temperature of 25^{0}C for 8 hours and 55^{0}C for two days (Spierto *et al,* 1997). In the above mentioned studies a loss of creatinine between 0.5~3% was reported, while in this study, the loss by comparing control and dried filter paper at two temperatures, varies between 6~8%, over ten days, without any preservation. Thus, since these data affirm the stability of creatinine in urine specimens stored on filter paper, normalising the concentrations of other urine constituent to creatinine, in most cases, should be unaffected by sample storage conditions. The results of the ratios, show no significant difference between temperatures and between temperatures and control (Table 11 and 12), except for K/Cr between temperatures and the control in artificial urine. In goat urine significant differences were present with P/Cr, and Ca/Cr between temperatures (30^{0}C and 22^{0}C), and K/Cr between control and the temperatures of 22^{0}C. The temperature seems not to affect the stability and reliability of the DFPM, illustrated by the insignificant difference between the two temperatures (30^{0}C and 22^{0}C) and between both temperatures and the reference (Table 10).

9.5. - Testing the Effect of Different Volumes of Impregnation and Different Urine Dilutions on the Method

An assumption has been made that any amount of discharged urine can be enough, as a sample for urine mineral/electrolyte analysis, as long as the mineral/electrolyte to creatinine ratio are kept invariable. Since a ratio can be obtained by taking measurements with the same volume of diluent even if the amount of specimen in unknown, it is assumed that there is not need to absorb a fixed amount of urine onto the filter paper when urine is collected using the dried filter paper method (Takemori, 1980). However, in the experiment using different urine volumes using the dried filter paper method, the results show significant differences between volumes 0.175 ml and 0.35 ml; 0.175 ml and 0.7 ml; 0.175 ml and 0.525 ml, but none of the ratios were significantly affected when comparing volumes 0.7 ml with 0.35 ml and 0.525 ml. It may be concluded, from the experiment that a dipping volume (0.7 ml), which is the easiest way of collecting the urine

sample with filter paper, could replace the volumes 0.35 ml and 0.525 ml without any effect on the mineral/electrolyte to creatinine ratio. Comparing the results of volume urine for ratios on undiluted urine and a dilution (1:5), the results show no significant difference, for Cl/Cr (Table 17), Mg/Cr (Table 18), Ca/Cr (Table 19), and K/Cr ratios (Table 21), between all volumes. At a dilution of 1:10, however, results show a significant difference for P/Cr (Table 16), and Na/Cr ratios (Table 20) between volumes.

A further experiment, for each urine volume when comparing different urine dilutions, significant differences exist between undiluted and 1:10 dilution, and between 1:5 dilution and 1:10 dilution, for most of the ratios. It should be emphasised that the 1:10 dilution of urine produces very low molecular concentrations, and that interactions between them and other molecules in the diluent may keep them unavailable, and could thus be the reason for the unreadable results in some ratios (P/Cr, Cl/Cr).

Further experiments to determine whether the dried filter paper method will show significant differences between fresh urine samples (used as a control) at different volumes and dilutions are presented Tables 13-15. The ratios indicate no statistically significant difference between the method and the control. On this basis it is assumed that the method is robust for using as a preservation and transportation method for urine with the advantage of not needing to use preservative or refrigeration. In addition a volume of 0.35 ml could be used for the method, without any changes of minerals/electrolyte to creatinine ratio, as long as the urine samples are not diluted less 1:5.

9.6. Testing the Effect of Different Water Intakes on Mineral/Electrolyte to Creatinine Ratio in Goat Urine by Using the Method

The coefficient of correlation of mineral/electrolyte to creatinine ratio between control (fresh urine sample, preserved in freezer) and the dried filter paper methods on Group 1 (goats given water *ad libitum*), was very high (Table 22). Interclass correlation agreement for the two methods were R_I>0.75. The high variance of P/Cr ratio has been show in other studies (Neiger *et al*, 1985; Scott, 1972). It has been shown that sheep and calves

excrete more phosphate and acid in urine when fed concentrate diets (Neiger *et al*, 1985; Payne, 1977; Scott, 1972) This would not explain the variation between animals in this study, because they were fed the identical complete ration. A possible explanation for the variation is that alkaline urine might form phosphate crystals that would settle to the floor of the urinary bladder (Neiger *et al*, 1985; Payne, 1977). Therefore, if just the first part of an alkaline voidance was sampled, the sample would have a falsely lowered phosphate concentration. In this study the urine samples were from completely voided samples, and they were further acidified to reduce this effect. Therefore this can not explain the low P/Cr ratio and the high variation between animals. However, in ruminants, Phosphorous is excreted principally via the gastrointestinal tract, which may influence renal excretion (Lackey *et al*, 1995), and may cause these differences (Belonje *et al*, 1971).

An experiment using goat urine to elucidate the relationship between the estimated influence of water deprivation on mineral/electrolyte to creatinine ratio was perfomed over a period of time. Examination of the data, Tables 26-31, demonstrates that concentrations and excretion of electrolytes vary from animal to animal, but the mineral/electrolyte to creatinine ratios hardly vary, even in water deprived animals. Comparison between treatment groups 1, 2, and 3, show no significant effect of water intake, except for Cl/Cr on days 2, 3, and 6 and day effect on Cl/Cr ratio for Group 2 (Table 27). Studies on llamas with (Lackey *et al*, 1995) concentrated urine, did not change significantly over time.

10. CONCLUSION

Preservation of urine samples on filter paper for mineral and electrolyte analysis by using mineral/electrolyte to creatinine ratio has thus proven itself to be a simple, cheap, and reliable technique, which is probably especially useful for large scale tests of a population of animals in remote parts with tropical and subtropical climates. The dried filter papers do not take up much space and the sample can not spill during transportation. The samples can also be transported through the mail effectively. A diluent is necessary to determine goat urine analytes in order to bring a final mineral/electrolyte concentration to fall into the linear and controlled/standard range of the particular mineral and electrolyte for routine laboratory analysers.

It has been shown that calculated mineral/electrolyte to creatinine ratios by the single-sample technique is an reliable indication of true urinary mineral and electrolyte excretion in goats.

The refinement of techniques and proper application of mineral/electrolytes to creatinine ratio in small ruminants could be very useful as a clinical diagnostic tool as well as an aid in renal physiology research. More important, however, is the evidence obtained from these experiments which suggest that the DFPMethod is robust and reliable and introduces a novel opportunity for monitoring and research into mineral nutrition in small ruminants in rural Africa.

However, further studies on determining mineral/electrolyte excretion against varying intake by using mineral and electrolyte ratio on dried filter paper need to be conducted in order to fully exploit the DFPMethod.

APPENDIX

11. APPENDIX – TABLES

Table 1: Analytes concentration of artificial urine using different filter papers type Whatman®[a] (**) and Schleicher & Schuell[b] (**) and the control (ref), expressed as mean of three replicates. Filter papers are identified by numbers.

FP no / Analytes	Ref	2**	3**	5**	6**	42**	43**	44**	542**	860*	593*	595*	597*	598*
Creatinine	16672	10605	10305	8422	10889	10105	9172	8005	9272	9189	8939	9455	9205	8989
Phosphate	4.48	2.313	2.647	2.65	3.147	2.647	2.813	2.647	2.813	2.147	2.313	3.647	1.813	1.647
Chloride	216	117.7	167.67	168	134.33	201	234.3	251	201	267.7	234.3	217.7	217.7	234.3
Magnesium	19.54	10.873	11.54	10.2	12.207	12.21	12.54	10.21	12.21	-0.46	12.21	12.04	10.87	10.54
Calcium	5.84	2.503	3.003	3.5	3.503	3.67	3.67	3.337	3.337	3.003	6.17	3.67	3.337	2.337
Sodium	132	182	182	165	91.33	115.3	248.7	198.7	282	165.3	165.3	165.3	165.3	132
Potassium	526.2	298.2	296.53	283	294.87	293.2	298.2	258.2	301.5	303.2	298.2	306.5	299.9	298.2

[a] Merck™, NT Laboratory supplies (Pty) Ltd, Midrand, Gauteng, South Africa
[b] Schleicher & Schuell filter papers, Lasec, Laboratory & Scientific Equipment, Pty, Ltd, Cape Town, South Africa

Table 2 : Mineral/electrolyte concentrations of artificial urine used to calculate mineral/electrolyte to creatinine for determination of the reproducibility of the DFPM, expressed as mean. Ten replicates were read at the artificial urine undiluted (AU) and ten at the one fifth of that concentration (1:5).

Ratios	Phosphate		Chloride		Magnesium		Calcium		Sodium		Potassium	
	A.U.	1:5	A.U.	1:5	A.U.	1:5	A.U	1:5	A.U.	1:5	A.U	1:5
1	2.352	1.602	399.333	224.333	11.785	5.035	4.693	4.193	138.833	27.863	262.583	52.583
2	2.502	1.352	424.333	324.333	11.785	4.685	4.193	3.693	138.833	28.833	267.583	52.583
3	2.602	1.602	474.333	274.333	11.785	4.858	4.193	2.693	128.833	27.864	262.583	52.583
4	2.602	1.602	424.333	274.333	12.785	4.535	3.693	4.193	128.833	28.833	267.583	52.583
5	2.602	1.602	424.333	224.333	12.285	7.785	4.193	3.993	128.833	26.860	262.583	52.583
6	2.602	1.602	424.333	324.333	12.285	4.735	4.193	3.693	128.833	28.833	267.583	52.583
7	2.602	1.602	374.333	224.333	11.785	5.035	4.193	4.193	128.833	26.833	267.583	52.583
8	2.552	1.352	349.333	224.333	12.785	4.785	5.193	3.443	128.833	28.833	262.583	52.583
9	2.352	1.352	474.333	274.333	12.785	4.785.	4.693	4.193	138.833	28.833	262.583	53.583
10	2.602	1.602	524.333	274.333	12.285	4.785	5.693	3.943	128.833	26.833	262.583	52.583
Mean	**2.562**	**1.527**	**429.333**	**264.333**	**12.285**	**4.77**	**4.493**	**2.823**	**131.833**	**28.042**	**264.583**	**52.683**

Table 3 : Descriptive statistics comparing analyte concentrations of artificial urine with control (Ref) at two different temperature (22^{0}C and 30^{0}C) over a period of ten days. Expressed as Mean and Standard deviation (std).

	Creatinine			Phosphate			Chloride			Magnesium			Calcium			Sodium			Potassium		
	µmol/l			mmol/l			mmol/l			mmol/l			mmol/l			mmol/l			mmol/l		
	Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp. (°C)		
Days	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref
1	9134.83	9168.16	9648.6	2.55	2.21	3.72	120.67	95.67	417.07	9.72	9.8	11.11	5.05	4.63	6.03	176.73	176.73	-56.4	257.52	257.52	253.22
2	9210.8	9094.17	9564.2	3.43	3.51	2.27	333.83	417.17	235.75	10.45	10.29	7.65	3.52	4.02	2.78	-66.34	-83	139.67	267.62	260.12	267.7
3	9276.85	9260.18	9478.7	3.04	3.29	3.86	475.5	367.17	242.8	11.02	11.27	11.78	5.9	5.06	4.64	31.67	31.67	-134.1	260.07	259.23	222.33
4	9475.5	9392.17	9472.2	2.89	3.22	3.15	235.48	260.48	274.2	11.6	12.02	10.3	7.12	6.95	3.25	94.83	111.5	-42.8	271.63	270.8	254.49
5	9310.5	9427.67	9414.8	3.31	3.39	3.10	328	394.67	138.4	11.85	10.35	11.51	7.26	7.51	4.79	154.5	179.5	-5.8	266.8	283.72	252.45
6	9151.2	9259.5	9588.2	2.96	3.12	3.05	548.34	615	301.4	10.36	11.19	11.11	5.9	5.23	1.56	95.17	103.5	101.8	262.47	266.63	263.14
7	9402.5	9585.83	9449.1	3.12	3.46	2.70	554.67	279.67	83.6	1.32	12.98	10.71	4.27	4.1	6.8	71	79.33	-104.8	262.57	268.4	249.32
8	9170.67	9437.33	10010.1	3.02	3.60	3.51	436.83	336.83	308.6	10.71	11.55	11.55	4.11	4.02	5.4	-197.17	-197.2	39.8	261.73	268.4	270.46
9	8967.8	9201.17	9688.3	2.87	3.03	3.47	289.33	239.33	375.4	9.11	8.94	10.52	3.77	2.84	4.45	63.67	63.67	196.05	255.07	254.23	269.25
10	9278.17	9292.83	9721.6	3.1	2.44	3.94	251.48	276.48	442.95	10.58	11.42	10.4	4.53	4.37	5.95	146.33	121.33	45.2	263.4	261.73	268.6
Mean	**9237.682**	**9341.902**	**9603.610**	**3.027**	**3.127**	**3.276**	**367.41**	**328.25**	**282.02**	**10.77**	**10.98**	**10.66**	**5.143**	**4.873**	**4.766**	**57.036**	**58.702**	**14.262**	**264.89**	**266.08**	**257.09**
Std	**146.722**	**146.863**	**177.178**	**0.244**	**0.460**	**0.528**	**142.91**	**136.63**	**114.36**	**0.871**	**1.162**	**1.175**	**1.345**	**1.410**	**1.616**	**113.24**	**117.37**	**11.93**	**9.009**	**8.496**	**14.606**

Table 4 : Descriptive statistics comparing analyte concentrations of goat urine with control (Ref) at two different temperature (22^{0}C and 30^{0}C) over a period of ten days. Expressed as a Mean and Standadrd Deviation (std)

	Creatinine			Phosphate			Chloride			Magnesium			Calcium			Sodium			Potassium		
	µmol/l			mmol/l			mmol/l			mmol/l			mmol/l			mmol/l			mmol/l		
	Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp. (°C)			Temp.			Temp. (°C)			Temp. (°C)		
Days	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref	22	30	Ref
1	14929.5	14687.8	14403.5	22.99	24.74	9.26	337.33	715.83	328.67	11.1	12.35	12.35	13.42	12.26	10.96	175.83	134.17	32	152.65	141.82	142.04
2	14953	14562	14346.9	22.36	22.61	8.83	329.33	346.5	181.34	10.03	7.45	7.45	8.24	8.37	4.91	256.5	389.83	182.17	152.02	155.35	147.34
3	14668.5	14951	10201	21.57	24.74	16.13	375	466.67	106.83	10.57	11.9	11.9	10.66	10.0	6.19	43.0	43.0	-78	145.88	145.05	101.14
4	14401.5	14584.8	14519.9	21.57	24.58	22.52	360.8	352.47	408.5	10.41	12.08	12.08	9.62	10.12	6.23	159.13	167.47	74.5	149.23	147.57	145.35
5	15126.8	15343.5	14655.9	22.02	23.35	22.62	276.03	209.37	228.5	11.11	12.45	12.45	8.37	7.37	8.26	237	237.83	-138	152.57	152.57	136.94
6	14893.5	15426.8	15080.9	21.92	22.83	24.64	327.5	302.5	306.5	10.15	11.40	11.40	9.45	8.78	5.54	254.17	295.83	282	155.22	161.83	152.3
7	14685.5	14802.2	14299.2	22.52	23.02	22.62	179	270.67	138	9.26	12.18	12.18	5.66	7.57	3.71	213.03	221.37	148.5	158.8	157.67	148.85
8	14572.2	15802.2	14707.2	21.32	23.91	22.93	432.83	349.5	269.5	10.7	12.03	12.03	8.71	11.13	10.32	251.7	210.03	135	155.07	144.19	145.35
9	14968.3	15423.3	12146.1	22.83	24.58	19.02	450	475	542	10.94	12.69	12.69	8.27	10.6	6.72	424.82	399.82	277.65	163.37	160.87	123.22
10	14817.7	15334.3	14871.8	22.66	24.16	22.33	506.18	422.85	539.85	10.85	10.69	10.69	8.17	10.09	6.02	195.33	212	120	156.67	160.9	155.56
Mean	**14819.6**	**15092**	**13923.69**	**22.18**	**23.96**	**19.09**	**367.40**	**391.14**	**304.97**	**10.61**	**11.62**	**11.62**	**9.06**	**9.63**	**6.88**	**221.06**	**231.14**	**103.38**	**154.16**	**152.78**	**139.81**
Std	**203.36**	**428.64**	**1638.9**	**0.670**	**0.76**	**5.81**	**92..65**	**141.34**	**163.54**	**0.68**	**1.54**	**1.54**	**2.01**	**1.68**	**2.3**	**96.26**	**109.36**	**136.94**	**4.91**	**7.64**	**16.29**

Table 5 : Analyte (creatinine and phosphate) concentrations of goat urine, comparison between four different volumes (0.175 ml; 0.35 ml; 0.525 ml; 0.7 ml) with three different dilutions (undiluted; dilution 1:5; dilution (1:10).

	Analytes											
	Creatinine (µmol/l)						Phosphate (mmol/l)					
Goat Urine	**Undiluted**		**Dilution 1:5**		**Dilution 1:10**		**Undiluted**		**Dilution 1:5**		**Dilution 1:10**	
Concent.	**Goat Urine**		**1:5**		**1:10**		**Goat Urine**		**1:5**		**1:10**	
Volume	**Control**	**DFPM**	**Control**	**DFPM**	**Control**	**DFPM**	**Control**	**DFPM**	**Control**	**DFPM**	**Control**	**DFPM**
0.175	4308	4158	708	608	608	408	1.902	1.402	1.902	1.402	0.902	1.402
	4658	4258	958	758	558	558	1.902	2.902	1.902	1.902	0.402	-0.098
	4258	4308	958	1008	258	508	1.402	1.402	0.902	1.402	0.902	-0.098
	4408	4058	908	658	458	508	1.902	0.902	1.402	1.402	0.902	1.402
	4408	4708	808	1058	508	458	1.402	1.902	1.402	0.902	-0.098	-0.098
Mean	**4408**	**4298**	**868**	**818**	**478**	**488**	**1.702**	**1.702**	**1.502**	**1.402**	**0.602**	**0.502**
Std dev	**154.110**	**248.495**	**108.397**	**204.328**	**135.093**	**57.009**	**0.274**	**0.758**	**0.418**	**0.354**	**0.447**	**0.822**
0.35	8658	8758	1858	1958	808	808	0.402	0.902	0.902	0.902	1.902	1.402
	8758	8558	1708	1608	758	758	0.902	1.902	1.402	1.402	0.402	0.402
	8708	9008	1858	1758	808	808	0.902	1.402	1.4024	1.902	-0.098	0.902
	8758	9058	1658	1958	858	758	1.402	0.902	1.402	0.902	0.902	0.902
	8808	8708	1658	1758	708	858	0.402	1.402	0.0902	1.402	0.902	0.402
Mean	**8738**	**8818**	**1748**	**1808**	**788**	**798**	**0.802**	**1.302**	**1.202**	**1.302**	**0.802**	**0.802**
Std dev	**547.009**	**210.357**	**104.470**	**150.000**	**57.009**	**41.833**	**0.418**	**0.418**	**0.274**	**0.418**	**0.742**	**0.418**
0.525	13308	13558	2608	2358	2258	1608	1.902	1.402	1.902	1.402	0.902	2.652
	13058	13208	2358	2358	2058	1808	1.402	1.152	1.402	1.402	1.402	1.902
	13508	13308	2458	2508	1558	2008	1.402	1.402	1.402	0.902	0.902	1.402
	13208	13658	2758	2558	15508	2008/	1.902	1.902	0.902	0.902	0.902	1.402
	13108	13658	2308	2458	1508	1608	1.402	2.402	0.902	1.402	0.402	1.902
Mean	**12238**	**13478**	**2498**	**2448**	**1788**	**1808**	**1.602**	**1.652**	**1.302**	**1.202**	**0.902**	**1.852**
Std dev	**178.885**	**207.966**	**185.068**	**89.443**	**345.688**	**200.00**	**0.274**	**0.5**	**0.418**	**0.274**	**0.354**	**0.512**
0.7 (Dipping)	17608	16508	3308	3108	1808	2508	1.902	1.402	1.902	1.902	-0.098	-0.098
	17508	17658	3458	3208	1908	2208	1.902	2.402	0.902	2.402	0.902	-0.098
	17358	17708	2908	3008	1858	1608	4.402	4.402	0.902	0.902	-0.098	0.402
	17608	17558	2758	3208	1758	1308	4.402	3.402	1.402	0.902	0.402	0.902
	17708	15958	3258	3258	1758	1408	2.902	2.902	1.402	0.902	-0.098	0.402
Mean	**17558**	**17078**	**3138**	**3158**	**1818**	**1808**	**3.102**	**2.902**	**1.302**	**1.402**	**0.202**	**0.302**
Std dev	**132.288**	**797.339**	**292.831**	**100.00**	**65.192**	**524.404**	**1.255**	**1.118**	**0.418**	**0.707**	**0.447**	**0.418**

Table 6 : Analyte (chloride and magnesium) concentrations of goat urine, comparison between four different volumes (0.175 ml; 0.35 ml; 0.525 ml; 0.7 ml) with three different dilutions (undiluted ; dilution 1:5; dilution (1:10).

	Analytes											
	Cloride (mmol/l)						Magnesium (mmol/l)					
Goat Urine	Undiluted		Dilution 1:5		Dilution 1:10		Undiluted		Dilution 1:5		Dilution 1:10	
Concent.	Goat Urine		1:5		1:10		Goat Urine		1:5		1:10	
Volume	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
0.175	205.8	255.8	305.8	255.8	105.8	95.8	14.236	12.236	5.236	6.236	1.236	0.236
	255.8	205.8	155.8	305.8	155.8	55.8	14.2362	13.236	6.236	5.736	1.236	1.736
	205.8	255.8	255.8	205.8	105.8	205.8	12.236	12.236	4.236	5.236	1.736	1.236
	305.8	255.8	255.8	195.8	155.8	155.8	14.236	13.736	4.236	6.236	1.736	1.236
	155.8	255.8	255.8	255.8	205.8	205.8	14.236	12.236	4.736	5.736	1.736	0.736
Mean	**225.8**	**245.8**	**245.8**	**243.8**	**145.8**	**143.8**	**13.836**	**12.936**	**4.936**	**5.836**	**1.536**	**1.036**
Std dev	**57.009**	**22.361**	**54.772**	**44.385**	**41.833**	**66.858**	**0.894**	**0.671**	**0.837**	**0.418**	**0.274**	**0.570**
0.35	260.8	255.8	205.8	155.8	205.8	155.8	27.236	24.736	5.236	6.736	2.736	-0.764
	255.8	2.55.8	255.8	205.8	255.8	205.8	25.236	26.736	5.736	7.736	2.236	1.736
	255.8.	255.8	255.8	205.8	255.8	200.8	27.236	27.736	7.736	7.736	2.736	2.236
	255.8	250.8	205.8	255.8	205.8	195.8	27.236	27.236	6.736	6.736	2.236	2.236
	255.8	255.8	155.8	205.8	105.8	255.8	25.236	26.736	4.736	7.236	-1.264	1.736
Mean	**256.8**	**254.8**	**215.8**	**205.8**	**205.8**	**202.8**	**26.536**	**26.636**	**6.036**	**7.236**	**1.736**	**1.436**
Std dev	**2.236**	**2.236**	**41.833**	**35.355**	**61.237**	**35.637**	**0.975**	**1.140**	**1.204**	**0.500**	**1.696**	**1.255**
0.525	355.8	305.8	255.8	155.8	205.8	195.8	40.236	38.236	6.736	8.736	4.736	3.236
	205.8	305.8	305.8	255.8	105.8	200.8	40.236	40.236	7.736	6.236	3.736	3.736
	305.8	305.8	355.8	255.8	260.8	205.8	41.236	41.236	8.236	6.736	4.736	3.736
	305.8	255.8	305.8	255.8	205.8	205.8	39.736	38.736	8.236	6.236	2.736	3.736
	305.8	305.8	305.8	505.58	270.8	200.8	40.736	41.236	8.236	6.736	0.736	2.736
Mean	**295.8**	**295.8**	**305.8**	**285.8**	**209.8**	**201.8**	**40.436**	**39.936**	**7.836**	**6.936**	**3.3336**	**3.436**
Std dev	**54.772**	**22.361**	**35.355**	**130.384**	**65.517**	**4.183**	**0.570**	**1.396**	**0.652**	**1.037**	**1.673**	**0.447**
0.7 (Dipping)	255.8	405.8	255.8	255.8	155.8	205.8	53.236	51.736	10.736	8.736	4.236	2.736
	305.8	605.8	305.8	355.8	255.8	205.8	53.236	51.236	10.236	9.736	3.236	3.236
	305.8	205.8	355.8	305.8	205.8	205.8	52.236	51.236	7.736	8.236	3.736	3.736
	305.8	155.8	305.8	305.8	305.8	255.8	52.736	50.236	10.236	8.736	3.736	3.736
	305.8	155.8	355.8	255.8	205.8	205.8	51.736	46.236	10.736	8.236	5.736	5.736
Mean	**295.8**	**305.8**	**315.8**	**295.8**	**225.8**	**215.8**	**52.636**	**50.136**	**9.936**	**8.736**	**4.136**	**3.836**
Std dev	**22.361**	**196.850**	**41.833**	**41.833**	**57.009**	**22.361**	**0.652**	**2.247**	**1.255**	**0.612**	**0.926**	**1.140**

Table 7 : Analyte (calcium and sodium) concentrations of goat urine, comparison between four different volumes (0.175 ml; 0.35 ml; 0.525 ml; 0.7 ml) with three different dilutions (undiluted; dilution 1:5; dilution (1:10).

	Analytes											
	Calcium (mmol/l)						Sodium (mmol/l)					
Goat Urine	Undiluted		Dilution 1:5		Dilution 1:10		Undiluted		Dilution 1:5		Dilution 1:10	
Volume	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
0.175	3.1	7.1	1.1	4.6	-0.4	0.6	24.75	24.75	24.75	-25.25	0.000	0.000
	3.6	-0.4	4.6	4.6	0.1	2.1	124.75	74.75	24.75	24.75	0.000	0.000
	4.6	11.1	3.6	3.6	3.6	2.1	69.75	74.75	-25.25	24.75	0.000	0.000
	4.1	6.1	4.6	4.6	3.6	2.6	74.875	74.75	-25.25	24.75	0.000	0.000
	6.1	6.1	3.1	2.6	1.1	1.1	24.75	74.75	54.75	24.75	0.000	0.000
Mean	**4.3**	**6**	**3.4**	**4**	**1.6**	**1.7**	**63.75**	**64.75**	**10.75**	**14.75**	**0.000**	**0.000**
Std dev	**1.151**	**4.129**	**1.440**	**0.894**	**1.904**	**0.822**	**41.593**	**22.361**	**35.071**	**22.361**	**0.000**	**0.000**
0.35	10.1	6.1	4.6	4.6	2.6	2.1	124.75	124.75	74.75	24.75	0.000	0.000
	8.1	6.1	3.6	8.6	-0.9	0.1	74.75	24.75	24.75	74.75	0.000	0.000
	4.6	6.6	4.6	5.6	0.6	-0.4	24.75	74.75	24.75	-25.25	0.000	0.000
	4.6	6.6	5.1	5.1	4.1	4.6	74.75	74.75	74.75	24.25	0.000	0.000
	4.6	6.1	3.6	4.6	7.1	7.6	74.75	24.75	24.75	74.75	0.000	0.000
Mean	**6.4**	**6.3**	**4.3**	**5.7**	**2.7**	**2.8**	**74.75**	**64.75**	**44.75**	**34.75**	**0.000**	**0.000**
Std dev	**2.564**	**0.274**	**0.671**	**1.673**	**3.110**	**3.328**	**35.355**	**41.833**	**27.386**	**41.833**	**0.000**	**0.000**
0.525	10.6	6.6	5.1	4.6	2.6	4.6	124.75	74.75	74.75	-25.25	0.000	0.000
	8.6	6.1	4.1	6.1	2.1	5.6	74.75	74.75	24.75	74.75	0.000	0.000
	7.1	9.1	5.1	5.1	7.1	2.1	74.75	74.75	24.75	24.75	0.000	0.000
	6.6	9.1	8.1	8.1	3.1	1.1	74.75	124.75	24.75	74.75	0.000	0.000
	6.1	6.1	4.6	5.1	3.6	3.6	124.75	74.75	24.75	24.75	0.000	0.000
Mean	**7.8**	**7.4**	**5.4**	**5.8**	**3.7**	**3.4**	**94.75**	**84.75**	**34.75**	**34.75**	**0.000**	**0.000**
Std dev	**1.823**	**1.565**	**1.565**	**1.396**	**1.981**	**1.823**	**27.386**	**22.361**	**22.361**	**41.833**	**0.000**	**0.000**
0.7 (Dipping)	7.1	8.6	10.6	8.6	3.6	4.1	74.75	74.75	74.75	74.75	0.000	0.000
	10.6	9.1	6.1	5.1	3.6	4.1	124.75	24.75	124.75	24.75	0.000	0.000
	10.1	7.6	5.1	5.1	2.6	4.6	124.75	174.75	-25.25	24.75	0.000	0.000
	6.6	7.1	5.1	6.6	4.1	2.6	124.75	124.75	74.75	24.75	0.000	0.000
	7.6	9.1	4.1	6.1	4.1	1.6	124.75	74.75	-50.75	24.75	0.000	0.000
Mean	**8.4**	**8.3**	**6.2**	**6.3**	**3.6**	**3.4**	**11.475**	**94.75**	**39.75**	**34.75**	**0.000**	**0.000**
Std dev	**1.823**	**0.908**	**2.559**	**1.440**	**0.612**	**1.255**	**22.361**	**57.009**	**74.162**	**22.361**	**0.000**	**0.000**

Table 8 : Analyte (potassium) concentrations of goat urine, comparison between four different volumes (0.175 ml; 0.35 ml; 0.525 ml; 0.7 ml) with three different dilutions (undiluted; dilution 1:5; dilution (1:10).

	Analytes					
	Potassium (mmol/l)					
Goat Urine	Undiluted		Dilution 1:5		Dilution 1:10	
Volume	Control	DFPM	Control	DFPM	Control	DFPM
0.175	186.24	186.24	71.24	71.24	26.24	20.24
	181.24	186.24	71.24	71.24	26.24	21.74
	181.24	191.24	71.24	71.24	21.24	21.24
	181.24	186.24	71.24	66.24	16.24	26.24
	181.24	181.24	71.24	66.24	26.24	21.24
Mean	**182.24**	**186.24**	**71.24**	**69.24**	**23.24**	**22.14**
Std dev	**2.236**	**3.536**	**0.000**	**2.739**	**4.472**	**2.356**
0.35	351.24	356.24	76.24	86.24	36.24	31.24
	346.24	351.24	81.24	81.24	34.24	41.24
	346.24	356.24	81.24	81.24	34.24	36.24
	346.24	351.24	76.24	81.24	31.24	36.24
	351.24	356.24	81.24	81.24	36.24	36.24
Mean	**348.24**	**354.24**	**79.24**	**82.24**	**35.24**	**36.24**
Std dev	**2.739**	**2.739**	**2.739**	**2.236**	**2.236**	**3.536**
0.525	551.24	551.24	106.24	101.24	41.24	41.24
	556.24	556.24	106.24	101.24	41.24	41.24
	566.24	566.24	106.24	103.74	41.24	41.24
	561.24	571.24	101.24	101.24	41.24	41.24
	571.24	566.24	106.24	116.24	41.24	41.24
Mean	**561.24**	**562.24**	**105.24**	**104.74**	**41.24**	**41.24**
Std dev	**7.906**	**8.216**	**2.236**	**6.519**	**0.000**	**0.000**
0.7 (Ddipping)	711.24	691.24	136.24	126.24	61.24	61.24
	716.24	721.24	136.24	141.24	61.24	61.24
	691.24	721.24	141.24	126.24	61.24	61.24
	969.24	681.24	136.24	131.24	61.24	61.24
	681.24	701.24	136.24	121.24	61.24	61.24
Mean	**699.24**	**703.24**	**137.24**	**129.24**	**61.24**	**61.24**
Std dev	**14.405**	**17.885**	**2.236**	**7.583**	**0.000**	**0.000**

Table 9: Mineral/electrolyte:creatinine ratio of goat urine, from three different dilutions (a=undiluted; b=dilution 1:5; c=dilution 1:10) impregnated with four different volumes (V1=0.175 ml; V2=0.35 ml; V3=0.525 ml; V4=0.7 ml), express as the *p<0.05* value, comparison factor dilution; factor volume ; interrelation Factor dilution vs factor volume

	Dilutions	Volumes	Dilutions vs volumes
P/Cr	$<0.001^{a,c;\ b,c}$	$<0.001^{1,2;\ 1,3;\ 1,4}$	$<0.001^{c}$
Cl/Cr	$<0.001^{a,c;\ b,c}$	$0.016^{1,2;\ 1,3;\ 1,4}$	<0.001
Mg/cr	$<0.001^{a,c;\ b,c}$	$0.001^{1,2;\ 1,4}$	0.074
Ca/Cr	$<0.001^{a,c;\ b,c}$	$<0.001^{1,3;\ 1,4}$	<0.001
Na/Cr	$<0.001^{a,\ b,\ c}$	$0.005^{1,2;\ 1,3;\ 1,}$	0.046
K/Cr	$<0.0107^{a,c;\ b,c}$	<0.08	0.002

$^{1,\ 2,\ 3,\ 4}$ stastical difference between volumes

$^{a,\ b,\ c}$ stastical difference between dilutions

Table 10: Analyte concentrations of goat urine. Comparison of the Dry Filter Paper Method (DFPM) and goat urine (control). Each value shows the mean of a duplicate pool of goat urine samples. The concentration were used to determine mineral/electrolyte to creatinine ratio.

	Analytes													
	Creatinine (µmol/l)		Phosphate (mmol/l)		Chloride (mmol/l)		Magnesium (mmol/l)		Calcium (mmol/l)		Sodium (mmol/l)		Potassium (mmol/l)	
	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM	Ctrl	DFPM
1	5343.8	5393.8	0.5	1	69.5	69.5	11.37	12.87	6.09	7.09	60.63	10.63	187.49	192.49
2	17093.8	17243.8	1	1	219.5	219.5	58.37	60.87	18.09	18.09	210.63	260.63	447.49	487.49
3	5893.8	5993.8	1.25	0.5	114.5	119.5	19.87	19.37	11.59	11.09	160.63	160.63	277.49	282.49
4	8297.2	9547.2	-0.098	0.902	-13.8	66.2	21.5	23.5	3.56	6.06	109.4	159.4	316.14	371.14
5	17747.2	18047.2	-0.598	-0.098	186.2	136.2	36	34	8.06	6.06	209.4	159.4	606.14	616.14
6	19747.2	20297.2	-0.098	0.902	336.2	286.2	50.5	47.5	12.06	10.06	259.4	259.4	946.14	946.14
7	25397.2	24347.2	-1.098	1.402	236.2	236.2	47.5	49.5	5.56	6.56	259.4	259.4	926.14	916.4
8	4747.2	5047.2	-0.098	1.402	236.2	236.2	28	27.5	5.56	2.06	209.4	259.4	238.2	218.2
9	5966.8	5366.8	0.01	0.01	454	165	13	14	10.64	10.14	109.4	109.4	546.14	396.14
10	17717	17417	0.01	-0.99	345	245	36	34	9.14	11.64	109.4	159.4	1096.1	1071.1
11	24817	24317	-0.49	-0.49	495	495	47.5	46.5	15.14	17.64	259.4	209.4	806.14	1096.1
12	23467	22567	-0.49	0.51	295	345	52	52.5	12.14	11.64	159.4	209.4	275.16	280.16
13	1566.8	2216.8	0.51	0.01	145	95	18	21	4.14	4.64	59.4	109.4	620.16	555.16
14	9313.8	9413.8	-0.98	1.02	328	198	15.66	18.16	5.81	7.81	259.4	259.4	755.16	765.16
15	21713.8	21913.8	-1.98	-0.48	348	323	35.16	39.66	11.81	12.81	209.4	309.4	319.2	313.2
16	6813.8	6963.8	7.02	-0.98	398	398	34.66	36.66	4.81	6.31	309.4	409.4	643.2	603.2
17	25413.8	25663.8	-0.98	-0.48	273	398	50.66	46.66	16.31	12.81	259.4	259.4	933.2	963.2
18	1263.8	963.8	0.52	-0.98	248	298	16.66	17.16	1.81	2.31	89.8	89.8	968.2	1030.7
19	9465.8	9265.8	-1.078	-0.578	115.6	265.6	15.59	21.598	5.04	5.54	489.8	139.8	258.2	248.2
20	17466	17315.8	-0.578	-1.078	215.6	165.6	41.098	45.598	6.04	6.54	239.8	139.8	523.2	563.2
21	20466	20615.8	-5.078	-0.078	315.6	265.6	42.598	44.098	1.54	12.04	189.8	189.8	1268.2	1218.2
22	33466	33665.8	-1.578	-0.578	315.6	265.6	66.598	71.598	14.04	13.54	70.2	20.2	903.2	873.2
23	5947.2	6047.2	-0.284	-0.284	497	247	12	14.5	2.14	7.64	159.4	159.4	453.2	473.2
Mean	**14310.1**	**14331.8**	**-0.2**	**0.07**	**268.39**	**240.82**	**33.49**	**34.73**	**8.31**	**9.14**	**193.58**	**187.06**	**622.34**	**629.59**
Std. Dev	**9111.5**	**8996.1**	**2.02**	**0.81**	**130.78**	**108.58**	**16.63**	**16.42**	**4.85**	**4.28**	**98.25**	**91.88**	**312.99**	**324.50**

Table 11 : Analyte concentrations of goat urine in group 1 (*ad libitum* water intake), comparison between control (urine) and dry filter paper method (DFPM) over a period of time, expressed as a mean of five replicates of goat urine collected each day.

	Group 1													
	Creatinine		Phosphate		Chloride		Magnesium		Calcium		Sodium		Potassium	
Day	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	8493.75	9543.75	2.666	0.8333	119.5	136.17	21.37	31.03	10.42	12.09	110.63	143.96	272.49	320.82
2	15187.2	15457.2	-0.398	0.902	196.2	146.2	36.7	36.4	6.96	6.16	209.4	249.4	682.14	691.14
3	14706.8	14377	-0.09	-0.09	265	245	33.3	33.6	8.34	11.14	139.4	209.4	603.55	524.16
4	11623.8	12983.8	0.72	-0.38	198	328	30.56	31.66	5.81	8.41	239.4	329.4	560.16	671.16
5	18578.3	20215.8	-2.078	-0.578	240.6	240.6	41.47	45.72	8.79	11.29	177.3	114.8	668.2	727.58
6	15997.2	15477.2	-1.084	-0.984	357	307	38.6	38.2	7.74	7.54	201.56	161.56	681.2	675.2

Table 12 : Analyte concentrations of goat urine in group 2 (50% water intake), comparison between control (urine) and dry filter paper method (DFPM) over a period of time, expressed as a mean of five replicates of goat urine collected each day.

	Group 2													
	Creatinine		Phosphate		Chloride		Magnesium		Calcium		Sodium		Potassium	
Day	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	14573.75	15523.8	0.2	0.3	219.5	179.5	30.37	32.17	11.13	11.98	140.63	180.63	490.49	513.49
2	16377.2	19497.2	0.02	0.005	186.2	226.2	37.8	41.7	3.93	6.96	189.4	249.4	646.14	762.14
3	19556.8	19517	1.61	-0.09	345	355	44.5	44.8	7.9	8.14	199.4	239.4	759.55	774.05
4	20153.8	22003.8	-1.08	-0.68	218	398	41.76	45.46	7.36	6.15	219.4	349.4	66.16	781.16
5	19485.8	21865.8	-0.778	-0.778	295.6	255.5	46.6	51.1	6.87	8.61	2793.8	139.8	717.2	797.2
6	19897.2	20787.2	-0.284	-1.284	237	207	45	46.7	6.92	6.22	241.56	181.56	727.2	779.2

Table 13 : Analyte concentrations of goat urine in group 3 (30% water intake), comparison between control (urine) and dry filter paper method (DFPM) over a period of time, expressed as a mean of five replicates of goat urine collected each day.

	Group 3													
	Creatinine		Phosphate		Chloride		Magnesium		Calcium		Sodium		Potassium	
Day	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM	Control	DFPM
1	19473.75	20163.8	0.7	-0.4	229.5	259.5	34.77	36.07	15.89	16.39	200.63	220.63	468.49	583.49
2	18537.2	21057.2	-0.198	0.02	126.2	146.2	33.8	40.4	6.06	5.96	209.4	259.4	586.14	625.14
3	22516.8	22577	-0.09	0.09	265	235	34.1	35.8	6.74	10.44	179.4	259.4	671.55	681.55
4	25480.47	27213.8	2.69	0.353	181.33	331.33	34.33	35.82	4.14	6.14	259.4	342.7	638.49	688.49
5	22865.8	25235.8	1.42	-0.778	205.6	205.6	45.2	50.1	8.34	10.44	219.8	159.8	767.7	823.7
6	22947.2	23797.2	-0.484	-0.384	327	487	45.9	47.5	6.14	7.24	291.56	221.56	668.79	790.79

Table 14 : Mineral/electrolyte ratio s of goats urine of three groups of water intake, over six (6) days, expressed as $p<0.05$ value, comparing the effect of time (days) on the ratio between groups of treatment.

	Ratios					
Day	**P/Cr**	**Cl/Cr**	**Mg/Cr**	**Ca/Cr**	**Na/Cr**	**K/Cr**
1	0.088	0.189	0.6930	0.068	0.638	0.134
2	0.134	0.044	0.693	0.346	0.954	0.419
3	0.111	0.001	0.414	0.095	0.954	0.367
4	0.404	0.0120	0.357	0.145	0.374	0.353
5	0.405	0.089	0.547	0.780	0.564	0.362
6	0.093*	0.001	0.531	0.208	0.454	0.398

Table 15 : Mineral/electrolyte to creatinine ratios of goat urine of three groups of water intake, over six (6) days, expressed as the $p<0.05$ value, comparing the effect different water intake on the ratio during a period of time.

Ratios	**Water Intake** (*p value*)	**Day** (*p value*)	**Water Intake x Day** (*p value*)
P/Cr	0.142	<0.001	0.371
Cl/Cr	<0.001	0.057	0.167
Mg/Cr	0.321	0.593	0.833
Ca/Cr	0.109	<0.001	0.230
Na/Cr	0.984	0.021	0.947
K/Cr	0.204	0.067	0.877

Table 16: Constituents concentrations and pure chemicals used to prepare the diluent for the impregnation volume experiment.

	$MgCl_26H_2O$ (0.10895 g/l)	$CaCl_2$ (0.1221 g/l)	KCl (0.26098 g/l)	NaCl (5.2027 g/l)	$NH_4H_2PO_4$ (0.0577 g/l)	Na_2HCO_3 (4.0508 g/l)	$C_4H_7N_3O$ (0.000905 g/l)	Total (Mmol/l)
Mg	0.536 mmol/l							0.536
Ca		1.1 mmol/l						1.1
PO_4^{-2}					0.502 mmol/l			0.502
Na				89.028 mmol/l		48.222 mmol/l		137.248
Cl	1.072 mmol/l	2.2 mmol/l	3.5 mmol/l	89.028 mmol /l				95.8
K			3.5 mmol/l					3.5
Cr							0.008 mmol/l	0.08

Where:
Electr.=Electrolytes; D. Rate = dilution rate; AU = Artificial urine minerals concentration; Conc. = Concentration; Mg = Magnesium; Ca = Calcium; PO^-_4 = Phosphate; Na = Sodium; Cl = Chloride; K = Potassium; Cr = Creatinine

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