การเปรียบเทียบระดับของ เอ็น-อะซีติล-เบต้า-ดี-กลูโคสามินิเดส ในปัสสาวะแมวที่เป็นโรคไตวายเรื้อรัง กระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ และนิ่วในกระเพาะปัสสาวะ

นางสาวอิษฎี ปานบุญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ภาควิชาอายุรศาสตร์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS IN CATS WITH CHRONIC KIDNEY DISEASE, FELINE IDIOPATHIC CYSTITIS AND CYSTIC CALCULI

Miss Isadee Panboon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2015

Thesis Title	COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS IN CATS WITH CHRONIC KIDNEY DISEASE, FELINE IDIOPATHIC CYSTITIS AND CYSTIC CALCULI
By	Miss Isadee Panboon
Field of Study	Veterinary Medicine
Thesis Advisor	Associate Professor Rosama Pusoonthornthum, D.V.M., M.Sc., Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Veterinary Science
(Professor Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Somsak Pakpinyo, D.V.M., Ph.D.)

.....Thesis Advisor
(Associate Professor Rosama Pusoonthornthum, D.V.M., M.Sc., Ph.D.)

.....Examiner
(Woraporn Sukhumavasi, D.V.M., Ph.D.)

.....Examiner
(Associate Professor Achara Tawatsin, B.Sc., M.S.)

.....External Examiner
(Associate Professor Ratanaporn Brahmasa, D.V.M., M.P.H.)

อิษฎี ปานบุญ : การเปรียบเทียบระดับของ เอ็น-อะซีติล-เบต้า-ดี-กลูโคสามินิเดส ในปัสสาวะแมวที่เป็นโรคไตวายเรื้อรัง กระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ และนิ่วในกระเพาะปัสสาวะ (COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS IN CATS WITH CHRONIC KIDNEY DISEASE, FELINE IDIOPATHIC CYSTITIS AND CYSTIC CALCULI) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. สพ.ญ. ดร.รสมา ภู่สุนทรธรรม, 68 หน้า.

ศึกษาระดับเอนไซม์ N-acetyl-beta-D-glucosaminidase (NAG) ในปัสสาวะแมวที่เป็นโรคไตวายเรื้อรังจำนวน 19 ตัว แมวกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุจำนวน 19 ตัว และแมวที่มีนิ่วในกระเพาะปัสสาวะจำนวน 9 ตัว โดยวัดปริมาณของ NAG ด้วยวิธี colorimetric method คำนวณผลเป็นค่า NAG index พบว่าแมวที่มีช่วงอายุ 3 ถึง 7 ปี น้ำหนักตัวมากกว่า 4 กิโลกรัม หรือบริโภคอาหารสำเร็จรูปชนิดเม็ดเพียงอย่างเดียว มีความเสี่ยงต่อการเกิดโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ และการเกิดนิ่วในกระเพาะปัสสาวะ แมวที่ป่วยเป็นโรคไตวายเรื้อรังมีค่าเฉลี่ยของ NAG index (8.32 ± 2.16 U/g) สูงกว่าในแมวปกติ (2.14 ± 0.48 U/g) (p<0.01) NAG index ของแมวกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ (4.79 ± 1.53 U/g) และแมวที่มีนิ่วในกระเพาะปัสสาวะ (3.53 ± 2.08 U/g) มีแนวโน้มสูงขึ้นกว่าแมวปกติ 2 เท่า และ 1.5 เท่าตามลำดับ แต่ไม่มีความแตกต่างทางสถิติ เมื่อหาความสัมพันธ์ของค่า log urine protein to creatinine ratio กับ log NAG index พบว่ามีความสัมพันธ์ระดับปานกลางในแมวกลุ่มกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ (r^2 = 0.512, p < 0.05) สามารถสรุปได้ว่า NAG index ไม่สามารถใช้เป็นตัวบ่งชี้ระยะแรกของโรคไตวายเรื้อรังในแมวระยะแรกได้ แต่อาจเกี่ยวข้องกับภาวะโปรตีนที่เพิ่มขึ้นในปัสสาวะก่อนเกิดภาวะ azotemia และอาจเกี่ยวข้องในแมวป่วยด้วยโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ ซึ่งต้องมีการศึกษาในแมวกลุ่มนี้ต่อไปในอนาคต

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์สัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558

5675335631 : MAJOR VETERINARY MEDICINE
KEYWORDS: CAT / CHRONIC KIDNEY DISEASE / CYSTIC CALCULI / IDIOPATHIC CYSTITIS / CYSTIC CALCULI / N-ACETYL-BETA-D-GLUCOSAMINIDASE

ISADEE PANBOON: COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS IN CATS WITH CHRONIC KIDNEY DISEASE, FELINE IDIOPATHIC CYSTITIS AND CYSTIC CALCULI. ADVISOR: ASSOC. PROF. ROSAMA PUSOONTHORNTHUM, D.V.M., M.Sc., Ph.D., 68 pp.

Comparative study was performed to measure urinary N-acetyl-beta-D-glucosaminidase (NAG) in cats with chronic kidney disease (CKD), idiopathic cystitis and cystic calculi. Information regarding age, gender, breed, type of food consumed and environment factors were asked through standard questionnaire. Urine samples were collected from 19 clinically normal cats, 19 cats with CKD, 19 cats with idiopathic cystitis and 9 cats with cystic calculi. The urinary NAG activity was quantified by colorimetric method. NAG index was calculated by NAG activity to urine creatinine ratio. The results showed that the risk factors of FIC and cystic calculi were age (3 to 7 years old), weighing more than four kilograms, and/or consuming only dry commercial food. CKD cats had significantly higher NAG index (8.32 ± 2.16 U/g) than the clinically normal cats (2.14 ± 0.48 U/g) ($p < 0.01$) while the average of NAG index in feline idiopathic cystitis (FIC) (4.79 ± 1.53 U/g) and cystic calculi group (3.53 ± 2.08 U/g) had an increased trend of 2 times and 1.5 times when compared with the clinically normal cat group, respectively ($p > 0.05$). In FIC group, log urine protein to creatinine ratio was positively correlated with log NAG index at moderate level ($r^2 = 0.512$, $p < 0.05$). Based on the results, NAG index could not be used to indicate early CKD. In FIC cats, the increased NAG index may be related to the increased proteinuria before azotemia occur. Further study is needed to address NAG role in the pathological abnormalities in cats with idiopathic cystitis.

Department: Veterinary Medicine Student's Signature .....
Field of Study: Veterinary Medicine Advisor's Signature .....
Academic Year: 2015

ACKNOWLEDGEMENTS

First of all, I would like to thank Associate Professor Dr. Rosama Pusoonthornthum, my advisor and Assistant Professor Sariya Asawakarn for invaluable advice, intensive review, and suggestion on several techniques.

My gratitude is to Associate Professor Achara Tawatsin for her guidance in statistical analysis.

I would like to thank lab demonstrators especially Mrs. Sujin Sirisawat, for advice and sharing all source information of laboratory technique and thanks to Biochemistry unit, Department of Physiology, Faculty of Veterinary Sciences, Chulalongkorn University for laboratory instruments.

Special thanks to Dr. Krissda Bunaramrueng, Dr. Jeerayu Koolprasoot and veterinary nurses of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University for their help to collect data.

Finally, I also would like to thank the 90th Anniversary of Chulalongkorn University, Rachadapisek Sompote Fund for supporting grant.

CONTENTS

Page

Page

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AKI	Acute kidney insufficiency
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
BUN	Blood urine nitrogen
CBC	Complete blood count
CFU	Colony forming units
CI	Confidence interval
CKD	Chronic kidney disease
dl	Deciliter
DSH	Domestic short hair
ε	Molar absorptivity
EDTA	Ethylenediaminetetraacetic acid
FIC	Feline idiopathic cystitis
Fig.	Figure
FLUTD	Feline lower urinary tract diseases
FUS	Feline urological syndrome
g	Gram
GAGs	Glycosaminoglycans
GFR	Glomerular filtration rate
GP51	Glycoprotein with molecular weight of 51 kilodaltons
HCT	Hematocrit
IC	Idiopathic cystitis
IQR	Interquartile range
IRIS	International Renal Interest Society
L	Liter
log	Logarithm
mg	Milligram

min	Minute
ml	Milliliter
μl	Microliter
NAG	N-acetyl-β-D-glucosaminidase
nm	Nanometer
OD	Optical density
OD_{SA}	Optical density of sample
OD_{ST}	Optical density of standard
OR	Odds ratio
pH	Power of hydrogen ion
PD	Polydipsia
PU	Polyuria
RBC	Red blood cell
RCF	Relative centrifugal force
sCr	Serum creatinine
SEM	Standard error of mean
TFF2	Trefoli factor 2
U	Unit
UCr	Urine creatinine
UPC	Urine protein to creatinine ratio
USG	Urine specific gravity
WBC	White blood cell

CHAPTER I
INTRODUCTION

Importance and Rationale

The urinary system is divided into upper urinary tract and lower urinary tract. The most common upper urinary tract disease in cats is chronic kidney disease (CKD). The prevalence of CKD in cats (1.6 – 20 %) is greater than dogs (0.5 – 7%) (Grauer, 2008). Interestingly, Siamese, Abyssinian, Persian and Maine Coon are breeds at risk for CKD (Reynolds and Lefebvre, 2013). Another important risk factor for cats with CKD is aging. The prevalence of CKD increased to 31% in cat with more than 15 years old (Lulich et al., 1992).

The lower urinary tract abnormalities in cats are known as feline lower urinary tract diseases (FLUTD) or formerly called feline urological syndrome (FUS). The common clinical signs of these abnormalities are irritative voiding such as dysuria, stranguria, hematuria, pollakiuria and/or periuria (inappropriate urination) (Buffington et al., 1996).

FLUTD can be classified into two causes; obstruction uropathy and non-obstructive uropathy. First, obstructive uropathy are urethral plug, obstructive-idiopathic cystitis and urolithiasis. Second, non-obstructive uropathy is known as non-obstructive feline idiopathic cystitis (FIC), urolith (non-obstruction) neurologic disorder, anatomical abnormality and bacterial urinary infection (Gunn-Moore, 2014). The two most common causes of FLUTD were FIC (55% - 69%) and urolithiasis (13% - 28%) (Hostutler et al., 2005). In Europe, 57% of cats with lower urinary tract signs were caused by FIC. Other causes were 22% uroliths, 10% urethral plug and 8% urinary tract infection (Gerber et al., 2005). In Thailand, 2.22 % of the general population cats had FLUTD (Pusoonthornthum et al., 2012).

There are several diagnostic methods to diagnose upper and lower urinary tract diseases in cats such as history taking, physical examination, blood analysis, urinalysis

and/or imaging. Most of the time, it is very late before cats are diagnosed with urinary tract diseases. Therefore, the methods to identify early pathological change are needed. At present, urinary enzymes are of interested in both human and animal patients as one possible indicator for the urinary tract diseases. The urinary enzymes may be one of a sensitive indicator for early detection, severity and progression of renal and urinary tract diseases (Cobrin et al., 2013).

N-acetyl-beta-D-glucosaminidase (NAG) is an enzyme which has been widely used to measure tubular function in human patient (Skalova, 2005; Ali et al., 2014). NAG was increased in patient with kidney disease and its complications (Skalova, 2005). NAG has been proposed to be an early indicator when compared with serum creatinine concentration in cat with glomerulonephritis (Bishop et al., 1991). Other studies have showed that NAG was elevated in cats with CKD (Sato et al., 2002; Jepson et al., 2010).

However, there are little studies about urinary NAG that might be related to lower urinary system. A normal urinary bladder has glycosaminoglycans (GAGs) lining on its urothelium. One hypothesis for the cause of FLUTD is the increase degradation of endogenous urine GAGs in cats (Pereira et al., 2004). Elevation of NAG might degrade GAGs lining on urinary bladder and bacteria or crystal in urine will contact easily with pain receptor on urothelium and cause FIC.

Objectives of the study

To compare urinary N-acetyl- beta -D- glucosaminidase concentrations in cats with chronic kidney disease, idiopathic cystitis and cystic calculi.

Hypothesis

Cats with chronic kidney disease, idiopathic cystitis and cystic calculi have different urinary N-acetyl-beta -D- glucosaminidase concentration from the normal client-owned cat.

Keywords (Thai): แมว, ไตวายเรื้อรัง, นิ่วในกระเพาะปัสสาวะ, กระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ, เอ็น-อะซีติล-เบต้า-ดี-กลูโคสามินิเดส

Keywords (English): cats, chronic kidney disease, cystic calculi, idiopathic cystitis, N-acetyl-beta-D- glucosaminidase

Advantages of Study

Urinary NAG can be used as an alternative non-invasive marker for cats with CKD, idiopathic cystitis and cystic calculi.

CHAPTER II
LITERATURE REVIEW

2.1 Chronic kidney disease (CKD)

Chronic kidney disease (CKD) is an irreversible and progressive loss function of kidney of more than three month (Polzin et al., 2005). CKD is a common cause of illness and death in aged cats (Syme et al., 2006). A common cause of cats with CKD is tubulointerstitial nephritis whereas, glomerular disease is a major cause in humans and dogs with CKD (DiBartola et al., 1987). Aging and breeds were reported as the risk factors of CKD.

2.2 Feline idiopathic cystitis (FIC)

2.2.1 Nosology

Since 1970, cats with hematuria, urolithiasis and/or urethal obstruction reported as “feline urologic syndrome” (FUS) (Osbaldiston and Taussig, 1970). In 1984, the term “feline lower urinary tract diseases (FLUTD)” was used to describe cats with the clinical sign of FUS. The cause of FLUTD may be urinary bladder or urethra problems which might be from infection, urolithiasis, metabolic disorder, neoplasia, and/or congenital or neurological of lower urinary tract disorder (Osborne et al., 1984). FLUTD is classified to two causes by obstructive condition. First, obstructive uropathy such as urolithiasis, urethral plug, and obstructive-idiopathic cystitis. This is rare in female cat but commonly found in male cat because of a small diameter of male’s urethra. Second, non-obstructive uropathy such as non-obstructive idiopathic cystitis or feline idiopathic cystitis (FIC), bacterial urinary infection, neurologic disorder and anatomical abnormality. The two most common causes of FLUTD were FIC (55% - 69%) and urolithiasis (13% - 28%) (Hostutler et al., 2005)

In 1999, there were several reports of cats with chronic irritative voiding. These cats had petechial hemorrhage on submucosa of urinary bladder, negative culture urine and underlying cause should be investigation. Therefore, the term “feline

idiopathic cystitis or feline interstitial cystitis" was introduced (Buffington et al., 1999). Pandora syndrome is another new term for FIC in 2011. These clinical signs are underlying causes or organs. It may associate with other organs or other systems to developing FIC (Buffington, 2011; Buffington et al., 2014).

2.2.2 Pathophysiology

The cause of FIC is unknown. Some postulated causes of FIC might be the lumen of urinary bladder abnormality (local external abnormality), layer of urinary bladder abnormality (intrinsic abnormality) and other organs (internal abnormality) (Buffington, 2011). The pathophysiology of FIC can be caused by that variety of central nervous system change, adrenal hypofunction during chronic stress, and permeability of bladder changing (Buffington and Chew, 2007).

Stress can induce sympathetic nervous system stimulation and release neurotransmitter to C-fiber that is sensory nerve fibers on urinary bladder. On the other hand, C-fiber can induce from urinary bladder irritation by urine constituent or bacteria. The C-fibers release neurotransmitter; substance P to impulse mast cell in vessels on submucosa of bladder. Histamine is excreted from mast cell, which will stimulates vasodilatation of blood vessels and leads to submucosa petechial hemorrhage. Besides, substance P can induce smooth muscle of urinary bladder contraction.

One research reported about feline calicivirus could induce cystitis in cats (Kruger et al., 1996). They reported that virus-like particle was found in urine samples of cats with urethral obstruction by transmission electron microscope. FCV strains were isolated from that urine sample of cats with idiopathic cystitis (Rice et al., 2002). However, persistence of viral or bacterial DNA in bladder of human with interstitial cystitis were found but it did not associate with the symptom of interstitial cystitis (Al-Hadithi et al., 2005). Therefore, viral infection is unlikely to be one common cause of FIC. Normal urinary bladder has glycosaminoglycans (GAGs) lining on its urothelium. Cats with lower urinary tract disease have increase urinary bladder permeability by

having decrease GAGs lining of the bladder urothelium. Decrease of GAGs might be one of the cause of feline idiopathic cysititis (FIC) (Buffington et al., 1996; Byrne et al., 1999; Panchaphanpong et al., 2011). However, the mechanism of decreased urine GAGs in FIC cats is unclear.

2.3 Glycosaminoglycan (GAG)

Glycosaminoglycans (GAGs) are mucopolysaccharide with long unbranched polysaccharides consisting of a repeating N-acetyl hexosamine and alduronic acid in extracellular matrix (Fig.1). When GAGs are linked to protein, proteoglycan is formed (Hurst, 1994). The proteoglycan that is synthesized in endoplasmic reticulum and golgi, is transferred to extracellular matrix, cell surface or intracellular organelles. It is depended on recognition and sorting of glycosaminoglycans or proteoglycans (Prydz and Dalen, 2000). Proteoglycan are classified into five groups; chondroitin sulfate, dermatan sulfate, heparin sulfate, keratin sulfate and heparin (Pereira et al., 2004) (Table 1). Chondroitin sulfate, dermatan sulfate, heparin sulfate are glycosaminoglycans that are found in the cat urine (Pereira et al., 2004).

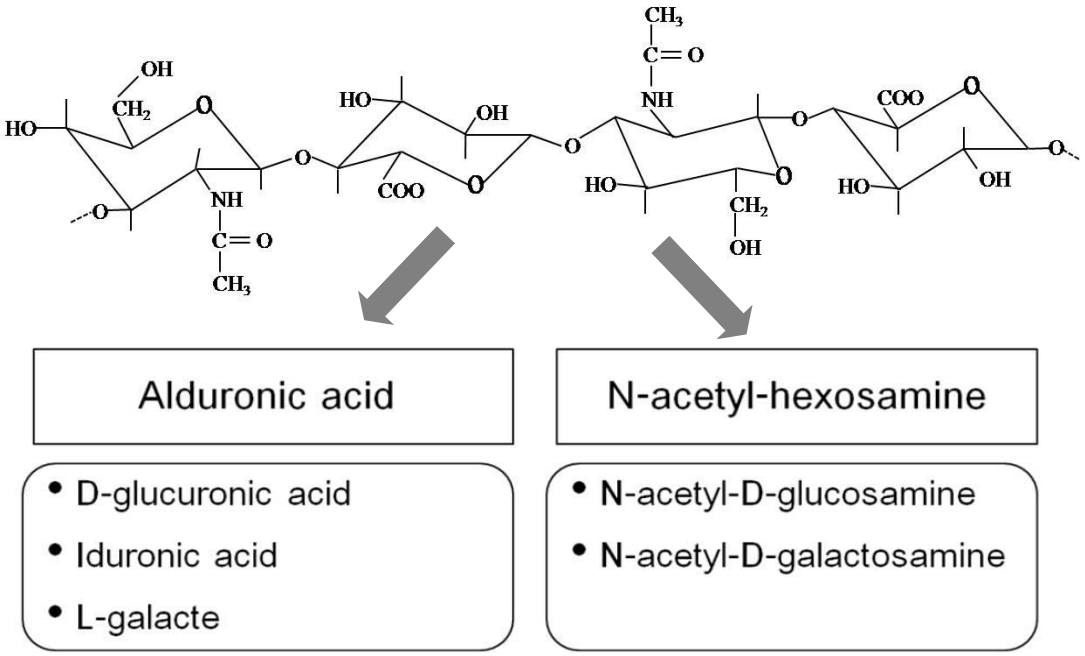

Fig. 1 Structure of glycosaminoglycans

(Adapted from Gandhi and Mancera, 2008)

Table 1 Type of glycosaminocans

Glycosaminoglycan	Compositions	Location
Chondroitin sulfate	GlcUA - GalNAc	cartilage, tendons, ligaments, wall of aorta
Dermatan sulfate	GlcUA/IdUA - GalNAc	skin, blood vessels, cornea, heart valves
Heparan sulfate	GlcUA/IdUA - GlcNAc	arterial wall, lung, liver, skin
Keratin sulfate	Gal - GlcNAc	cornea, cartilage, bone
Hyaluronic acid	GlcUA - GalNAc	synovial fluid, vitreous eye

Gal – Galactosamine, IdUA – Iduronic acid, GlcUA – Glucuronic acid, GalNAc – N-acetyl-D-galactosamine, GlcNAc - N-acetyl-D-glucosamine

Normal urinary bladder has glycosaminoglycans (GAGs) lining on its urothelium (Fig.2). Cats with lower urinary tract diseases has increased urinary bladder permeability by having decrease GAGs lining of the bladder urothelium (Buffington et al., 1996). These properties can protect bacteria, crystal or noxious urine constituent to directly contact with bladder urothelium (Hurst, 1994). The failure of urothelial permeability barrier may be one important factor causing feline interstitial cystitis (Buffington et al., 1996).

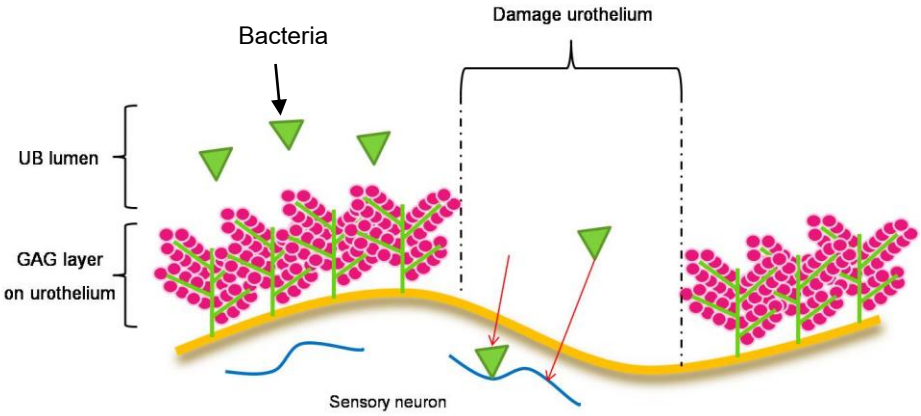

Fig. 2 Glycosaminoglycan lining in urinary bladder

(Adapted from Hurst, 2003)

Another study reported that bladder urothelium is lined by specific glycosaminoglycans called GP51 (Byrne et al., 1999). It is a urinary glycoprotein with molecular weight of 51 kilodaltons. This urinary glycoprotein is produced and secreted from the transitional epithelium of genitourinary tract. There was one study using GP51 as a clinical marker for diagnosis human with interstitial cystitis by a non-invasive urinary assay (Byrne et al., 1999). In cat with idiopathic cystitis, several studies have found a decrease in urinary glycosminoglycans levels (Buffington et al., 1996; Pereira et al., 2004; Panchaphanpong et al., 2011). The mechanism of decreased urine GAGs is unclear. One hypothesis is that the increased permeability of urinary bladder and GAG will be absorbed (Lavelle et al., 2000). In contrast, the degradation of endogenous urine GAGs (Pereira et al., 2004). GAGs was degraded by N-acetyl-beta-D-glucosaminidase (NAG) in circulation (Komosinska-Vassev et al., 2005). NAG is an enzyme that can degrade mucopolysaccharide and glycoproteins in the renal tubular epithelium (Ogawa et al., 1982). Elevation of NAG may be one major factor of feline idiopathic cystitis. The failure of urothelial permeability barrier may be important factor causing feline interstitial cystitis (Buffington et al., 1996).

2.4 N-acetyl- beta-D-glucosaminidase (NAG)

One major diagnosis in cats with CKD in clinic is the increase in plasma creatinine concentration. However, the increase in creatinine level is not detected until nephron is damaged up to 75% of its normal function (Ross and Finco, 1981). Therefore, various parameters were studied to be an early indicator for renal diseases in cats. Urinary enzymes are widely used in human medicine as an indicator for renal insufficiency. One of the urinary enzymes, N-acetyl- beta -D- glucosaminidase (NAG) is the biomarker for detection of renal tubular damage (Skalova, 2005). This enzyme is a lysosomal glycosidase produce from the proximal convoluted tubule of the kidney. It is found in various tissue such as liver, nervous tissue, and synovial fluid; nevertheless, NAG does not infiltrate glomerular basement membrane because of its large molecular weight of NAG is 130,000 - 140,000 daltons (Skalova, 2005). NAG is stable even though

there is circadian variation in humans, dogs and cats. Besides, it is stable in pH and temperature changing. (Uechi et al., 1994; Uechi et al., 1998; Skalova, 2005). No difference of NAG levels between sexes was reported (Sato et al., 2002).

In human, NAG is widely used as tubular function and kidney damage marker from secondary diseases such as diabetes mellitus (Kordonouri et al., 1998; Sheira et al., 2015) and hypertension (Semczuk-Sikora et al., 2003)

In cats, NAG is used as early detection for renal damage. Increased NAG is detected earlier than the changes in serum creatinine concentration (Bishop et al., 1991). There were elevated NAG levels in cats with CKD (Jepson et al., 2009; Sato et al., 2002) and hyperthyroid cats with azotemia (Lapointe et al., 2008).

The purposes of the present study were to compare urinary NAG levels in cats with CKD, idiopathic cystitis and cystic calculi on the first day of diagnosis and to find out whether the changes in NAG levels can be used an early indicator of CKD, FIC and cystic calculi in cats.

CHAPTER III
MATERIALS AND METHODS

3.1 Animals

Eighty-four cats from the Small Animal Hospital, Chulalongkorn University, Bangkok, Thailand between April 2014 and August 2015 were included. The owner of each cat was interviewed regarding the cat's age, gender, breed, previous food consumed and environmental factors using the standard questionnaire on the first day of diagnosis to determine risk and protective factors. Cats were divided into four groups; 19 clinically normal cats, 24 cats with CKD, 25 cats with idiopathic cystitis and 16 cats with cystic calculi.

The criteria of cats in this study were shown in Fig. 3. All clinically normal cats were cats with normal physical examination, hematology, blood chemistry and urinalysis. CKD cats group were cats with azotemia (blood urea nitrogen (BUN) > 35 mg/dl and/or serum creatinine > 1.6 mg/dl), urine protein to creatinine ratio (UPC) concentration above 0.4 according to reference range of International Renal Interest Society (IRIS) in 2009, and/or urine specific gravity (USG) range between 1.008 – 1.012 (Lulich et al., 1992). Cats with irritative voiding signs such as dysuria stranguria pollakiuria, hematuria or periuria were included. Cats with cystitic calculi group were cats diagnosed by survey abdominal radiography, double contrast cystography and/or ultrasound. Cats with bladder tumor or other congenital abnormality were excluded. Cats with clinical signs of FLUTD but without calculi, tumor, congenital abnormality and urinary tract infection were included as FIC group.

Cats with special diets or receive supplement diets within 30 days before the diagnosis were be excluded. The owners of each cat were asked to allow their cats to be studied and request to sign a consent statement. The study protocol has approved by the Ethic Committee for the Human and/or Animal Experimentation, Faculty of Veterinary Science, Chulalongkorn University No.1431057.

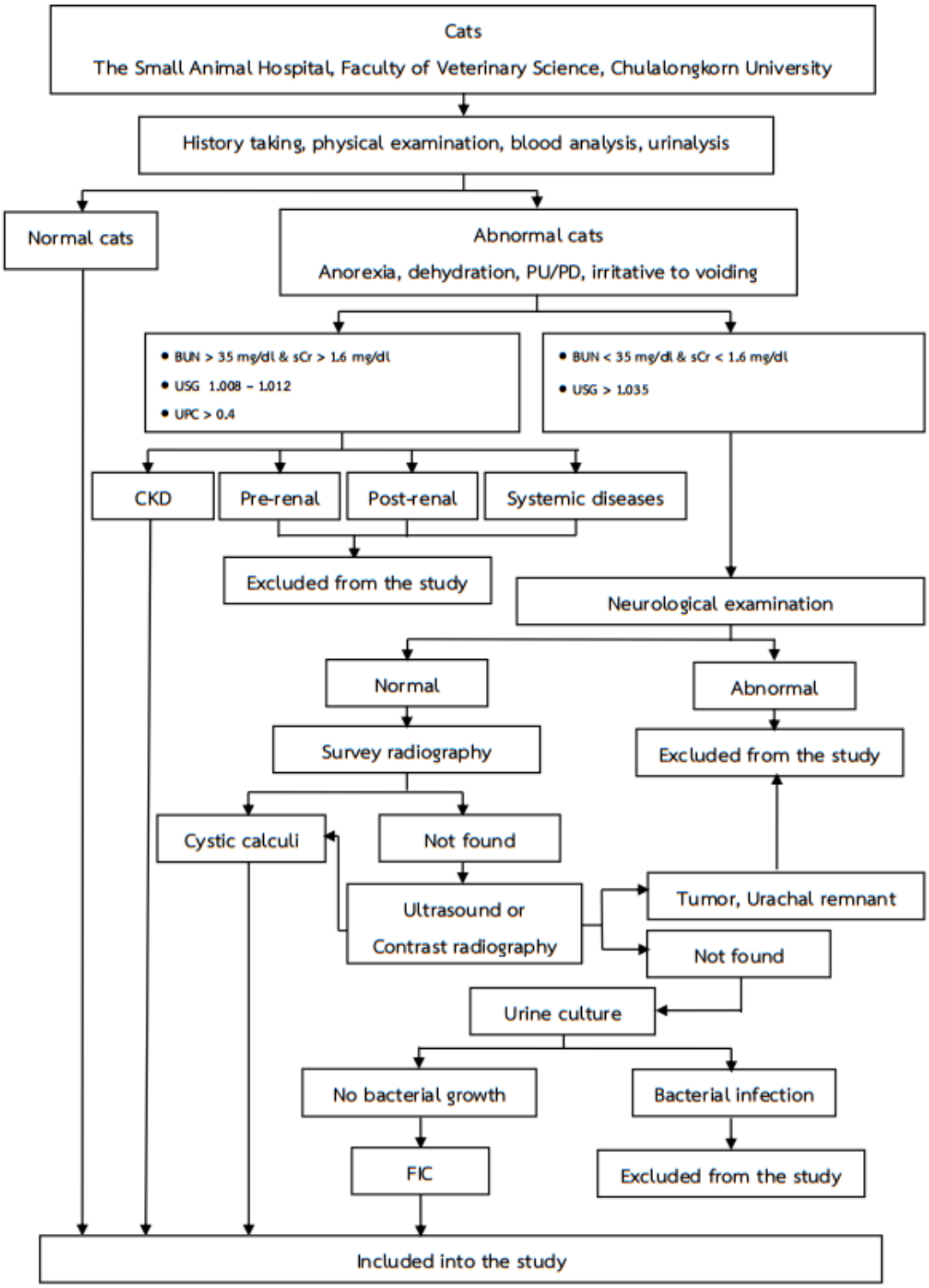

Fig. 3 Inclusion and exclusion criteria of the cats in the study

3.2 Study design

From eighty-four cats, only sixty-six cats' blood and urine samples were studied. Sixty-six cats divided into four groups consisting of 19 clinically normal cats, 19 cats with CKD, 19 cats with idiopathic cystitis and 9 cats with cystic calculi. Blood and urine samples were collected at the time of initial examination. Concentration of NAG, protein and creatinine concentration were measured from urine samples. NAG index was calculated by dividing NAG concentration into urine creatinine concentration ratio and UPC was calculated by dividing urinary protein concentration ratio (Fig. 4).

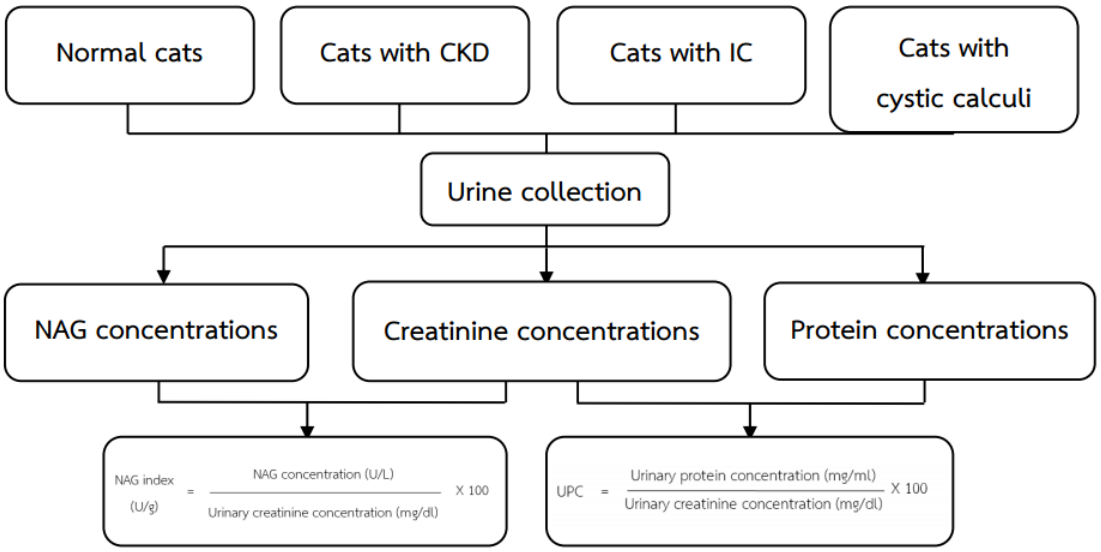

Fig. 4 Study design

3.3 Clinical examination

All cats in this study were diagnosed by complete history taking and physical examination. Two milliliters of blood sample was collected from cephalic or femoral vein in anticoagulant (ethylenediaminetetraacetic acid; EDTA) aliquot for complete blood count (CBC) and heparin aliquot for blood chemistry. The CBC were measured by automated blood count (Cell-Dyn® 3700) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine by automated clinical chemistry analyzer (ILab 650) at the Pathology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University.

Five milliliters of urine sample was collected by voiding (midstream), catheterization and/or cystocentesis. Commercial dipstick (Combur 9 test®) was used for analysis of pH, protein, glucose, ketone, bilirubin, leukocyte and erythrocyte, immediately. Urine specific gravity was measured by refractometer. Urine sediment (cast, red blood cell, white blood cell and crystals) was examined by microscope. Urine was collected in a transport media and determined urine culture colony count by Department of Pathology, Faculty Veterinary Science, Chulalongkorn University. The cut point for positive urine bacterial culture is $>10^5$ CFU/ml for urine collection by cystocentesis. Catheterization and midstream of voiding urine sample was considered positive when bacterial culture is more than $>10^4$ CFU/ml (Wamsley and Allenman, 2007).

Cats with lower urinary tract signs were analyzed by survey radiography. Moreover, contrast radiography or ultrasonography was used to confirm abnormal lower urinary tract diseases such as urolith, tumor and/or urachal remnant.

3.4 Laboratory examination

3.4.1. Urine collection

1. Urine samples were collected by voiding during midstream, catheterization or cystocentesis.
2. Urine supernatant was separated by centrifugation at RCF 1519 x g for 5 minutes.
3. Urine supernatant was stored at -80°C for further urine creatinine and NAG analysis (Fig. 5).

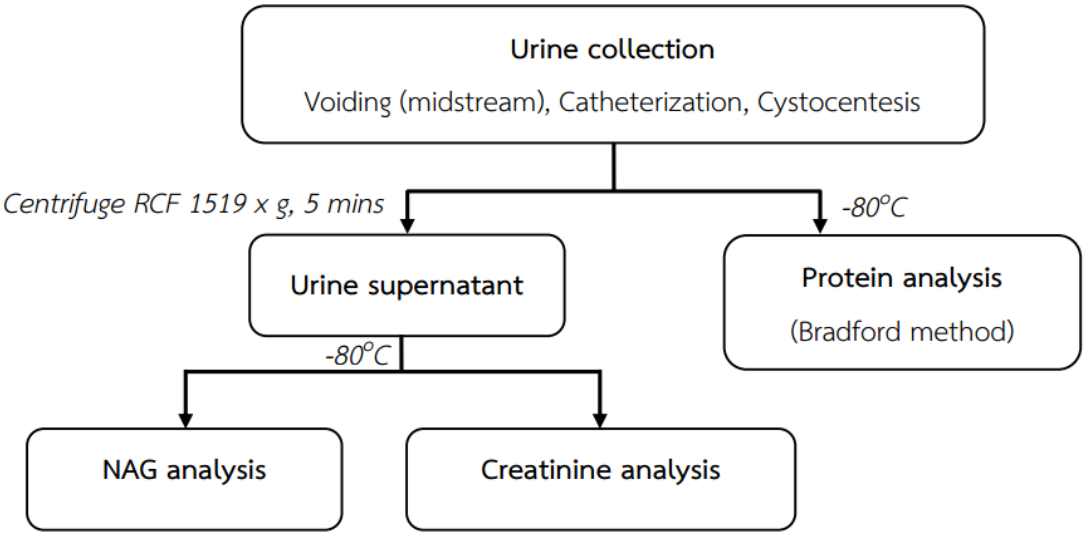

Fig. 5 Preparation of urine NAG, creatinine and protein analysis

3.4.2. Urinary creatinine quantitation

Urinary creatinine was determined by Alkaline Picrate-end Point Reaction method (colorimetric method) (Jaffé, 1886). Picrate-Creatinine complex is form by creatine and picrate ion reaction at alkaline condition.

1. Put 1 ml precipitating reagent (commercial reagent) in 125 µl dilute urine (urine : distilled water = 1 : 99).
2. Separated urine supernatant by centrifugation at RCF 547 x g for 10 minutes.
3. Mixed 750 µl urine supernatant and 250 µl picrate acid.
4. Added 125 µl alkaline solution.
5. Mixed and leave 15 minutes.
6. Measured an absorbance of changed color of urine sample at 520 nanometer (nm) (Fig. 6) and the result were calculated as creatinine concentration (mg/dl).

Standard curve for reference method was prepared with creatinine standard solution (calibration 2 mg%, 5 mg%, 8 mg%) set no. 10401-S from Life Science Dynamic

Division, Arnaparn CO., LTD. Finally, urinary creatinine concentration was calculated using reference value and presented in mg/dl. According to following equation:

$$\text{Concentration urine creatinine sample (mg/dl)} = \frac{OD_{SA} \times \text{Concentration standard}}{OD_{ST}} \times \text{Dilution Factor}$$

OD_{SA} = Optical density of sample

OD_{ST} = Optical density of standard

Dilution Factor = 100

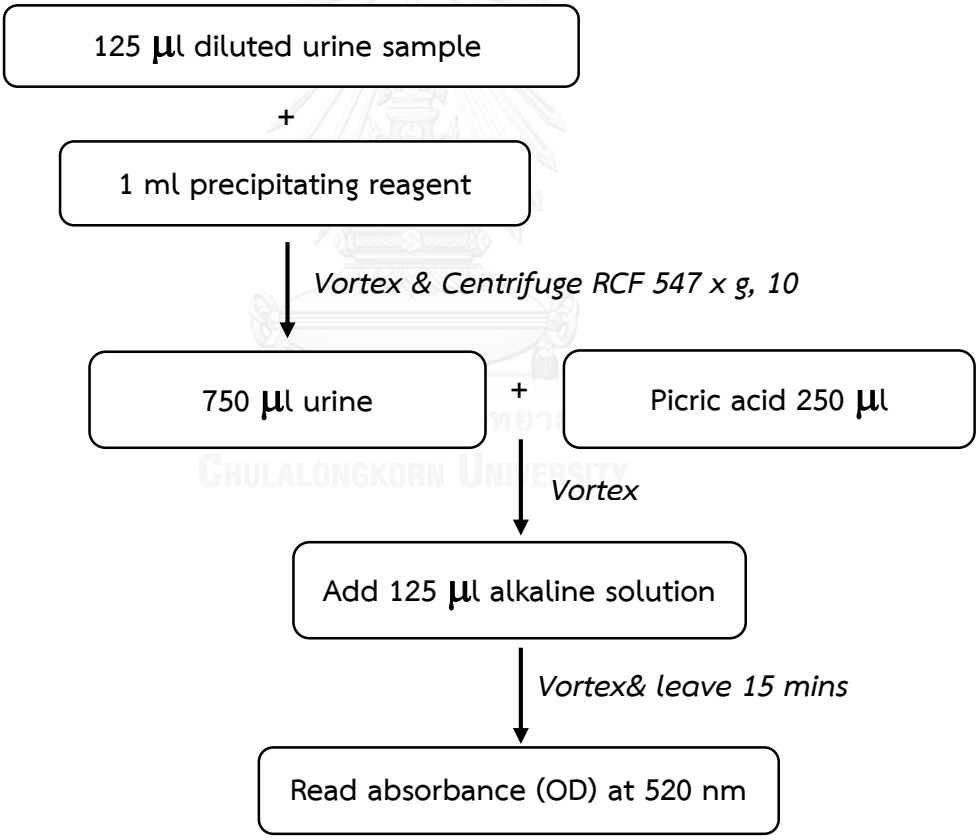

Fig. 6 Procedure of urine creatinine analysis

3.4.3. Urinary protein quantitation

Urinary protein was analyzed by Bradford method (colorimetric method) (Bradford, 1976). This assay is dyed-binding assay which a changing of the Coomassie Brilliant Blue G-250 (Bio-Rad Protein Assay) at acidic condition depended on concentration of protein in urine sample.

1. Percolated diluted Coomassie blue dye (dye reagent : distilled water = 1 : 4) by filter paper (Whatman #1).
2. Prepared five dilutions of a protein standard (bovine serum albumin standard; BSA). The linear range of protein solution was tested for standard.
3. Put 50 μl of each protein standard dilution and diluted urine sample (urine : distilled water = 1 : 3) in test tube.
4. Added 2.5 ml of diluted dye reagent to each tube and vortex.
5. Leave 5 minutes and measured an absorbance at 595 nm (Fig. 7).
6. Calculated urinary protein concentration by using slope of protein standard and presented in mg/ml. According to following equation:

$$\text{Concentration urine protein sample (mg/ml)} = \frac{OD_{SA}}{\text{Slope of protein standard}} \text{ X Dilution Factor}$$

OD_{SA} = Optical density of sample

Dilution Factor = 4

7. The finally, UPC was calculated with formula:

$$\text{UPC} = \frac{\text{Urinary protein concentration (mg/ml)}}{\text{Urinary creatinine concentration (mg/dl)}} \text{ X } 100$$

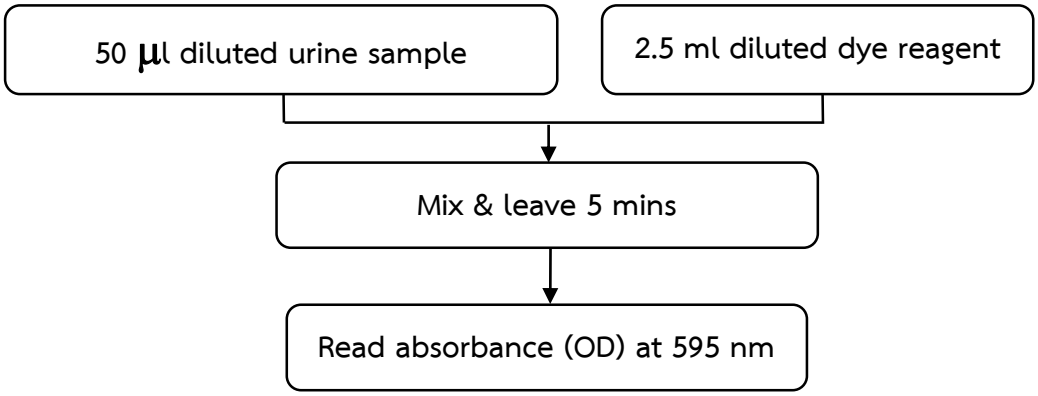

Fig. 7 Procedure of urine protein analysis

3.4.4. Urinary NAG quantitation

Urinary NAG concentration was quantified by colorimetric (Yakata et al., 1983). 3-Cresolsulfonphthaleinyl-N-acetyl- beta-D-glucosaminidase (substrate) is hydrolysed by NAG with release of 3-cresolsulfonphthalein (3-cresol purple) at alkaline condition.

1. Put 500 µl 3-Cresolsulfonphthaleinyl-N-acetyl- beta-D-glucosaminidase in test tube and incubated at 37°C for 5 minutes.
2. Added 25 µl urine NAG samples in the same test tube and incubated at 37°C for 15 minutes.
3. Stopped reaction by add 1 ml alkaline stopping buffer (sodium carbonate).
4. Mixed and leave 10 minutes.
5. Measured an absorbance at 580 nm (Fig. 8).
6. Urinary NAG concentration was calculated using reference value provided by the company.

Standard curve for reference method was prepared with lyophilised NAG enzyme standard obtained from beef kidney catalog number 10982962001 from Roche Diagnostics Corporation. The final urinary NAG concentration was calculated using

reference value and present in Unit/Litre (U/L) and molar absorptivity (ε) of 3-cresolsulfonphthalein at 580 ηm is 40.67 L x $mmol^{-1}$ x cm^{-1}. According to following equation:

$$\text{Urine NAG activity (U/L)} = \frac{1000 \times V}{40.67 \times 1 \times v \times t} \times A_{(sample)}$$

V = total volume measured (ml)

v = volume of urine sample (ml)

t = incubation time (minute)

A = absorbance measured at 580 nm

$\varepsilon_{580\ nm}$ of 3-cresolsulfonphthalein = 40.67 L x $mmol^{-1}$ x cm^{-1}

7. The finally, NAG index was calculated with formula:

$$\text{NAG index (U/g)} = \frac{\text{NAG concentration (U/L)}}{\text{Urinary creatinine concentration (mg/dl)}} \times 100$$

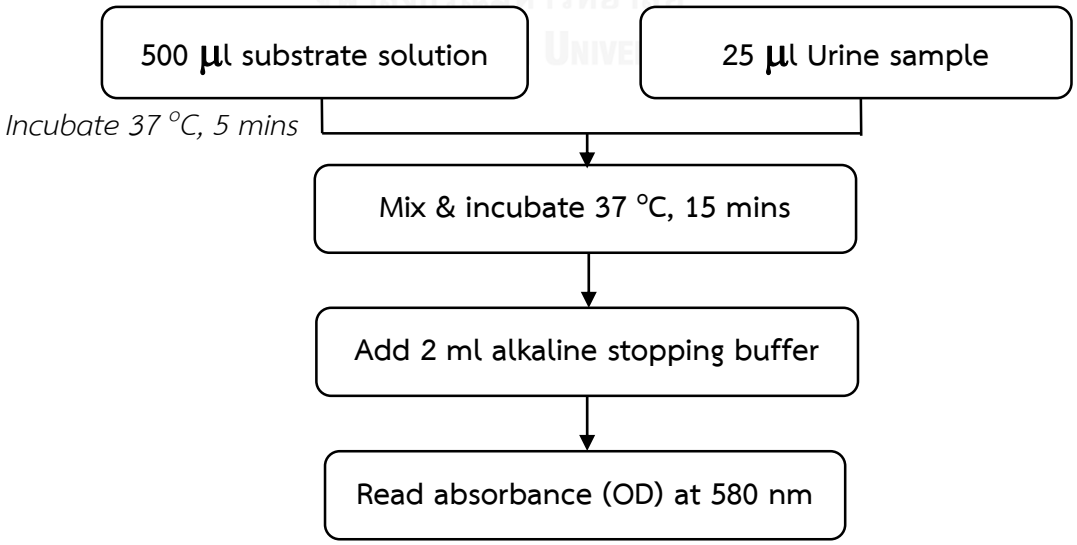

Fig. 8 Procedure of urine NAG analysis

(Substrate solution: 3-Cresolsulfonphthaleinyl-N-acetyl- beta-D-glucosaminidase)

3.5 Statistical analysis

All statistical data were analyzed by SPSS Statistics version 17.0 program. The significant level was considered at $p < 0.05$. Descriptive statistics were reported for signalments, and urinalysis. The association of predisposing factors was tested by Chi-square at $p < 0.05$. Fisher's exact test was used when expected value was small frequency. Odds ratio (OR) and 95% confidence intervals was used to measure the association of risk factors and developing FIC or cystic calculi.

Shapiro-Wilk test was used to evaluate the data distribution and Levene test was used to test homogeneity of variances. Quantitative data of NAG concentration, NAG index, Urine protein, and UPC were taken logarithm (log) for normalize distribution. Quantitative data reported as mean ± standard of error (SEM). Difference in between group NAG index was tested by one way ANOVA; furthermore, Tukey's test was analyzed NAG index when the differences between clinically normal cats group, CKD group, FIC group and cystic calculi were assessed to be significant. Correlation between log NAG index and serum creatinine or log UPC was performed by using the Pearson's correlation.

CHAPTER IV

REESULTS

4.1 Signalments

Data of eighty-four cats were included into the study. They were composed of 4 groups; 19 clinically normal cats, 24 cats with chronic kidney disease, 25 cats with idiopathic cystitis and 16 cats with cystic calculi. Mean age of clinically normal cats, FIC and cystic calculi group were 3.42 ± 0.81, 3.55 ± 0.56 and 4.44 ± 0.52 years old, respectively. Mean age of CKD cats was 8.21 ± 0.65 years old. CKD cats were older than other groups. Cats aging 3 to 7 years old had higher risk for CKD (OR = 16.10, 95% CI 3.40 – 76.21), FIC (OR = 13.53, 95% CI 2.78 – 65.88) and cystic calculi (OR = 36.14, 95% CI 6.76 – 193.28) than other age group (Table 2, 3, 4). The average weight was 3.58 ± 0.22 kg in clinically normal cats, 3.34 ± 0.20 kg in CKD cats, 4.38 ± 0.21 kg in cats with idiopathic cystitis and 4.49 ± 0.28 kg in cats with cystic calculi. Cats weighing more than four kilograms had significantly higher risk of developing FIC (OR = 18.90, 95% CI 3.85 – 92.68) and cystic calculi (OR = 14.15, 95% CI 2.80 – 71.50) than cat weighing 1 to 4 kilograms (Table 3, 4). Domestic short hair (86.9%; 73/84) was the most prevalence breed in this study. There were 9.5% of Persian (6/84) and 3.6% (3/84) of other breeds (American short hair, Scottish fold and Maincoon) (Fig. 9 - 10). Reproductive status of these cats were 46.4% (39/84) intact male, 35.7% (30/84) castrated male, 3.6% (3/84) intact female and 14.3% (12/84) sterile female (Fig. 11). Intact male were mostly found with idiopathic cystitis and cystic calculi (Fig.12). Moreover, Intact male was a protective factor of CKD cats (OR = 0.28, 95% CI 0.10 – 0.76) (Table 2). Sixty-eight point four percentage of the clinically normal cat, 79.2% cats with CKD 64% cats with idiopathic cystitis and 68.8% cats with cystic calculi lived indoor (Fig. 13). Cats in this study lived in the same household with more than one cat (Fig.14). There were various kinds of food consumed in each group; 84.2% clinically normal cats and 50.0% CKD cats consumed can and dry food. Fifty-six percent of cats with idiopathic cystitis and 63.0% cats with cystic calculi consumed dry food only (Fig. 15). Cats ate dry food had significantly higher risk for FIC (OR = 14.15, 95% CI 2.80 – 71.50) and cystic calculi (OR = 14.15, 95% CI 2.80 – 71.50) (Table 2, 3). Conversely, the result showed that consumed

dry food only (OR = 0.17, 95% CI 0.31 – 0.97) and/or consumed canned and dry food (OR = 0.25, 95% CI 0.08 – 0.78) had a lower risk to developed CKD (Table 2, 3, 4). All cats received water ad libitum. In FIC group, 68% of cats in the same house-hold had greater than or equal number of litter boxes. Fifty-seven point nine percent clinically normal cats and 58.3% CKD cats had a number of litter boxes greater than the number of cats in that household (Fig. 16).

Table 2 Odds ratio (OR), 95% confidence interval (CI) and chi-square of age, weight, breed, reproductive status, life style, number of cat in same household, type of food, number of litter boxes in cats with chronic kidney disease and clinically normal cats

Characteristic	No. of CKD cats n/N (%)	No. of clinically normal cats n/N (%)	OR	95% CI	Chi-square
Age					
< 3 yrs*	0/24 (0)	13/19 (52.0)	ND	ND	ND
3 - 7 yrs	14/24 (58.3)	2/19 (6.7)	16.10	3.40 – 76.21	0.001
> 7 yrs	10/24 (41.7)	5/19 (26.3)	1.071	0.40 – 2.87	0.891
Weight					
1 - 4 kg	19/24 (79.2)	17/19 (92.0)	0.33	0.06 – 1.64	0.200
> 4 kg	5/24 (20.8)	2/19 (8.0)	3.03	0.61 – 15.05	0.200
Breed					
DSH*	23/24 (95.8)	18/19 (94.7)	0.96	0.08 – 11.11	1.000
Persian*	1/24 (4.2)	1/19 (5.3)	1.04	0.09 – 12.10	1.000
Other breeds	0/24 (0)	0/19 (0)	ND	ND	ND
Reproductive Status					
Intact male	7/24 (29.0)	13/19 (68.4)	0.28	0.10 – 0.76	0.011
Castrated male	12/24 (50.0)	5/19 (26.3)	1.78	0.66 – 4.80	0.254
Intact female*	1/24 (4.0)	1/19 (5.3)	1.04	0.09 – 12.10	1.000
Sterile female*	4/24 (17.0)	0/19 (0)	ND	ND	ND
Life style					
Indoor	19/24 (79.2)	13/19 (68.4)	1.48	0.48 – 4.51	0.492
Outdoor*	2/24 (8.3)	2/19 (10.5)	0.67	0.14 – 3.24	0.685
Indoor & Outdoor*	3/24 (12.5)	4/19 (21.1)	0.75	0.19 – 2.95	0.727
Number of cats					
n = 1	11/24 (46.0)	6/19 (31.6)	1.80	0.65 – 4.96	0.254
n = 2*	2/24 (8.0)	6/19 (31.6)	0.36	0.08 – 1.50	0.259
n ≥ 3	11/24 (46.0)	7/19 (36.8)	1.50	0.56 – 4.07	0.420
Type of Food					
Can food	11/24 (45.8)	0/19 (0)	ND	ND	ND
Dry food*	1/24 (42.0)	3/19 (15.8)	0.17	0.31 – 0.97	0.042
Can & Dry food	12/24 (12.0)	16/19 (84.2)	0.25	0.08 – 0.78	0.011
Homemade*	0/24 (0)	0/19 (0)	ND	ND	ND
Number of litter boxes					
cats ≥ litter boxes	10/24 (41.7)	8/19 (42.1)	0.77	0.29 – 2.05	0.63
cats < litter boxes	14/24 (58.3)	11/19 (57.9)	1.29	0.49 – 3.42	0.63

*Fisher's exact test, n - number of cats in each group characteristic; N – total number of cats with chronic kidney disease or clinically normal cats; ND – not determined

Table 3 Odds ratio (OR), 95% confidence interval (CI) and chi-square of age, weight, breed, reproductive status, life style, number of cat in same household, type of food, number of litter boxes in cats with idiopathic cystitis and clinically normal cats

Characteristic	No. of FIC cats n/N (%)	No. of clinically normal cats n/N (%)	OR	95% CI	Chi-square
Age					
< 3 yrs	13/25 (52.0)	13/19 (68.4)	0.50	0.18 – 1.41	0.187
3 - 7 yrs	10/25 (40.0)	1/19 (5.3)	13.53	2.78 – 65.88	0.001
> 7 yrs	2/25 (8.0)	5/19 (26.3)	0.18	0.05 – 0.67	0.007
Weight					
1 - 4 kg	11/25 (44.0)	17/19 (89.5)	0.53	0.11 – 0.26	0.001
> 4 kg	14/25 (56.0)	2/19 (10.5)	18.90	3.85 – 92.68	0.001
Breed					
DSH*	19/25 (76.0)	18/19 (94.7)	0.11	0.013 – 0.95	0.038
Persian*	5/25 (20.0)	1/19 (5.3)	6.62	0.77 – 56.73	0.072
Other breeds*	1/25 (4.0)	0/19 (0)	ND	ND	ND
Reproductive Status					
Intact male	11/25 (44.0)	13/19 (68.4)	0.41	0.14 – 1.15	0.086
Castrated male	7/25 (28.0)	5/19 (26.3)	0.66	0.22 – 1.96	0.452
Intact female*	1/25 (4.0)	1/19 (5.3)	1.37	0.12 – 15.99	1.000
Sterile female	6/25 (24.0)	0/19 (0)	ND	ND	ND
Life style					
Indoor	16/25 (64.0)	13/19 (68.4)	0.64	0.21 – 1.91	0.422
Outdoor*	2/25 (8.0)	2/19 (10.5)	0.65	0.12 – 3.50	0.678
Indoor & Outdoor	7/25 (28.0)	4/19 (21.1)	2.22	0.62 – 8.00	0.216
Number of cats					
n = 1	6/25 (24.0)	6/19 (31.6)	0.59	0.19 – 1.85	0.360
n = 2	6/25 (24.0)	6/19 (31.6)	1.29	0.37 – 4.42	0.690
n ≥ 3	13/25 (52.0)	7/19 (36.8)	2.09	0.74 – 5.93	0.162
Type of Food					
Can food	0/25 (0)	0/19 (0)	ND	ND	ND
Dry food	14/25 (56.0)	3/19 (15.8)	4.22	1.31 – 13.65	0.013
Can & Dry food	8/25 (32.0)	16/19 (84.2)	0.15	0.05 – 0.50	0.001
Homemade*	3/25 (12.0)	0/19 (0)	ND	ND	ND
Number of litter boxes					
cats ≥ litter boxes	17/25 (68.0)	8/19 (42.1)	2.26	0.80 – 6.41	0.12
cats < litter boxes	8/25 (32.0)	11/19 (57.9)	0.44	0.16 – 1.26	0.12

*Fisher's exact test, n - number of cats in each group characteristic; N – total number of cats with idiopatic cystitis or clinically normal cats; ND – not determined

Table 4 Odds ratio (OR), 95% confidence interval (CI) and chi-square of age, weight, breed, reproductive status, life style, number of cat in same household, type of food, number of litter boxes in cats with cystic calculi and clinically normal cats

Characteristic	No. of cystic calculi cats n/N (%)	No. of clinically normal cats n/N (%)	OR	95% CI	Chi-square
Age					
< 3 yrs	3/16 (18.8)	13/19 (68.4)	0.11	0.03 – 0.45	0.001
3 - 7 yrs	11/16 (68.7)	1/19 (5.3)	36.14	6.76 – 193.28	0.001
> 7 yrs	2/16 (12.5)	5/19 (26.3)	0.24	0.06 – 0.90	0.028
Weight					
1 - 4 kg	8/16 (50.0)	17/19 (89.5)	0.07	0.01 – 0.35	0.001
> 4 kg	8/16 (50.0)	2/19 (10.5)	14.15	2.80 - 71.50	0.001
Breed					
DSH*	13/16 (81.0)	18/19 (94.7)	0.20	0.02 – 1.84	0.200
Persian*	1/16 (6.0)	1/19 (5.3)	1.78	0.15 – 20.86.	1.000
Other breeds	2/16 (13.0)	0/19 (0)	ND	ND	ND
Reproductive Status					
Intact male	8/16 (50.0)	13/19 (68.4)	0.71	0.24 – 2.11	0.542
Castrated male	6/16 (37.5)	5/19 (26.3)	1.09	0.36 – 3.29	0.884
Intact female	0/16 (0)	1/19 (5.3)	ND	ND	ND
Sterile female	2/16 (12.5)	0/19 (0)	ND	ND	ND
Life style					
Indoor	11/16 (68.8)	13/19 (68.4)	1.02	0.31 – 3.37	0.973
Outdoor	2/16 (12.5)	2/19 (10.5)	0.85	0.16 – 4.62	1.000
Indoor & Outdoor	3/16 (18.8)	4/19 (21.1)	1.09	0.26 – 4.61	1.000
Number of cats					
n = 1	7/16 (43.8)	6/19 (31.6)	1.73	0.57 – 5.26	0.335
n = 2	3/16 (18.8)	6/19 (31.6)	1.04	0.28 – 3.94	0.950
n ≥ 3	6/16 (37.5)	7/19 (36.8)	0.93	0.31 – 2.87	0.907
Type of Food					
Can food*	4/16 (25.0)	0/19 (0)	ND	ND	ND
Dry food	10/16 (62.5)	3/19 (15.8)	6.55	1.91 – 22.49	0.002
Can & Dry food	1/16 (6.3)	16/19 (84.2)	0.02	0.01 – 0.11	0.001
Homemade*	1/16 (6.3)	0/19 (0)	ND	ND	ND
Number of litter boxes					
cats ≥ litter boxes	8/16 (50.0)	8/19 (42.1)	0.88	0.30 – 2.57	0.816
cats < litter boxes	8/16 (50.0)	11/19 (57.9)	1.14	0.39 – 3.32	0.816

*Fisher's exact test, n - number of cats in each group characteristic; N – total number of cats with cystic calculi or clinically normal cats; ND – not determined

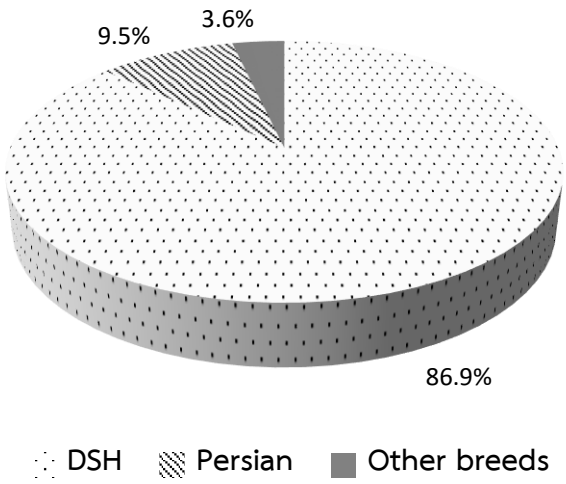

Fig. 9 Percentage of breed cats (total number of cats = 84)

DSH - domestic short hair, other breeds – American short hair, Scottish fold, or Maincoon

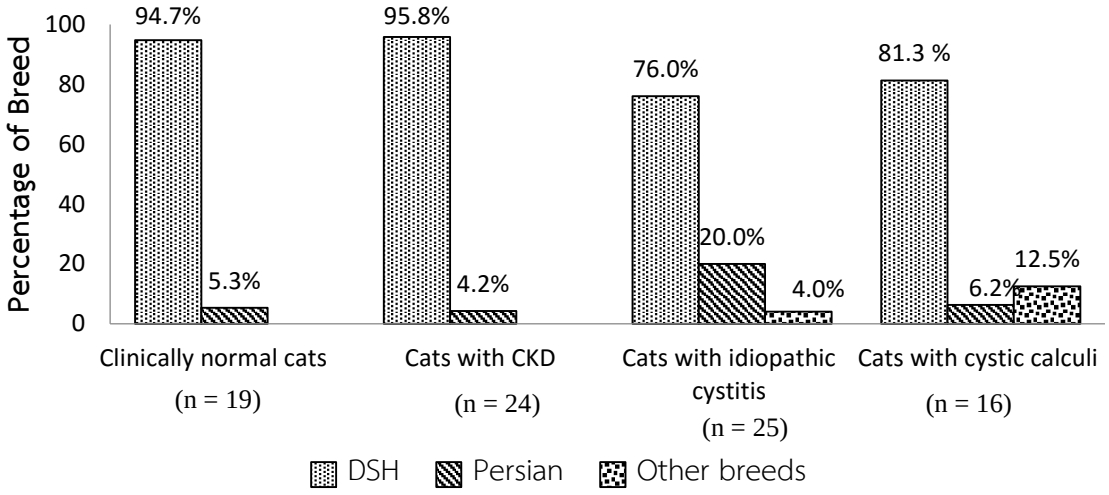

Fig. 10 Percentage of breed cats according to different groups

DSH - domestic short hair, other breeds – American short hair, Scottish fold, or Maincoon

n – number of cats in each group

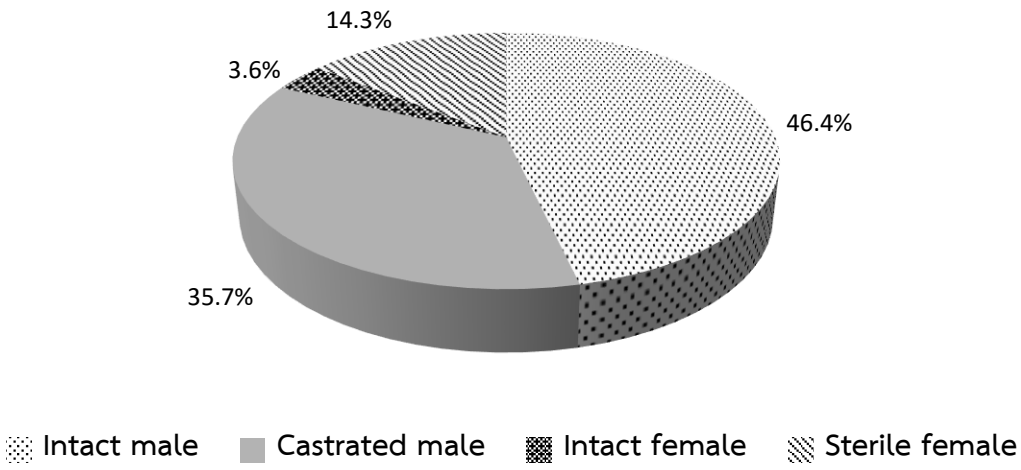

Fig. 11 Percentage of reproductive status (total number of cats = 84)

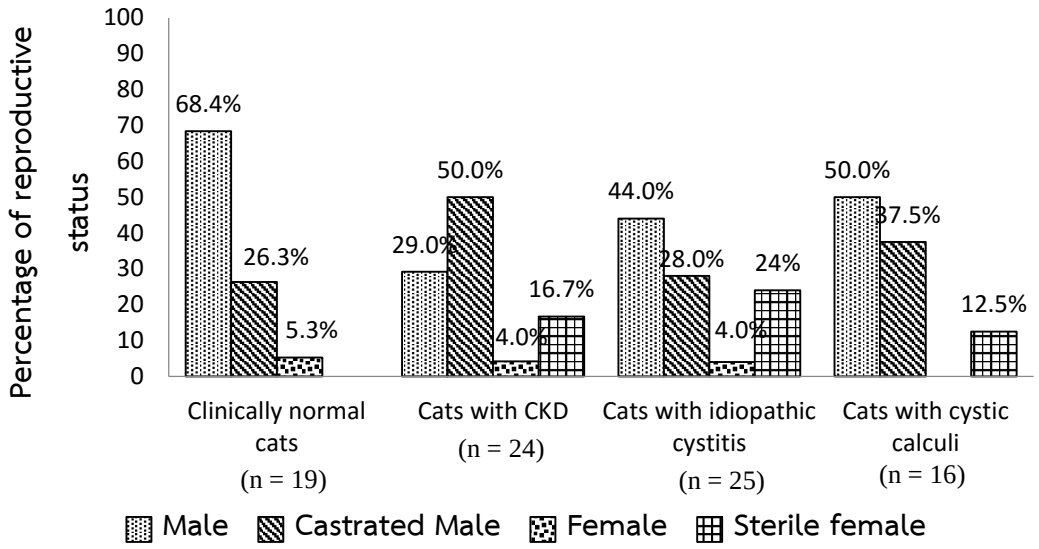

Fig. 12 Percentage of reproductive status of cats according to different groups
n – number of cats in each group

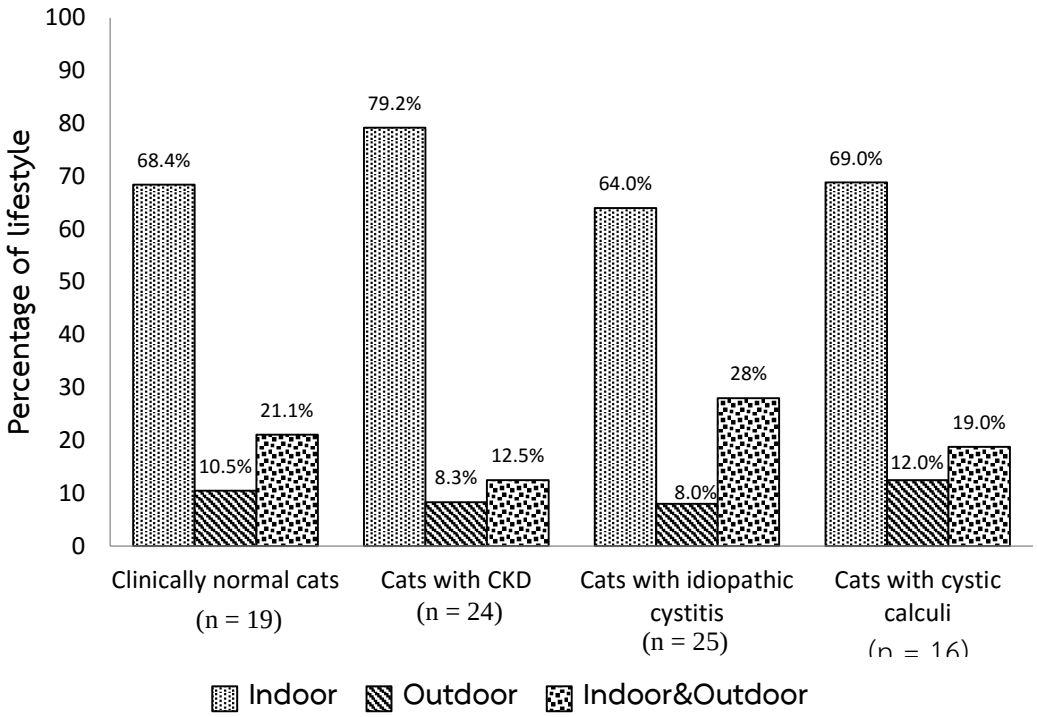

Fig. 13 Percentage of lifestyle of cats according to different groups

n – number of cats in each group

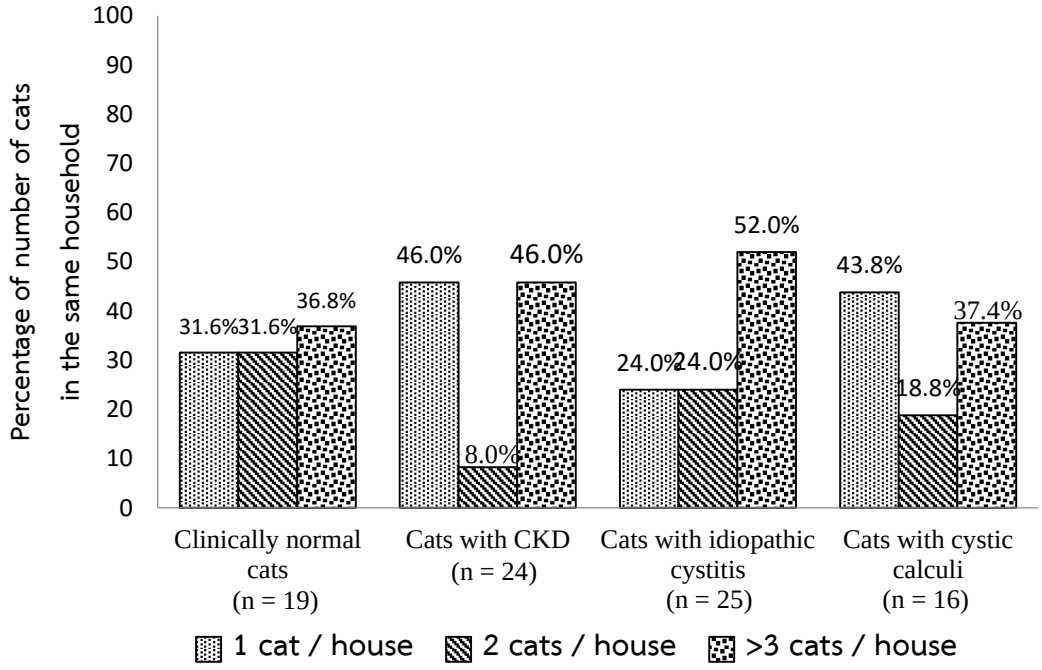

Fig. 14 Percentage of number cats in the same household according to different groups
n – number of cats in each group

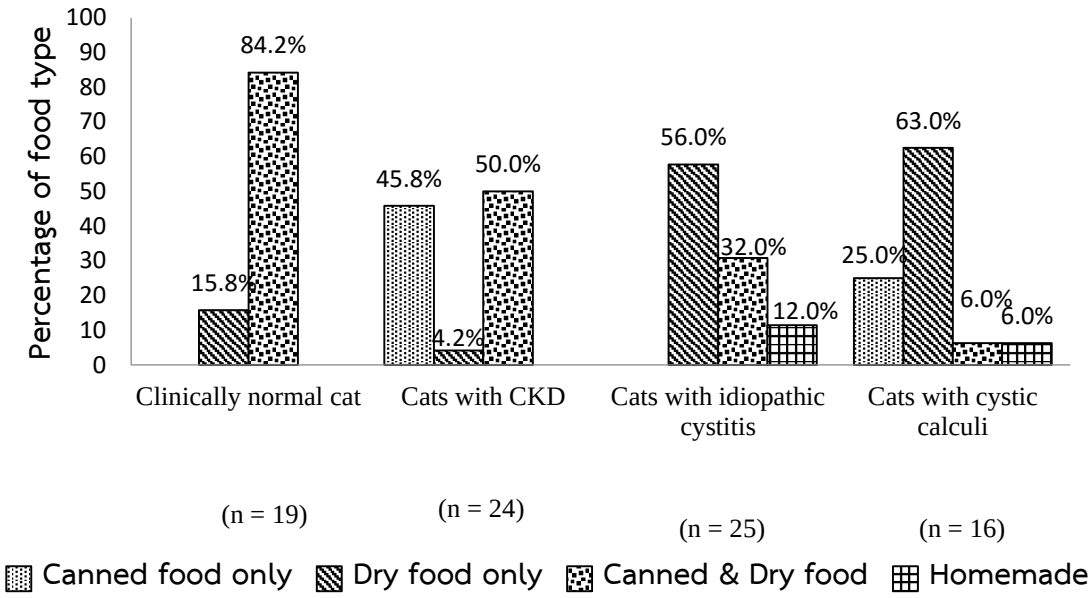

Fig. 15 Percentage of food type according to different groups

n – number of cats in each group

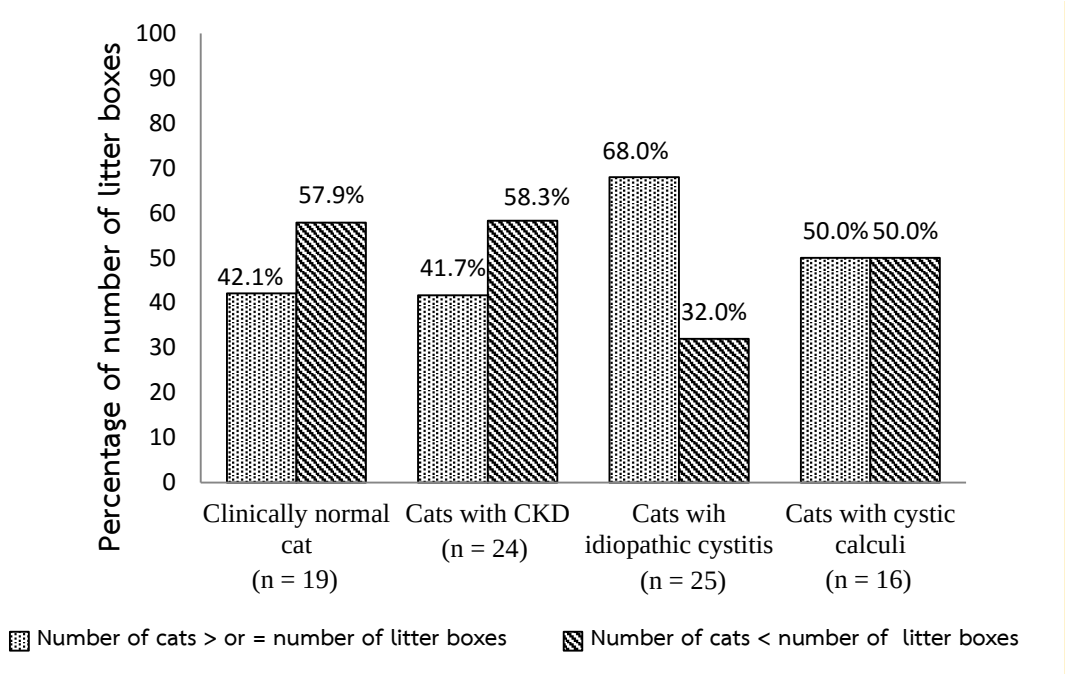

Fig. 16 Percentage of a number of litter boxes in the household according to different groups

n – number of cats in each group

4.2 Blood analysis

Six cats in the idiopathic cystitis group and seven cats in the cystic calculi group were excluded from this study because they had BUN more than 35 mg/kg and/or serum creatinine more than 1.6 mg/dl. Besides, five cats in CKD group were excluded because they had improper urination of the total eighty-four cats included, only sixty-six cats' blood and urine samples were analyzed.

The results of hematocrit, WBC count, serum BUN and serum creatinine values of the 66 cats were reported as mean ± standard error of mean (SEM) (Table 4). All 66 cats had blood profile within the normal range except CKD cats. CKD cats had statistically significant lower HCT (28.05 ± 1.97 %) and RBC count (5.14 ± 0.38 x 10^6/μl) than other groups ($p < 0.01$). BUN (53.93 ± 42.18) and sCr (3.35 ± 1.45) in this group were statistically significant higher than other groups ($p < 0.01$).

Table 5 Mean ± SEM of blood profile in the clinically normal cats, cats with CKD, idiopathic cystitis and cystic calculi.

Parameter	Normal value*	Normal cat		Cats with CKD		Cats with idiopathic cystitis		Cats with cystic calculi	
		Value	n	Value	n	Value	n	Value	n
HCT (%)	29.20-51.70	39.21 ± 1.70	19	28.05 ±1.97[aa]	19	45.05 ± 0.90[bb]	19	44.67 ± 2.36[bb]	9
RBC count (x10^6cell/μl)	5.24 – 10.89	8.06 ± 0.54	19	5.14 ± 0.38[aa]	19	9.19 ± 1.44[bb]	19	8.41 ± 0.53[bb]	9
WBC count (x10^3cell/μl)	4.20 – 17.50	10.89 ± 1.01	19	13.41 ± 1.37	19	10.82 ± 0.95	19	10.62 ± 1.31	9
BUN (mg/dl)	15.00 – 35.00	22.87 ± 1.88	16	70.31 ± 11.14[aa]	19	24.95 ± 1.43[bb]	19	23.24 ± 1.07[bb]	9
sCr (mg/dl)	< 1.6	1.48 ± 0.04	19	4.40 ± 0.71[aa]	19	1.41 ± 0.08[bb]	19	1.45 ± 0.11[bb]	9

* Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p. HCT – hematocrit; RBC - red blood cell; WBC - white blood cell; BUN - blood urea nitrogen; sCr - serum creatinine; n – number of cats in each group

[aa] $p < 0.01$ when compared with clinically normal cats.

[bb] $p < 0.01$ when compared between CKD cats.

4.3 Urinalysis

All cats in the clinically normal group, 84.0% of FIC group and 68.8% of cystic calculi group had urine specific gravity of more than 1.035. Four cats in FIC group and five cats in cystic calculi group had urine specific gravity between 1.013-1.034 because these cats had post renal azotemia. On the other hand, CKD cats had urine specific gravity between 1.008-1.016. Most cats had urine pH between 6 - 7 (77.4%; 65/84) (Fig.17). Three plus level of dipstick protein reaction in urine samples was found in cats with idiopathic cystitis (28%; 7/25) and cystic calculi (37.5%; 6/16) (Fig.18). However, most cats (72.6%; 61/84) had normal level (negative – 1+) of dipstick protein reaction in urine samples. Results WBC, RBC and crystal in urine samples was counted or analyzed by microscopic examination. All clinically normal cats and 75% of CKD cats had no WBC in urine sample, 40% of cats with idiopathic cystitis and 37.6% cystic calculi cats had more than 3 WBC per high power field in urine sample. Nevertheless, cats in idiopathic cystitis group (28%; 7/25) and cystic calculi group (31.3%; 5/16) had numerous WBC (more than 5 cell/high power field) in the urine samples (Fig. 19). Cats in idiopathic cystitis and cystic calculi group had 48% (12/25) and 56.3% (9/16) of numerous RBC (more than 5 cell/high power field), respectively (Fig. 20). Struvite was the most commonly found crystal in cats with idiopathic cystitis (16%; 4/25) and cats with cystic calculi (43.8%; 7/16).

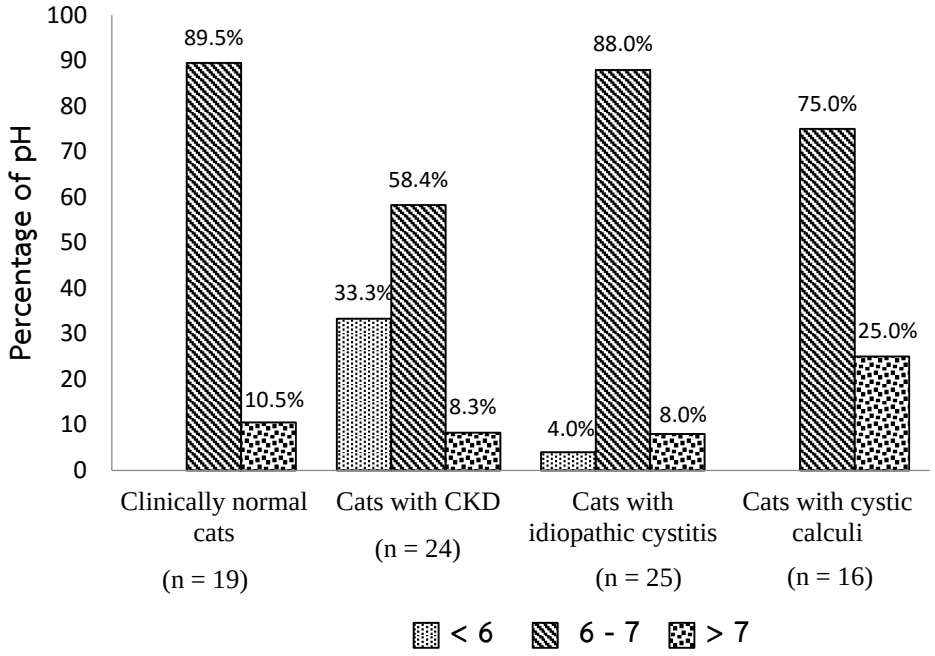

Fig. 17 Percentage of pH in urine samples according to different groups

n – number of cats in each group

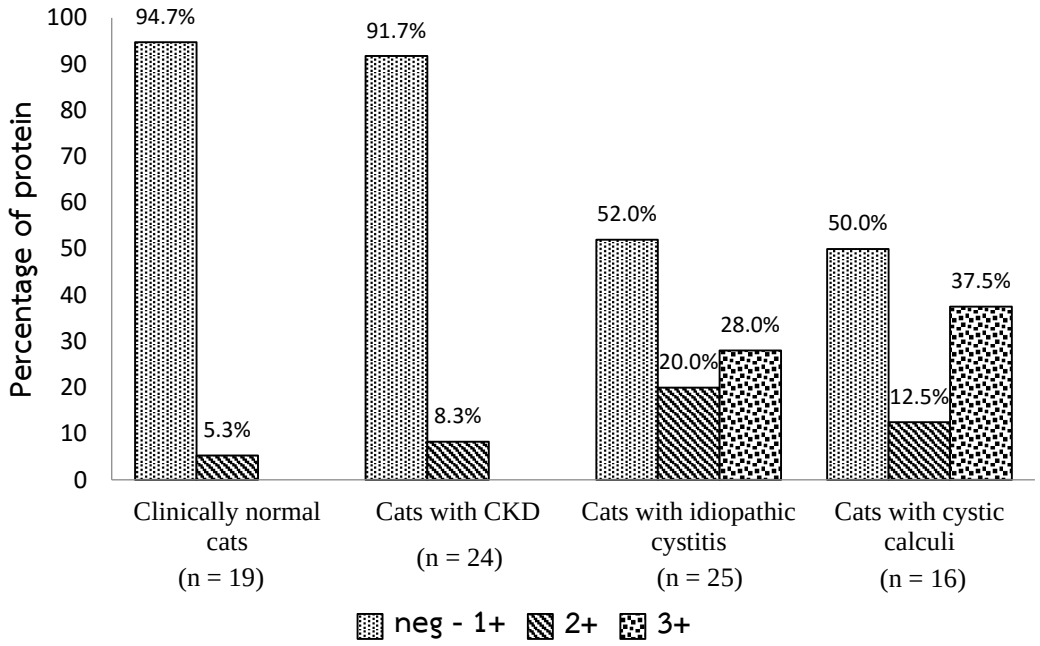

Fig. 18 Percentage of protein in urine samples using commercial strip test according to different groups

n – number of cats in each group

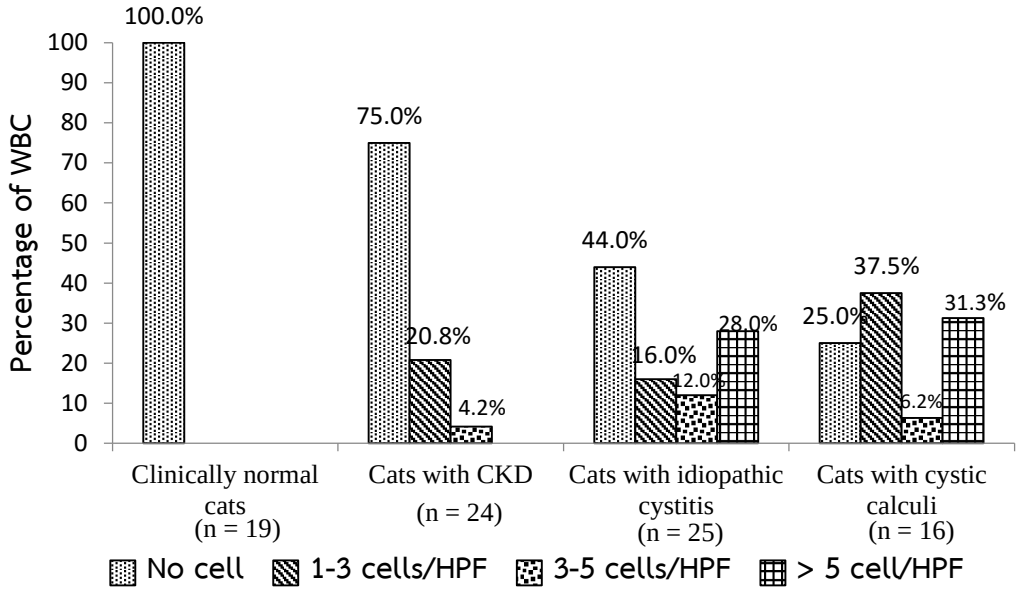

Fig. 19 Percentage of WBC in urine samples using microscopic examination according to different groups

n – number of cats in each group

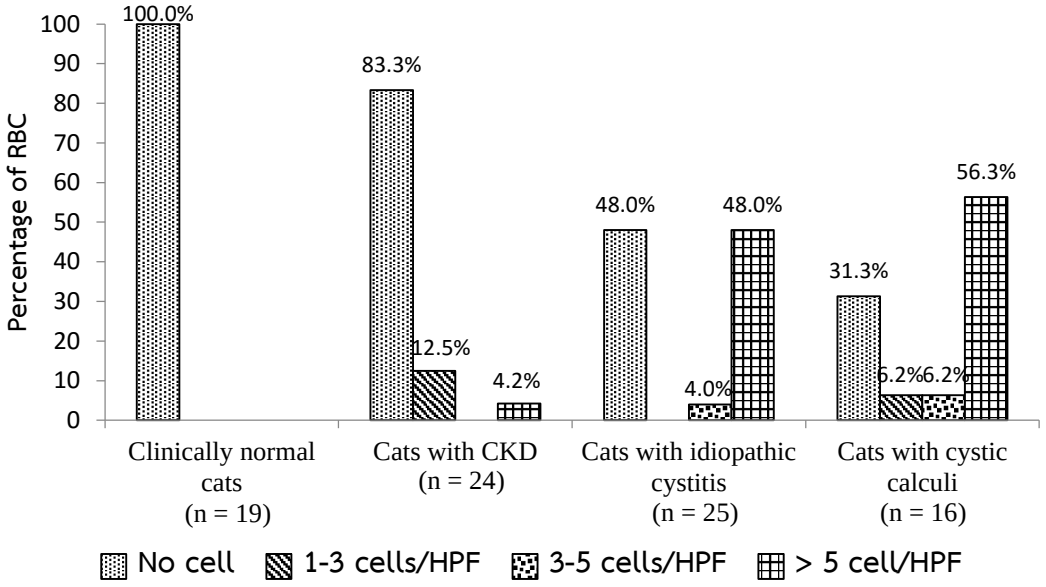

Fig. 20 Percentage of RBC in urine samples using microscopic examination according to different groups

n – number of cats in each group

4.4 Urine protein to creatinine ratio (UPC) and NAG index analysis

The urine creatinine (71.42 ± 7.71 mg/dl) of CKD cats was lower than the clinically normal cats, cats with idiopathic cystitis and cats with cystic calculi ($p < 0.01$). Mean ± SEM of NAG index of the clinically normal cats, CKD cats, cats with idiopathic cystitis and cystic calculi were 2.14 ± 0.48 U/g, 8.32 ± 2.16 U/g, 4.79 ± 1.53 U/g and 3.53 ± 2.08 U/g, respectively (Table 4). Mean ± SEM of NAG index of CKD cats was higher than the clinically normal cats ($p < 0.01$). CKD cats had lower mean ± SEM of urine protein than the clinically normal cats ($p < 0.01$). While, the FIC group or cystic calculi group had lower mean ± SEM of urine protein than the clinically normal cats ($p < 0.05$). Mean ± SEM of UPC in CKD cats (0.93 ± 0.23) was higher than the clinically normal cats ($p < 0.01$). Mean ± SEM of UPC in cats with idiopathic cystitis (0.70 ± 0.19) or cystic calculi (1.20 ± 0.69) was higher than clinically normal cats (0.14 ± 0.02) at $p < 0.05$.

Table 6 Mean ± SEM of UCr, NAG, NAG index, Uprotein, and UPC in clinically normal cats, CKD cats, cats with idiopathic cystitis (FIC) and cats with cystic calculi.

Parameter	Clinically normal cats		Cats with CKD		Cats with idiopathic cystitis		Cats with cystic calculi	
	Value	n	Value	n	Value	n	Value	n
UCr (mg/dl)	396.21 ± 43.19	19	71.42 ± 7.71^{aa}	19	362.32 ± 28.28^{bb}	19	294.33 ± 49.29^{bb}	9
NAG (U/l)	6.87 ±1.45	19	5.27 ± 1.52	19	14.79 ± 4.93	19	4.41 ± 0.97	9
NAG index (U/g)	2.14 ± 0.48	19	8.32 ± 2.16^{aa}	19	4.79 ± 1.53	19	3.53 ± 2.08	9
Uprotein (mg/dl)	56.13 ± 9.68	19	50.18 ± 10.39^{aa}	19	218.29 ± 58.95^{a}	19	162.11 ± 40.14^{a}	9
UPC	0.14 ± 0.02	19	0.93 ± 0.23^{aa}	19	0.70 ± 0.19^{a}	19	1.20 ± 0.69^{a}	9

UCr - urine creatinine; NAG - N-acetyl-β-D-glucosaminutesidase; Uprotein - urine protein; UPC - urine protein to creatinine ratio n – number of cats in each group

^{a} $p < 0.05$, ^{aa} $p < 0.01$ when compared with clinically normal cats.

^{bb} $p < 0.01$ when compared with CKD cats.

4.5 Relationship of log NAG index and serum creatinine or log UPC

All groups in this study did not have significantly relationship between log NAG index and serum creatinine or log UPC (Table 7 - 8), except log NAG index and log UPC in FIC group demonstrated significant moderate positive correlation (r^2 =0.512, $p < 0.05$) (Fig. 21).

Table 7 Relationship of log NAG index and serum creatinine

Group	Pearson's Correlation	Significant (2-tailed)
Normal cat	-0.003	0.990
CKD cat	-0.445	0.056
FIC cat	-0.284	0.239
Cystic calculi cat	0.434	0.244

Table 8 Relationship of log NAG index and log UPC

Group	Pearson's Correlation	Significant (2-tailed)
Normal	0.160	0.513
CKD	0.329	0.169
FIC	0.512	0.025*
Cystic calculi	0.302	0.429

*Correlation is significant at the 0.05 level (2-tailed)

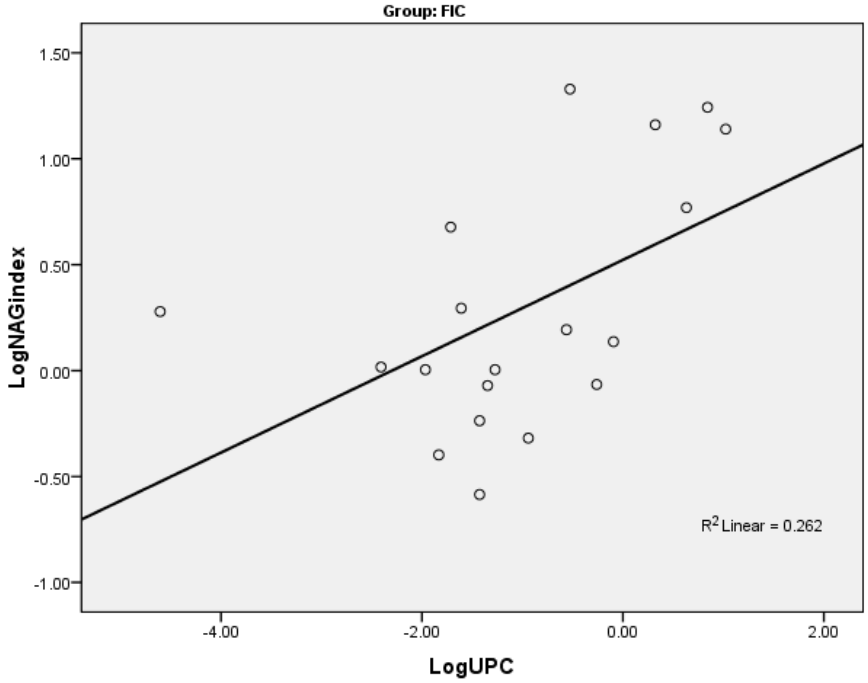

Fig. 21 Scatter plot of the Pearson's correlation between log urinary NAG index and log UPC in FIC cats (Pearson correlation, r^2 =0.512, $p < 0.05$)

CHAPTER V

DISSCUSSION

5.1 Signalments

Cats with aging 3 to 7 had higher risk to developing CKD which was lower age group than previous studies that observed age of CKD cats were more seven years (DiBartola et al., 1987; White et al., 2006). Cats aging 3 to 7 years old had higher risk of FIC (OR = 13.53, 95% CI 2.78 – 65.88) and cystic calculi (OR = 36.14, 95% CI 6.76 – 193.28) than other age. Same trend were found in the previous studies in cats with lower urinary tract diseases (Lekcharoensuk et al., 2000; Gunn-Moore and Shenoy, 2004), and idiopathic cystitis (2 to 7 years old; Panchaphanpong et al., 2011).

Cats of more than four kilograms body weight had higher risk for FIC (OR = 18.90, 95% CI 3.85 – 92.68) and cystic calculi (OR = 14.15, 95% CI 2.80 – 71.50). These results were consistent with previous studies (Defauw et al., 2011; Panchaphanpong et al., 2011; Pusoonthornthum et al., 2012; Lund et al., 2015). Overweight cats were at risk for developing FIC and cystic calculi (Lekcharoensuk et al., 2000; Gunn-Moore and Shenoy, 2004; Hostutler et al., 2005). Domestic short hair (DSH) was found as the most popular breed in all groups. DSH had lower risk of FIC. One research reported Siamese cats had decreased risk for FIC. However, several studies reported that breed was not a risk factor of FLUTD (Buffington et al., 2006b; Pusoonthornthum et al., 2012) or FIC (Cameron et al., 2004; Defauw et al., 2011; Panchaphanpong et al., 2011).

The observation of reproductive status in this study showed that intact male was associated with decreased risk of CKD. This finding was differ from the previous study that showed male cats were at risk of CKD (White et al., 2006). However, there was little research about relationship of reproductive status and CKD. The gender of cats with idiopathic cystitis or cystic calculi was not a risk factor in this study. Intact male cats were common in FIC group or cystic calculi group as shown in one previous study in Thailand (Pusoonthornthum et al., 2012). Male cats were at higher risk for idiopathic cystitis because their urethra are narrower than the female cats (Cameron

et al., 2004). Studies demonstrated that reproductive status was not predisposing factor (Willeberg, 1984; Buffington et al., 1997; Buffington et al., 2006b); in contrast, castrated male cats were at higher risk than other reproductive status in FLUTD and FIC (Lekcharoensuk et al., 2000; Panchaphanpong et al., 2011).

Cats with indoor lifestyle had the same risk as the cats living outdoor lifestyle in this study. Indoor lifestyle was a risk factor of FLUTD due to low activity and stress in cats (Buffington et al., 2006a). Environment and enrichment to stimulate cat's activity can decrease stress. Thus, the cats that lived indoor with enrichment environment had lower risk to develop FIC or cystic calculi (Hostutler et al., 2005).

More than one cat in the same household was reported to predispose cats to more stress and more conflict with other cats which can develop idiopathic cystitis or cystic calculi (Hostutler et al., 2005; Pusoonthornthum et al., 2012). Other research suggested that number of cat in the same household did not play a direct role but it was indirect effect whether the cat gets along well with their housemate (Cameron et al., 2004; Defauw et al., 2011). The results of this study also showed that number litter box and number cat in the same house had an equal chance of predispose cats to FIC and cystic calculi. Quality of litter box provided by the owners , such as cleaning of litter box, no odor like ammonia, position of litter box in quiet place, have been postulated to be important factors (Hostutler et al., 2005).

The results in this study also indicated that cats consumed only dry commercial cat food or cats consumed canned and dry commercial cat food combination had lower risk to developed CKD. Cats ate only dry commercial cat food was more associate with developing idiopathic cystitis and cystic calculi than cats ate other types of food. This finding resembled many previous studies on FIC (Buffington et al., 1997; Gunn-Moore and Shenoy, 2004; Gerber et al., 2005) and cystic calculi (Bartges and Kirk, 2006; Dru Forrester and Roudebush, 2007). Dry commercial cat food had moisture content less than canned commercial cat food or homemade diet. Consequently, increased urine concentration in urinary bladder of cats may lead to

supersaturation and predispose cats to cystitis or urolith (Dru Forrester and Roudebush, 2007).

5.2 Blood analysis

RBC count and HCT of cats in CKD group were slightly lower than the normal references (Table 3) and were significantly difference from other groups. Generally, kidney has peritubular fibroblast type-1 interstitial cells that produce erythropoietin hormone. Erythropoietin stimulates production of red blood cell at bone marrow (Reynolds and Lefebvre, 2013). Thus, cats with CKD had low RBC count and HCT. In addition, average BUN concentration and serum creatinine concentration of CKD groups were significantly higher than other groups. Cats with chronic kidney disease had tubulointerstitial fibrosis of nephrons that result in decrease glomerular filtration rate (GFR). Therefore, there was an increased levels of BUN concentration and serum creatinine concentration in cats with CKD (Salem, 2002).

5.3 Urinalysis

Most cats in the FIC and cystic calculi group had urine specific gravity of more than 1.035 which mean that they can concentrate urine. Four cats with idiopathic cystitis and five cats with cystic calculi had urine specific gravity between 1.013 - 1.034 due to post renal azotemia.

Urine samples of CKD cats were isostheuria (1.008 - 1.012) because glomeruli loss their function to concentrate urine properly (Wamsley and Alleman, 2007). In general, urine pH of normal cats is between 6 to 7 (Whitbred, 2015). From the results, eight CKD cats in this study presented with acidic urine (pH < 6) due to metabolic acidosis (Wamsley and Alleman, 2007). On the other hand, two cats in FIC group and four cats in cystic calculi group had urine pH of higher than 7 because of cystitis. Urinary bladder increased permeability when these cats had cystitis and plasma protein can leak into urine. Struvite crystal formation was found in four cats with idiopathic cystitis and seven cats with cystic calculi in this study which may due to alkaline urine

(Westropp et al., 2005). However, food and time to collect urine samples might affect the urine pH (Wamsley and Alleman, 2007). Cats in FIC group and cats in cystic calculi group in this study had proteinuria which indicated that these cats had hematuria or inflammation of the urinary bladder. Urine of cats that had macroscopic hematuria often presents at least 3 plus level of dipstick protein reaction (Wamsley and Alleman, 2007).

5.4 Urinary creatinine concentration

Urine creatinine concentration in CKD cats in this study was lower than other groups**.** Nevertheless, CKD cats had low body mass index that the body mass index associated to urine creatinine concentration (Di Micco et al., 2013).

5.5 Urine protein to creatinine ratio (UPC)

Cats in CKD group had high significantly difference UPC from the clinically normal cats ($p < 0.01$). The increased UPC in this group may be due to tubular and glomerular impairment and plasma protein leaked into urine. While, cats in FIC group and cats in cystic calculi had significantly difference from the clinically normal cats ($p < 0.05$). Increased UPC in both FIC and cystic calculi may be caused by inflammation or hemorrhage in the lower urinary tract disease of cats. Besides, increased permeability of urinary bladder often result in plasma protein leakage into the urine (Wamsley and Alleman, 2007). In our opinion, identifying urine protein in FIC is very important and was postulated to be the important characteristic of FIC.

One study found that the cats with idiopathic cystitis had significantly lower Trefoli factor 2 (TFF2) concentrations than the control cats. They believed that TFF2 might relate to healing process of the epithelium and to stabilize the bladder mucous layer (Lemberger et al., 2011b). Other studies analyzed increasing levels of fibronectin in cats with idiopathic cystitis and compared it with normal cats, cats with urolithiasis, or cats with bacterial urinary tract infection (Lemberger et al., 2011a; Treutlein et al., 2013). Fibronectin is used as an indicator of urinary bladder fibrosis. The fibronectin

would lose from layer and leak into the urine, when the urinary bladder epithelium was damaged (Lemberger et al., 2011a). GAGs, another protein, also has been reported to protect the urinary bladder epithelium in cats with FIC (Panchaphanpong et al., 2011).

5.6 NAG index in urine

5.6.1 CKD group

CKD cats in this study had the mean NAG index higher than the clinically normal cats. The same trend was also observed by Sato et al. in 2002. That research performed NAG index in four CKD cats using p-nitrophenyl N-acetyl- beta-D-glucosaminide (PNP) as the substrate ranged 6.2 to 35.5 U/g and received the mean±SEM of NAG index of 20.18 ± 6.32 U/g (Sato et al., 2002). The mean value of NAG index in that study was higher than the present study because of the small sample size and using different substrate for NAG measurement. However, the present study used 3-cresolsulphonphthaleinyl-N-acetyl- beta -D-glucosaminide as the substrate which has high sensitivity to detect NAG and low overlapping urine color. (Noto et al., 1983; Wen and Kellum, 2012).

Studies in cats with various diseases also demonstrated higher NAG index. Seven cats with euthyroid and azotemia had significantly higher median NAG index (13.12 U/g) than the aged-matched healthy control cats with aged-matched (1.38 U/g) (Lapointe et al., 2008). They suggested that the cause of increased NAG was tubular damage. Generally, function of the proximal tubular cell is reabsorbing protein. Tubular cell saturation of transport protein mechanism induce proximal tubular damage (D'Amico and Bazzi, 2003). One interesting research observed association between NAG index and development of azothemic cats with prospective longitudinal study. They found that median ± IQR of NAG index on date at entry to the study in developed azotemic cats group (1.40 ± 1.65 U/g) had higher than non-azotemic cats (0.85 ± 0.95 U/g) ($p=0.05$) and NAG index associated with development of azothemia when test by univariable analysis ($p \leq 0.05$) (Jepson et al., 2009).

In contrast, other study argued that the increased NAG index in rat was not involved with tubular damage but the cause was an increased lysosomal activity of the tubular cell (Bosomworth et al., 1999). Rats in that study were induced renal tubular injury by puromycin aminonucleoside (PAN) injection. NAG index was high on day two of the study and then decline on day four and remained stable. Proteinuria was increased and protein droplet was found on tubular cell on day four. The researcher suggested that NAG in rats will be increased if activity of lysosomal on tubular cell increased its function. When more nephrons are absence, lowered levels of NAG will be released (Bosomworth et al., 1999) The urinary NAG has high sensitivity of changing tubular cell but low specificity of tubular injury (Wen and Kellum, 2012).

The present research was cross-sectional clinical study which could not control characterization of nephron injury in each cat at the time of entry. Thus, the increased urinary NAG levels in the CKD cats in this study might result from; firstly, samples were collected on first day of diagnosed which CKD cats and lysosome of tubular cell might still active that related to proteinuria in CKD cats of this study. NAG can still present in urine for 12 hours to 4 days before increased regular parameters of renal function (Wen and Kellum, 2012). Secondly, NAG might filtrate from the circulation in glomerular damage or lost function and when average serum creatinine of CKD cats was in stage 3 of IRIS stage. Generally, NAG has large molecule and cannot filtrate from the renal glomeruli in normal cats (Skalova, 2005).

In CKD cats, the association of log NAG index and serum creatinine; association of log NAG index and log UPC were not significantly associated with each other in this study. Jepson et al. (2010) showed that log NAG index associated with plasma creatinine but the association was poor correlation (r^2=0.025, $p < 0.05$) and there was little correlation of log NAG index and log UPC (r^2=0.249, $p<0.001$) (Jepson et al., 2010). Moreover, they found that NAG index were not associated with neither progression of CKD nor plasma creatinine. Serum creatinine was lately indirect indicator of glomerular filtration and kidney can compensate itself to loss of function more than 75% (Skalova,

2005). Later reports also supported the findings that increased urinary NAG excretion was associated with proteinuria (Jepson et al., 2010). The same trend also observed in human patients (Sheira et al., 2015). In a study of patient with diabetes mellitus, it was found that the patient with normoalbuminuria had significantly higher urinary NAG levels than the normal control group. They suggested that increase urinary NAG excretion resulted from the increased in lysosomal activity of tubular cell to uptake protein. Therefore, changing urinary NAG levels in human patient could detect early glomerular damage than albuminuric condition (Sheira et al., 2015).

5.6.2 FIC group and cystic calculi group

In FIC and cystic calculi group, NAG index of these two groups were 2 times and 1.5 time of the NAG index levels in the clinically normal cats, respectively. However, the statistical testing were performed, the values were not significant difference from the clinical normal cats. Our findings were contradictory to one study which found that cats with lower urinary tract diseases had NAG index within the range of normal cat (Sato et al., 2002). In that study, only five FLUTD cats were studied and NAG index range between 1.1 and 3.2 U/g with the NAG index (1.78± 0.38 U/g) (Sato et al., 2002). Panchaphanpong et al. (2011) indicated that FIC cats had significantly lower urinary GAG to creatinine ratio. The trend of increase NAG index in the present study may explain why lower GAG to creatinine ratio was observed in the FIC cats. NAG was proposed to be involved with the degradation process of GAGs in circulation (Komosinska-Vassev et al., 2005) and one characteristic of FIC is proteinuria. The association between proteinuria and increased NAG index was demonstrated in experimental rats (Bosomworth et al., 1999), human patient (Sheira et al., 2015), and in cats (Jepson et al., 2010). Further study is needed to find the association between urine protein and NAG index that increase in the FIC cats.

In conclusion, urinary NAG cannot be used as an early indicator of CKD in cats. However, it might be early marker of proteinuria before the development of azotemia. Changing in urinary NAG levels were associated with proteinuria and may be related to

the pathological abnormalities in cats with idiopathic cystitis. Further study is needed to characterize the urine protein and to investigate NAG role in FIC group.

REFERENCES

Al-Hadithi HN, Williams H, Hart CA, Frazer M, Adams EJ, Richmond DH and Tincello DG. 2005. Absence of bacterial and viral DNA in bladder biopsies from patients with interstitial cystitis/chronic pelvic pain syndrome. The Journal of urology. 174(1): 151-154.

Ali RJ, Al-Obaidi FH and Arif HS. 2014. The Role of Urinary N-acetyl Beta-D-glucosaminidase in Children with Urological Problems. Oman Med J. 29(4): 285-288.

Bartges JW and Kirk CA. 2006. Nutrition and lower urinary tract disease in cats. Vet Clin North Am Small Anim Pract. 36(6): 1361-1376.

Bishop SA, Lucke VM, Stokes CR and Gruffydd-Jones TJ. 1991. Plasma and urine biochemical changes in cats with experimental immune complex glomerulonephritis. J Comp Pathol. 104(1): 65-76.

Bosomworth MP, Aparicio SR and Hay AW. 1999. Urine N-acetyl-beta-D-glucosaminidase a marker of tubular damage? Nephrol Dial Transpl. 14(3): 620-626.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1–2): 248-254.

Buffington CA and Chew DJ. 2007. Management of non-obstractive idiopathic/interstitial cystitis in cats. In: BASAVA Manual of canine and feline nephrology and urology. BASAVA, England. 264-281.

Buffington CA, Chew DJ and DiBartola SP. 1999. On the definition of feline interstitial cystitis. J Am Vet Med Assoc. 215(2): 186-188.

Buffington CAT. 2011. Idiopathic cystitis in domestic cats--beyond the lower urinary tract. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine. 25(4): 784-796.

Buffington CAT, Blaisdell JL, Binns Jr SP and Woodworth BE. 1996. Decreased Urine Glycosaminoglycan Excretion in Cats with Interstitial Cystitis. J Urol. 155(5): 1801-1804.

Buffington CAT, Chew DJ, Kendall MS, Scrivani PV, Thompson SB, Blaisdell JL and Woodworth BE. 1997. Clinical evaluation of cats with nonobstructive urinary tract diseases. J Am Vet Med Assoc. 210(1): 46-50.

Buffington CAT, Westropp JL and Chew DJ. 2014. From FUS to Pandora syndrome: where are we, how did we get here, and where to now? J Feline Med Surg. 16(5): 385-394.

Buffington CAT, Westropp JL, Chew DJ and Bolus RR. 2006a. Clinical evaluation of multimodal environmental modification (MEMO) in the management of cats with idiopathic cystitis. J Feline Med Surg. 8(4): 261-268.

Buffington CAT, Westropp JL, Chew DJ and Bolus RR. 2006b. Risk factors associated with clinical signs of lower urinary tract disease in indoor-housed cats. J Am Vet Med Assoc. 228(5): 722-725.

Byrne DS, Sedor JF, Estojak J, Fitzpatrick KJ, Chiura AN and Mulholland SG. 1999. The urinary glycoprotein GP51 as a clinical marker for interstitial cystitis. J Urol. 161(6): 1786-1790.

Cameron ME, Casey RA, Bradshaw JWS, Waran NK and Gunn-Moore DA. 2004. A study of environmental and behavioural factors that may be associated with feline idiopathic cystitis. J Small Anim Pract. 45(3): 144-147.

Cobrin AR, Blois SL, Kruth SA, Abrams Ogg ACG and Dewey C. 2013. Biomarkers in the assessment of acute and chronic kidney diseases in the dog and cat. J Small Anim Pract. 54(12): 647-655.

D'Amico G and Bazzi C. 2003. Pathophysiology of proteinuria. Kidney international. 63(3): 809-825.

Defauw PAM, Van de Maele I, Duchateau L, Polis IE, Saunders JH and Daminet S. 2011. Risk factors and clinical presentation of cats with feline idiopathic cystitis. J Feline Med Surg. 13(12): 967-975.

Di Micco L, Quinn RR, Ronksley PE, Bellizzi V, Lewin AM, Cianciaruso B and Ravani P. 2013. Urine creatinine excretion and clinical outcomes in CKD. Clin J Am Soc Neprol. 8(11): 1877-1883.

DiBartola SP, Rutgers HC, Zack PM and Tarr MJ. 1987. Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973-1984). J Am Vet Med Assoc. 190(9): 1196-1202.

Dru Forrester S and Roudebush P. 2007. Evidence-Based Management of Feline Lower Urinary Tract Disease. Vet Clin of N Am-Small. 37(3): 533-558.

Gandhi NS and Mancera RL. 2008. The structure of glycosaminoglycans and their interactions with proteins. Chem Biol Drug Des. 72(6): 455-482.

Gerber B, Boretti FS, Kley S, Laluha P, Muller C, Sieber N, Unterer S, Wenger M, Fluckiger M, Glaus T and Reusch CE. 2005. Evaluation of clinical signs and causes of lower urinary tract disease in European cats. J Small Anim Pract. 46(12): 571-577.

Grauer G. 2008. "Subject: Staging and management of chronic kidney disease" (cited 2013 December, 1). Available: http://veterinarycalendar.dvm360.com/staging-and-management-chronic-kidney-disease-proceedings-0?id=&pageID=1&sk=&date=.

Gunn-Moore DA. 2014. "Subject: Urethral obstruction in feline lower urinary tract disease" (cited 2014 September, 20). Available: http://www.vetsonline.com/publications/veterinary-times/archives/n-44-06/urethral-obstruction-in-feline-lower-urinary-tract-disease.html.

Gunn-Moore DA and Shenoy CM. 2004. Oral glucosamine and the management of feline idiopathic cystitis. J Feline Med Surg. 6(4): 219-225.

Hostutler RA, Chew DJ and DiBartola SP. 2005. Recent concepts in feline lower urinary tract disease. Vet Clin North Am Small Anim Pract. 35(1): 147-170.

Hurst RE. 1994. Structure, function, and pathology of proteoglycans and glycosaminoglycans in the urinary tract. World J Urol. 12(1): 3-10.

Hurst RE. 2003. A Deficit of Proteoglycans on the Bladder Uroepithelium in Interstitial Cystitis. Eur Urol Suppl. 2(4): 10-13.

Jaffé M. 1886. Über den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaktion des Kreatinins. Physiol Chem Phys Med. 10(5): 391-400.

Jepson RE, Brodbelt D, Vallance C, Syme HM and Elliott J. 2009. Evaluation of Predictors of the Development of Azotemia in Cats. J Vet Intern Med. 23(4): 806-813.

Jepson RE, Vallance C, Syme HM and Elliott J. 2010. Assessment of urinary N-acetyl-β-D-glucosaminidase activity in geriatric cats with variable plasma creatinine concentrations with and without azotemia. Am J Vet Res. 71(2): 241-247.

Komosinska-Vassev K, Olczyk K, Kozma EM, Olczyk P, Wisowski G and Winsz-Szczotka K. 2005. Alterations of glycosaminoglycan metabolism in the development of diabetic complications in relation to metabolic control. Clin Chem Lab Med. 43(9): 924-929.

Kordonouri O, Hartmann R, Müller C, Danne T and Weber B. 1998. Predictive Value of Tubular Markers for the Development of Microalbuminuria in Adolescents with Diabetes. Horm Res. 50(suppl 1)(Suppl. 1): 23-27.

Kruger JM, Osborne CA, Venta PJ and Sussman MD. 1996. Viral infections of the feline urinary tract. The Veterinary clinics of North America. Small animal practice. 26(2): 281-296.

Lapointe C, Bélanger MC, Dunn M, Moreau M and Bédard C. 2008. N-Acetyl-β-d-Glucosaminidase Index as an Early Biomarker for Chronic Kidney Disease in Cats with Hyperthyroidism. J Vet Intern Med. 22(5): 1103-1110.

Lavelle JP, Meyers SA, Ruiz WG, Buffington CA, Zeidel ML and Apodaca G. 2000. Urothelial pathophysiological changes in feline interstitial cystitis: a human model. American journal of physiology. Renal physiology. 278(4): 540-553.

Lekcharoensuk C, Lulich JP, Osborne CA, Koehler LA, Urlich LK, Carpenter KA and Swanson LL. 2000. Association between patient-related factors and risk of calcium oxalate and magnesium ammonium phosphate urolithiasis in cats. J Am Vet Med Assoc. 217(4): 520-525.

Lemberger SI, Deeg CA, Hauck SM, Amann B, Hirmer S, Hartmann K and Dorsch R. 2011a. Comparison of urine protein profiles in cats without urinary tract disease and cats with idiopathic cystitis, bacterial urinary tract infection, or urolithiasis. Am J Vet Res. 72(10): 1407-1415.

Lemberger SI, Dorsch R, Hauck SM, Amann B, Hirmer S, Hartmann K and Deeg CA. 2011b. Decrease of Trefoil factor 2 in cats with feline idiopathic cystitis. BJU Int. 107(4): 670-677.

Lulich J, Osborne C, O'Brien T and Polzin D. 1992. Feline renal failure: questions, answers, questions. Compend Contin Educ Pract Vet. 14(2): 127-152.

Lund HS, Saevik BK, Finstad OW, Grontvedt ET, Vatne T and Eggertsdottir AV. 2015. Risk factors for idiopathic cystitis in Norwegian cats: a matched case-control study. J Feline Med Surg. Published online: May 27, 2015

Noto A, Ogawa Y, Mori S, Yoshioka M, Kitakaze T, Hori T, Nakamura M and Miyake T. 1983. Simple, rapid spectrophotometry of urinary N-acetyl-beta-D-glucosaminidase, with use of a new chromogenic substrate. Clin Chem. 29(10): 1713-1716.

Osbaldiston GW and Taussig RA. 1970. Clinical report on 46 cases of feline urological syndrome. Vet Med Small Anim Clin. 65(5): 461-468.

Osborne CA, Johnston GR, Polzin DJ, Kruger JM, Poffenbarger EM, Bell FW, Feeney DA, Goyal S, Fletcher TF, Newman JA and et al. 1984. Redefinition of the feline urologic syndrome: feline lower urinary tract disease with heterogeneous causes. Vet Clin North Am Small Anim Pract. 14(3): 409-438.

Panchaphanpong J, Asawakarn T and Pusoonthornthum R. 2011. Effects of oral administration of N-acetyl-d-glucosamine on plasma and urine concentrations of glycosaminoglycans in cats with idiopathic cystitis. Am J Vet Res. 72(6): 843-850.

Pereira DA, Aguiar JAK, Hagiwara MK and Michelacci YM. 2004. Changes in cat urinary glycosaminoglycans with age and in feline urologic syndrome. Biochem Biophys Acta. 1672(1): 1-11.

Polzin DJ, Osborne CA and Ross SJ. 2005. Chronic Renal Failure. 6th ed. In: Textbook of Veterinary Internal Medicine, Philadelphia: W.B. Saunder Company. 1756-1763.

Prydz K and Dalen KT. 2000. Synthesis and sorting of proteoglycans. J Cell Sci. 113 (2): 193-205.

Pusoonthornthum R, Pusoonthornthum P and Osborne CA. 2012. Risk Factors for Feline Lower Urinary Tract Diseases in Thailand. Thai J Vet Med. 42(4): 517-522.

Reynolds BS and Lefebvre HP. 2013. Feline CKD: Pathophysiology and risk factors--what do we know? J Feline Med Surg. 15 suppl1: 3-14.

Ross LA and Finco DR. 1981. Relationship of selected clinical renal function tests to glomerular filtration rate and renal blood flow in cats. Am J Vet Res. 42(10): 1704-1710.

Salem MM. 2002. Pathophysiology of hypertension in renal failure. Semin Nephrol. 22(1): 17-26.

Sato R, Soeta S, Syuto B, Yamagishi N, Sato J and Naito Y. 2002. Urinary excretion of N-acetyl-beta-D-glucosaminidase and its isoenzymes in cats with urinary disease. J Vet Med Sci. 64(4): 367-371.

Semczuk-Sikora A, Sikora P, Biaduń U and Semczuk M. 2003. [Urinary N-acetyl-beta-D-glucosaminidase (NAG) excretion in women with pregnancy complicated with hypertension]. Ginekol Pol. 74(10): 1269-1275.

Sheira G, Noreldin N, Tamer A and Saad M. 2015. Urinary biomarker N-acetyl-β-D-glucosaminidase can predict severity of renal damage in diabetic nephropathy. J Diabetes Metab Disord. 14(4): 1-5.

Skalova S. 2005. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. Acta Medica (Hradec Kralove). 48(2): 75-80.

Syme HM, Markwell PJ, Pfeiffer D and Elliott J. 2006. Survival of Cats with Naturally Occurring Chronic Renal Failure Is Related to Severity of Proteinuria. J Vet Intern Med. 20(3): 528-535.

Treutlein G, Deeg CA, Hauck SM, Amann B, Hartmann K and Dorsch R. 2013. Follow-up protein profiles in urine samples during the course of obstructive feline idiopathic cystitis. Vet J. 198(3): 625-630.

Uechi M, Terui H, Nakayama T, Mishina M, Wakao Y and Takahashi M. 1994. Circadian variation of urinary enzymes in the dog. J Vet Med Sci. 56(5): 849-854.

Uechi M, Uechi H, Nakayama T, Wakao Y, Ogasawara T, Takase K and Takahashi M. 1998. The circadian variation of urinary N-acetyl-beta-D-glucosaminidase and gamma-glutamyl transpeptidase in clinically healthy cats. J Vet Med Sci. 60(9): 1033-1034.

Wamsley H and Alleman R. 2007. Complete urinalysis. 2 ed. In: BASAVA Manual of canine and feline nephrology and urology. BSAVA, England. 87-115.

Wen X and Kellum J. 2012. N-Acetyl-beta-D-Glucosaminidase (NAG). In: Encyclopedia of Intensive Care Medicine. ed. Vincent Jean-Louis and Hall JesseB (ed.). Springer Berlin Heidelberg. 1509-1510.

Westropp JL, Buffington CAT and Chew DJ. 2005. Feline lower urinary tract disease. 6 ed. In: Textbook of veterinary internal medicine. Elseveir Saunders, Missouri. 1828-1850.

Whitbred TJ. 2015. "Subject: Urinalysis" (cited 2015 June, 10). Available: http://www.merckvetmanual.com/mvm/clinical_pathology_and_procedures/diagnostic_procedures_for_the_private_practice_laboratory/urinalysis.html.

White JD, Norris JM, Baral RM and Malik R. 2006. Naturally-occurring chronic renal disease in Australian cats: a prospective study of 184 cases. Aust Vet J. 84(6): 188-194.

Willeberg P. 1984. Epidemiology of naturally occurring feline urologic syndrome. Vet Clin North Am Small Anim Pract. 14(3): 455-469.

Yakata M, Sugita O, Sakai T, Uchiyama K and Wada K. 1983. Urinary enzyme determination and its clinical significance. C. Enzyme derived from the kidney tubular epithelium--N-acetyl-beta-D-glucosaminidase. 4. Preclinical evaluation of the urinary NAG activity and changes in renal diseases. Jpn J Clin Pathol. 56: 90-101.

APPENDICES

Appendix 1 Questionnaire

วันที่ _____

ชื่อแมว_____**ชื่อเจ้าของ**_____**HN**_____

ชนิด_____ **พันธุ์**_____ **เพศ**__M__Mc__F__Fs **อายุ**_____ **น้ำหนัก** _____

ชนิดอาหาร ☐ อาหารเม็ด ยี่ห้อ_____ ☐ อาหารกระป๋อง ยี่ห้อ_____ ☐ ปรุงเอง _____

ความถี่ในการให้อาหาร ☐ 2 ครั้ง ☐ 3 ครั้ง ☐ มากกว่า 3 ครั้ง ☐ เททิ้งไว้ตลอด

อาหารชนิดนี้ให้มาเป็นระยะเวลา ☐ < 1 ปี ☐ 1-3 ปี ☐ 4-6 ปี ☐ > 7 ปี

แหล่งน้ำ ☐ น้ำประปา ☐ น้ำบาดาล ☐ น้ำกรอง ☐ น้ำต้ม ☐ อื่นๆ _____

ความถี่ในการให้น้ำ ☐ เป็นเวลา ☐ ตั้งทิ้งไว้

ลักษณะการเลี้ยง ☐ ในบ้านเท่านั้น ☐ เลี้ยงปล่อยบางเวลา ☐ นอกบ้านเท่านั้น

จำนวนสัตว์เลี้ยง ☐ แมว _____ ☐ สุนัข _____ ☐ อื่นๆ _____

สถานที่ขับถ่ายปัสสาวะ ☐ กระบะทราย จำนวน _____ ☐ หนังสือพิมพ์ ☐ ข้างนอกบ้าน

ประวัติการทำวัคซีน (ภายใน 3 ปี) ☐ พิษสุนัขบ้า ☐ หัด-หวัดแมว ☐ ลิวคีเมีย
☐ เยื่อบุช่องท้องอักเสบ ☐ ไม่เคยทำวัคซีน

ประวัติปัญหาระบบทางเดินปัสสาวะส่วนล่าง

☐ ปวดเบ่ง ☐ ปัสสาวะลำบาก ☐ ปัสสาวะเป็นเลือด ☐ ปัสสาวะบ่อย
☐ ปัสสาวะไม่เป็นที่ ☐ อื่นๆ _____

ประวัติให้ยารักษา glucosamine

☐ เคย เมื่อ _____ ☐ ไม่เคย

ประวัติการป่วยโรคอื่นๆ

Appendix 2 Signalment, life style, number of cats in the same household, type of food, receiving of water and number of litter boxes in clinically normal cat group

No.	Code	Age (yr.)	Weight (kg)	Breed	Gender	Life style	No. of cats	Type of food	Water	No. of litter boxes
1	FA001	2	3	DSH	M	In	3	Can&Dry	ADL	2
2	FA002	2	3.5	DSH	M	In	3	Can&Dry	ADL	2
3	FA004	1	3.2	DSH	F	Out	2	Can&Dry	ADL	1
4	FA005	4	4	DSH	M	Out	2	Can&Dry	ADL	1
5	FA006	2	3.1	DSH	Mc	In	4	Can&Dry	ADL	2
6	FA007	1	3.5	Persian	M	In	3	Can&Dry	ADL	1
7	FA008	1	2	DSH	M	In	3	Can&Dry	ADL	1
8	FA009	0.7	3.2	DSH	M	In&Out	2	Can&Dry	ADL	2
9	FA010	8	4.3	DSH	Mc	In	1	Dry	ADL	1
10	FA011	10	6	DSH	Mc	In	1	Dry	ADL	1
11	FA012	0.7	3.4	DSH	M	In&Out	2	Can&Dry	ADL	2
12	FA013	0.5	2.2	DSH	M	In&Out	1	Can&Dry	ADL	1
13	FA014	1	3.6	DSH	M	In	1	Dry	ADL	1
14	FA015	9	3.2	DSH	Mc	In	3	Can&Dry	ADL	1
15	FA016	10	3	DSH	Mc	In	3	Can&Dry	ADL	1
16	FA018	8	4.3	DSH	Mc	In	2	Can&Dry	ADL	1
17	FA019	2	5.2	DSH	M	In	1	Can&Dry	ADL	1
18	FA020	1	3.2	DSH	M	In&Out	1	Can&Dry	ADL	1
19	FA021	1	4.1	DSH	M	In	2	Can&Dry	ADL	2

ADL – Ad libitum, DSH – Domestic short hair, F – Intact female, Fs - Sterile female, M – Intact male, Mc – Castrated male, In – Indoor, Out – Outdoor, In&Out – Indoor & Outdoor, yr. - year

Appendix 3 Signalment, life style, number of cats in the same household, type of food, receiving of water and number of litter boxes in CKD cat group

No.	Code	Age (yr.)	Weight (kg)	Breed	Gender	Life style	No. of cats	Type of food	Water	No. of litter boxes
1	FB001	15	5.4	DSH	Mc	In	1	Can	ADL	1
2	FB002	14	2.2	DSH	Fs	In	1	Can	ADL	1
3	FB003	7	3.0	DSH	F	In	3	Can	ADL	1
4	FB004	6	4.5	DSH	M	In&Out	1	Can	ADL	1
5	FB005	8	3.1	DSH	M	In	3	Can	ADL	2
6	FB006	7	3.0	DSH	Mc	In	2	Can&Dry	ADL	2
7	FB007	5	3.8	DSH	Mc	In	3	Can&Dry	ADL	2
8	FB009	3	4.1	DSH	Mc	In	1	Can	ADL	1
9	FB010	8	2.0	DSH	M	In	3	Can&Dry	ADL	2
10	FB011	7	3.9	DSH	Fs	In	3	Can	ADL	2
11	FB013	7	2.9	DSH	Mc	Out	2	Can&Dry	ADL	2
12	FB016	10	3.0	DSH	M	In	4	Can&Dry	ADL	2
13	FB017	14	2.2	DSH	Fs	In	3	Can&Dry	ADL	2
14	FB018	11	3.2	DSH	Mc	In	3	Can&Dry	ADL	3
15	FB019	7	3.2	Persian	M	In	1	Can	ADL	1
16	FB020	7	3.5	DSH	Mc	In&Out	4	Can	ADL	1
17	FB021	14	3.7	DSH	Fs	In	1	Can&Dry	ADL	1
18	FB022	8	2.8	DSH	Mc	In	1	Can	ADL	1
19	FB023	6	2.0	DSH	Mc	In	1	Can&Dry	ADL	1
20	FB024	5	4.2	DSH	Mc	In	1	Can&Dry	ADL	1
21	FB025	7	3.5	DSH	Mc	In&Out	4	Can	ADL	1
22	FB026	8	2.9	DSH	Mc	Indoor	1	Dry	ADL	1
23	FB027	6	5.7	DSH	Mc	Indoor	4	Can&Dry	ADL	1
24	FB028	7	2.3	DSH	Mc	Outdoor	1	Can&Dry	ADL	2

ADL – Ad libitum, DSH – Domestic short hair, F – Intact female, Fs - Sterile female, M – Intact male, Mc – Castrated male, In – Indoor, Out – Outdoor, In&Out – Indoor & Outdoor, yr. - year

Appendix 4 Signalment, life style, number of cats in the same household, type of food, receiving of water and number of litter boxes in FIC cat group

No.	Code	Age (yr.)	Weight (kg)	Breed	Gender	Life style	No. of cats	Type of food	Water	No. of litter boxes
1	FC004	1	3.9	Persian	M	In	1	Dry	ADL	1
2	FC005	5	3.7	DSH	M	In&Out	2	Homemade	ADL	0
3	FC007	0.5	2.0	DSH	M	In	1	Homemade	ADL	0
4	FC008	4	4.1	DSH	Mc	Out	3	Dry	ADL	2
5	FC009	2	3.3	DSH	M	In	3	Dry	ADL	0
6	FC010	3	4.8	Persian	M	In	3	Can & Dry	ADL	2
7	FC014	5	4.5	DSH	Mc	In&Out	3	Dry	ADL	0
8	FC016	7	3.7	DSH	Fs	In&Out	6	Dry	ADL	4
9	FC017	0.8	3.6	DSH	M	In&Out	3	Can & Dry	ADL	1
10	FC018	9	5.3	DSH	M	In&Out	3	Can & Dry	ADL	1
11	FC019	6	5.5	DSH	Fs	In&Out	2	Homemade	ADL	1
12	FC020	2	4.6	DSH	Mc	In&Out	3	Dry	ADL	1
13	FC021	7	4.4	Persian	M	In	2	Can & Dry	ADL	1
14	FC025	2	5.8	DSH	M	In	3	Can & Dry	ADL	3
15	FC027	7	3.8	DSH	Fs	In	1	Can & Dry	ADL	1
16	FC028	1	7.3	Persian	Mc	In	3	Dry	ADL	2
17	FC029	2	4.0	DSH	Fs	In	1	Dry	ADL	1
18	FC031	2	3.9	DSH	Mc	In	3	Dry	ADL	1
19	FC032	2	3.6	Scottish Fold	Fs	In	1	Dry	ADL	1
20	FC033	1	4.0	DSH	Mc	In	2	Dry	ADL	1
21	FC034	1	4.5	DSH	M	In	3	Dry	ADL	3
22	FC035	10	4.1	DSH	F	In	2	Can & Dry	ADL	1
23	FC037	2	5.0	DSH	M	Out	2	Dry	ADL	2
24	FC038	1.5	6.0	DSH	Mc	In	1	Dry	ADL	1
25	FC039	5	4.2	Persian	M	In	3	Can & Dry	ADL	3

ADL – Ad libitum, DSH – Domestic short hair, F – Intact female, Fs - Sterile female, M – Intact male, Mc – Castrated male, In – Indoor, Out – Outdoor, In&Out – Indoor & Outdoor, yr. - year

Appendix 5 Signalment, life style, number of cats in the same household, type of food, receiving of water and number of litter boxes in cystic calculi cat group

No.	Code	Age (yr.)	Weight (kg)	Breed	Gender	Life style	No. of cats	Type of food	Water	No. of litter boxes
1	FC011	1	3.3	DSH	Mc	In&Out	3	Can	ADL	1
2	FC012	3	4.7	DSH	Mc	In	2	Dry	ADL	4
3	FC013	4	5.4	DSH	M	In&Out	3	Dry	ADL	0
4	FC026	5	4.0	DSH	Mc	Out	1	Homemade	ADL	1
5	FC030	8	5.2	DSH	M	In	3	Dry	ADL	2
6	FD001	6	5.3	DSH	Mc	In&Out	2	Dry	ADL	1
7	FD002	4	5.5	DSH	Mc	In	3	Dry	ADL	0
8	FD003	5	4.2	Persian	M	In	1	Can	ADL	1
9	FD004	8	3.5	DSH	Fs	In	1	Dry	ADL	1
10	FD005	5	4.9	ASH	M	In	1	Dry	ADL	1
11	FD006	5	6.5	DSH	M	In	3	Can	ADL	1
12	FD007	2	3.9	DSH	M	Out	1	Dry	ADL	0
13	FD008	3	4.0	DSH	Mc	In	1	Can	ADL	1
14	FD009	2	2.6	Maine Coon	Fs	In	3	Dry	ADL	1
15	FD010	3	3.0	DSH	M	In	2	Can & Dry	ADL	2
16	FD011	7	5.9	DSH	M	In	1	Dry	ADL	1

ADL – Ad libitum, DSH – Domestic short hair, F – Intact female, Fs - Sterile female, M – Intact male, Mc – Castrated male, In – Indoor, Out – Outdoor, In&Out – Indoor & Outdoor, yr. - year

Appendix 6 Complete blood count and blood chemistry values of clinically normal cat group

No.	Code	RBC (x 10^6)	Hct (%)	WBC (/μl)	BUN (mg/dl)	sCr (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	FA001	12.59	38	4,350	12.59	38.00	43.50	20.00
2	FA002	4.47	40	18,850	34.06	1.48	19.20	24.81
3	FA004	4.00	24	14,000	7.45	3.51	ND	ND
4	FA005	4.70	29	8,000	6.98	4.51	ND	ND
5	FA006	8.70	44	6,950	26.40	1.30	62.00	26.00
6	FA007	10.60	45	7,290	28.00	1.60	67.00	35.00
7	FA008	11.90	48	11,800	17.00	1.20	40.00	35.00
8	FA009	9.96	52	12,200	ND	1.52	32.62	ND
9	FA010	7.10	41	10,200	30.00	1.60	96.00	51.00
10	FA011	7.70	38	10,200	30.00	1.60	45.00	34.00
11	FA012	7.59	45	19,200	ND	1.68	37.03	ND
12	FA013	8.53	50	10,680	ND	1.10	54.00	ND
13	FA014	8.50	40	9,230	24.00	1.50	45.00	32.00
14	FA015	5.88	29	15,300	20.00	1.50	75.00	36.00
15	FA016	6.51	36	12,700	24.00	1.60	54.00	18.00
16	FA018	6.14	39	6,910	26.00	1.30	48.00	31.00
17	FA019	7.35	36	6,270	20.00	1.20	60.00	17.00
18	FA020	8.00	31	9,460	25.00	1.80	74.00	34.00
19	FA021	9.85	40	13,500	27.00	1.70	68.00	44.00

ND – Not determined

Appendix 7 Complete blood count and blood chemistry values of CKD cat group

No.	Code	RBC (x 10^6)	Hct (%)	WBC (/μl)	BUN (mg/dl)	sCr (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	FB001	7.30	40	13,900	32.00	3.40	38.00	21.00
2	FB002	4.70	23	17,300	99.00	3.40	72.00	49.00
3	FB003	6.60	32	9,780	52.00	3.60	42.00	20.00
4	FB004	2.41	16	54,500	74.70	3.70	75.00	34.00
5	FB005	5.25	30	12,300	35.50	3.10	34.00	12.00
6	FB006	5.70	30	31,600	46.10	4.70	28.00	9.00
7	FB007	6.90	32	5,200	106.60	4.30	109.00	22.00
8	FB009	4.60	25	50,300	48.50	3.70	52.00	5.00
9	FB010	4.54	38	10,100	50.86	4.16	19.20	23.00
10	FB011	6.80	38	8,450	19.92	2.50	7.20	26.19
11	FB013	3.00	19	9,370	223.00	14.70	24.00	7.00
12	FB016	5.28	25	9,580	74.00	3.70	67.00	15.00
13	FB017	4.04	23	16,400	47.00	2.40	33.00	12.00
14	FB018	5.27	25	19,000	61.00	5.30	31.00	27.00
15	FB019	7.44	30	6,040	30.00	3.20	40.00	27.00
16	FB020	3.03	24	10,400	21.00	2.60	105.00	22.00
17	FB021	6.70	41	16,000	57.00	3.30	57.00	23.00
18	FB022	3.70	19	21,700	140.00	10.00	49.10	49.30
19	FB023	3.80	17	22,000	60.00	2.60	77.00	24.00
20	FB024	6.30	34	7,200	47.2	3.10	45.00	16.00
21	FB025	6.00	34	8,230	37.00	2.20	89.00	30.00
22	FB026	4.60	23	6,910	119.10	7.10	88.00	12.00
23	FB027	8.30	43	8,490	47.60	2.10	58.00	26.00
24	FB028	5.87	30	27,100	84.00	2.60	64.50	50.30

ND – Not determined

Appendix 8 Complete blood count and blood chemistry values of FIC cat group

No.	Code	RBC (x 10^6)	Hct (%)	WBC (/μl)	BUN (mg/dl)	sCr (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	FC004	10.40	45	5,110	18.70	1.40	42.00	42.00
2	FC005	9.20	50	14,800	36.80	1.50	90.00	38.00
3	FC007	9.60	45	9,750	34.10	1.50	27.00	59.00
4	FC008	8.90	44	19,000	170.70	12.60	38.00	13.00
5	FC009	10.00	48	11,100	30.80	1.80	74.00	23.00
6	FC010	8.20	40	15,500	27.00	1.60	33.00	22.00
7	FC014	5.30	42	10,200	30.00	1.40	12.00	50.00
8	FC016	10.90	53	12,600	32.00	1.20	106.00	96.00
9	FC017	ND	ND	ND	ND	ND	ND	ND
10	FC018	ND	ND	ND	ND	ND	ND	ND
11	FC019	8.50	44	14,300	26.50	1.70	53.00	15.00
12	FC020	7.70	40	14,400	9.60	0.10	5.00	3.00
13	FC021	9.80	47	7,640	23.10	1.30	49.00	25.00
14	FC025	9.40	49	4,240	25.70	1.40	56.00	60.00
15	FC027	10.00	41	8,500	27.50	1.50	62.00	16.00
16	FC028	10.00	50	8,150	22.00	1.40	37.00	18.00
17	FC029	9.80	47	7,640	23.10	1.30	52.00	15.00
18	FC031	7.08	34	33,500	203.00	13.90	52.00	20.00
19	FC032	11.20	46	7,820	21.00	1.40	55.00	31.00
20	FC033	7.00	40	12,000	24.00	1.30	54.00	63.00
21	FC034	8.80	42	7,730	21.20	1.20	23.00	68.00
22	FC035	10.00	47	11,500	21.90	1.70	45.00	20.00
23	FC037	10.30	46	21,600	21.20	1.80	35.00	34.00
24	FC038	8.46	41	8,700	21.00	1.70	28.00	40.00
25	FC039	7.47	42	26,000	94.00	4.60	47.00	36.00

ND – Not determined

Appendix 9 Complete blood count and blood chemistry values of cystic calculi cat group

No.	Code	RBC (x 10^6)	Hct (%)	WBC (/μl)	BUN (mg/dl)	sCr (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	FC011	10.00	50	12,400	32.90	2.00	42.00	25.00
2	FC012	10.00	51	15,500	36.90	1.90	81.00	22.00
3	FC013	10.00	56	9,092	25.00	1.20	51.00	20.00
4	FC026	5.30	29	4,230	25.40	1.80	47.00	40.00
5	FC030	8.63	45	9,640	19.00	1.00	41.00	31.00
6	FD001	8.40	46	12,700	17.00	1.10	29.00	14.00
7	FD002	9.60	48	8,580	24.40	1.80	57.00	56.00
8	FD003	9.10	40	47,200	126.80	8.80	41.00	18.00
9	FD004	9.85	44	9,460	25.00	1.80	74.00	34.00
10	FD005	6.30	47	12,800	26.00	1.30	46.00	27.00
11	FD006	9.60	47	41,200	51.70	3.70	43.00	12.00
12	FD007	8.80	42	18,700	25.40	1.57	54.00	21.00
13	FD008	8.80	42	18,700	50.60	2.70	54.00	21.00
14	FD009	7.80	32	22,500	19.80	2.60	42.00	20.00
15	FD010	10.00	49	18,000	177.90	16.20	52.00	26.00
16	FD011	8.80	45	10,400	22.00	1.50	42.00	25.00

ND – Not determined

VITA

Miss Isadee Panboon was born on June 4, 1987 in Bangkok, Thailand. She finished high school from Triamudomsuksa Pattanakarn School in 2005. And then, she moved to Chiang Mai to study for Doctor of Veterinary Medicine (D.V.M.) from Faculty of Veterinary Medicine, Chiang Mai University and graduated in 2012. She studies for Master's degree in Veterinary Medicine at Faculty of Veterinary Science, Chulalongkorn University in 2013 and she also is clinician in private small animal hospital. She interest in feline medicine and lower urinary tract diseases.