การศึกษาเปรียบเทียบระดับเอ็น-อะซีติล-เบต้า-ดี-กลูโคซามินิเดส ระดับไกลโคซามิโนไกลแคน ในปัสสาวะและระดับไกลโคซามิโนไกลแคนในเลือดของแมวที่เป็นโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ

นางสาวจีรนันท์ เบ็ญจศิริวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2560

COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS, URINARY GLYCOSAMINOGLYCANS AND PLASMA GLYCOSAMINOGLYCANS LEVELS IN CATS WITH FELINE IDIOPATHIC CYSTITIS

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จีรนันท์ เบ็ญจศิริวรรณ : การศึกษาเปรียบเทียบระดับเอ็น-อะซีติล-เบต้า-ดี-กลูโคซามินิเดส ระดับไกลโคซามิโนไกลแคน ในปัสสาวะและระดับไกลโคซามิโนไกลแคนในเลือดของแมวที่เป็นโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ (COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS, URINARY GLYCOSAMINOGLYCANS AND PLASMA GLYCOSAMINOGLYCANS LEVELS IN CATS WITH FELINE IDIOPATHIC CYSTITIS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. สพ.ญ. ดร.รสมา ภู่สุนทรธรรม, 82 หน้า.

การศึกษาเปรียบเทียบระดับเอนไซม์ N-acetyl-β-glucosaminidase (NAG) ในปัสสาวะ, glycosaminoglycans (GAGs) ในปัสสาวะ และเลือด ในแมวที่เป็นโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ (Feline idiopathic cystitis; FIC) ร่วมกับการเก็บข้อมูลแมวโดยถาม-ตอบแบบสอบถาม ในหัวข้อ ข้อมูลพื้นฐานทั่วไปของแมว ลักษณะนิสัยแมว ลักษณะการเลี้ยงและสิ่งแวดล้อมของแมว ชนิดอาหารและการจัดการกระบะทรายของแมว เพื่อประเมินปัจจัยเสี่ยง (risk factor) ในการเกิดโรคในแมว FIC ทำการเก็บตัวอย่างเลือดและปัสสาวะจากแมว FIC จำนวน 19 ตัว และแมวปกติที่มีสุขภาพดีจำนวน 19 ตัว ที่มีอายุและเพศใกล้เคียงกัน จากนั้นตรวจวัดระดับเอนไซม์ NAG ในปัสสาวะ, ระดับ GAGs ในปัสสาวะและเลือด โดยวิธี colorimetric method และคำนวณผลเป็นค่า NAG index และ GAGs-to-creatinine ratio ผลพบว่าแมวที่มี body condition score >3/5 (OR = 4.96; 95% CI 0.873-28.152), แมวเพศผู้ทำหมันแล้ว (OR = 2.36; 95% CI 0.640-8.667) และ แมวขนยาว (OR = 8.31; 95% CI 0.890-77.568) มีแนวโน้มที่จะมีความเสี่ยงต่อการเกิดโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ ในทางกลับกันแมวพันธุ์ domestic shorthair (OR = 0.09; 95% CI 0.010-0.876) เป็นปัจจัยป้องกัน (protective factor) สำหรับโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุ แมว FIC มักพบการใช้กระบะทรายมากกว่าแมวสุขภาพดีอย่างมีนัยสำคัญ (OR = 14.57; 95% CI 2.566-82.732) แมว FIC มีค่าเฉลี่ยของ NAG index (2.36 ± 0.69 U/g) สูงกว่าแมวสุขภาพดี (1.00 ± 0.21 U/g) อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) และ ในแมว FIC มีค่าเฉลี่ยอัตราส่วน GAGs ต่อ ครีเอทินีน (GAGs-to-creatinine ratio) (3.84 ± 0.52 x10^3) น้อยกว่าแมวสุขภาพดี (4.52 ± 0.76 x10^3) แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ยังพบว่าค่าอัตราส่วนโปรตีนต่อครีเอทินีน (Urine protein to creatinine ratio; UPC) และ NAG index มีความสัมพันธ์เชิงบวกในระดับปานกลาง ($r = 0.511$, $p < 0.05$) ในกลุ่มแมว FIC จากการศึกษานี้สามารถสรุปได้ว่า NAG index สามารถใช้เป็นตัวบ่งชี้การเกิดโรคและประเมินการดำเนินไปของโรคกระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุในแมวได้ โดยเฉพาะในแมวป่วยที่มีระดับโปรตีนในปัสสาวะเพิ่มขึ้น และอาจมีกระบวนการเกิดพยาธิสภาพที่ไต ก่อนจะส่งผลมาที่การเกิดพยาธิสภาพของระบบขับถ่ายปัสสาวะส่วนล่าง ความเสียหายของผนังกระเพาะปัสสาวะชั้น GAGs ส่งผลให้เกิดการขับออกของ urinary GAGs ในปัสสาวะที่ลดลง และอาจเกี่ยวข้องกับการเพิ่มขึ้นของเอนไซม์ในไลโซโซม (lysosomal enzyme) โดยเฉพาะอย่างยิ่งเอนไซม์ NAG ที่ขับออกจากไต

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์สัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5875305031 : MAJOR VETERINARY MEDICINE
KEYWORDS: CAT / FELINE IDIOPATHIC CYSTITIS / N-ACETYL-BETA-D-GLUCOSAMINIDASE / GLYCOSAMINOGLYCANS

JEERANAN BENJASIRIWAN: COMPARATIVE STUDY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS, URINARY GLYCOSAMINOGLYCANS AND PLASMA GLYCOSAMINOGLYCANS LEVELS IN CATS WITH FELINE IDIOPATHIC CYSTITIS. ADVISOR: ASSOC. PROF. ROSAMA PUSOONTHORNTHUM, D.V.M., M.Sc., Ph.D, D.T.B.V.M., 82 pp.

Comparative study was conducted to measure urinary N-acetyl-β-glucosaminidase (NAG), urinary glycosaminoglycans (GAGs) and plasma glycosaminoglycans (GAGs) in cats with feline idiopathic cystitis (FIC). A standard questionnaire was designed to gather information for all cats including signalment, characteristics, environment, type of food and management of the cats' litter box to evaluate the risk factors for developing FIC. Blood and urine samples were collected from 19 clinically normal cats and 19 aged and sex matched cats with FIC. Concentration of urinary NAG, urinary GAGs and plasma GAGs were measured by colorimetric method. NAG index and GAGs-to-creatinine ratio were calculated. The results demonstrated that cats with body condition score >3/5 (OR = 4.96; 95% CI 0.873-28.152), castrated male (OR = 2.36; 95% CI 0.640-8.667) and longhaired-cats (OR = 8.31; 95% CI 0.890-77.568) tend to be the risk factor for developing FIC. On the contrary, domestic shorthair breed (OR = 0.09; 95% CI 0.010-0.876) was the protective factors for FIC. Cats with FIC were significantly more likely to use a litter box than clinically normal cats (OR = 14.57; 95% CI 2.566-82.732). Cats with FIC had significantly higher NAG index (2.36 ± 0.69 U/g) than clinically normal cats (1.00 ± 0.21 U/g) ($p < 0.05$). The cats with FIC had lower GAGs-to-creatinine ratio (3.84 ± 0.52 x10^3) than clinically normal cats (4.52 ± 0.76 x10^3) but the values were not significantly different. The Urine protein to creatinine ratio (UPC) and NAG index presented the significant moderate positive correlation (r = 0.511, $p < 0.05$) in cats with FIC. These finding suggested that the increased NAG index might play a role as a biomarker for identifying and assessing progressive idiopathic cystitis, particularly in cats with proteinuria condition. It was possibly that cats with FIC had some complications related to the kidney dysfunction prior to the development of FIC. This defective GAGs layer in cats with FIC resulting in decreased urinary GAGs excretion and GAGs-to-creatinine ratio might relate to the increased lysosomal enzyme such as NAG from the kidney.

Department:	Veterinary Medicine	Student's Signature	.....
Field of Study:	Veterinary Medicine	Advisor's Signature	.....
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LIST OF ABBREVIATIONS

A	Absorbance
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
BCS	Body condition score
BUN	Blood urea nitrogen
CBC	Complete blood count
CFU	Colony forming units
CI	Confidence intervals
CKD	Chronic kidney disease
Cr	Creatinine
CS	Chondroitin sulfate
dl	Deciliter
DS	Dermatan sulfate
EDTA	Ethylenediaminetetraacetic acid
FCVs	Feline calicivirus
FIC	Feline idiopathic cystitis
Fig.	Figure
FLUTD	Feline lower urinary tract disease
FUS	Feline urologic syndrome
g	Grams
GAGs	Glycosaminoglycans
GFR	Glomerular filtration rate
HA	Hyaluronic acid
Hct	Hematocrit
HPA	Hypothalamic pituitary axis
IC	Interstitial cystitis
Kg	Kilograms
KS	Keratan sulfate

l	Liter
LUTS	Lower urinary tract signs
M	Molar
MEMO	Multimodal environment modification
MIC	Minimum inhibitory concentration
ml	Milliliter
µg	Microgram
µl	Microliter
NAG	N-acetyl-β-D-glucosaminidase
nm	Nanometer
OAB	Overactive bladder
OD	Optical density of sample
OR	Odds ratio
PBS	Painful bladder syndrome
PGs	Proteoglycans
POD	Post-obstructive diuresis
RCF	Relative centrifugal force
sCr	Serum creatinine
SEM	Standard error of mean
TNTC	Too numerous to count
TTF2	Trefoil factor 2
U	Unit
UB	Urinary bladder
UPC	Urine protein to creatinine ratio
USG	Urine specific gravity
UT	Urinary tract
WBC	White blood cell

CHAPTER I
INTRODUCTION

Importance and Rationale

The feline lower urinary tract disease (FLUTD) is the condition that can affect urinary bladder or urethra of cats resulting in hematuria, stranguria, dysuria, periuria and pollakiuria (Defauw et al., 2011). FLUTD can be classified into 2 groups including obstructive FLUTD and non-obstructive FLUTD. The causes of non-obstructive FLUTD were 65.0% idiopathic, 15.0% uroliths, 10.0% anatomical defect or neoplasia, less than 10.0% behavioural problems and less than 2.0% bacterial infection. The causes of obstructive FLUTD were 59% urethral plug, 29.0% idiopathic, 10.0% uroliths and 2.0% bacterial infection (Gunn-Moore, 2003). The high percentage of cause of the FLUTD were an idiopathic cystitis called feline idiopathic cystitis (FIC). In contrast, Eggertsdottir et al. (2007) noted that the bacterial infection may have been underdiagnosed in Norwegian cats presenting the clinical signs of FLUTD. Because of the idiopathic cause, the only way to diagnoses is ruled out other causes (Buffington, 2011) which taking a long time and resulting in an increased the mortality rate.

In Thailand, Pusoonthornthum et al. (2012) noted that the most common cause of FLUTD was idiopathic cystitis (27.1%). Likewise, Segev et al. (2011) who investigate the prognosis of urethral obstructions, the overall mortality showed 8.5%. Cats with FIC usually showed the signs of severe stranguria and dysuria lead to systemic condition such as accumulation of uremic toxin, acid-base imbalanced, decreased glomerular filtration rate (GFR) and dead (Lee and Drobatz, 2003; Segev et al., 2011). This severe stranguria and dysuria condition lead to urethral obstruction. The long-termed prognosis in cats with urethral obstruction was guarded whether veterinarian can early diagnose and start the proper treatment immediately (Gerber et al., 2008).

In healthy cats, urinary bladder (UB) wall consisted of 3 layers including muscular layer, urothelium layer and glycosaminoglycans (GAGs) layer. The GAGs layer lined inside the UB wall for protect other layers from noxious substances (Buffington, 2011). The possible etiology of FIC was the defective GAGs resulting in decreased urinary GAGs excretion (Buffington et al., 1996). One hypothesis was low GAGs level might be due to absorption and/or degradation of endogenous urinary GAGs and indicated a damaged of UB surface (Pereira et al., 2004).

At present, the urinary biomarker play a role of early diagnostic tools in many diseases such as N-acetyl-β-D-glucosaminidase (NAG) in chronic kidney disease (Cobrin et al., 2013). The NAG was used as an early biomarker of tubular damage in several species as a NAG index (Bourbouze et al., 1984; Sato et al., 2002). Not only the tubular damaged but also the proteinuria condition can induce increased NAG index due to increased lysosomal turnover (Bosomworth et al., 1999). However, there are a few study reported about the biomarker related to FLUTD especially idiopathic cause. Reliable diagnostic markers for FIC in current clinical field are not yet available (Buffington, 2011). Elevation of NAG or decreased GAGs excretion might play a role of enzyme degraded the GAG layers lining inside the UB wall (Pereira et al., 2004; Panboon et al., 2017) lead to noxious substances in urine can stimulate the pain receptors easily in cats with FIC (Buffington et al., 2014)

Objectives of Study

1. To investigate the levels of urinary N-acetyl-β-D-glucosaminidase, urinary glycosaminoglycans and plasma glycosaminoglycans in cats with feline idiopathic cystitis compared to the clinically normal cats.

2. To evaluate the possible risk factors for developing feline idiopathic cystitis.

Hypothesis

N-acetyl-β-D-glucosaminidase (NAG) can be used as a biomarker for cats with feline idiopathic condition.

Keywords (Thai): แมว, กระเพาะปัสสาวะอักเสบโดยไม่ทราบสาเหตุในแมว, เอ็น-อะซีติล-เบต้า-ดี-กลูโคซามินิเดส, ไกลโคซามิโนไกลแคน

Keywords (English): cats, feline idiopathic cystitis, N-acetyl-β-D-glucosaminidase, glycosaminoglycans

Advantages of Study

Urinary NAG, urinary GAGs and plasma GAGs level can be used as biomarkers for monitoring cats with idiopathic cystitis.

CHAPTER II
LITERATURE REVIEW

2.1. Feline lower urinary tract disease (FLUTD)

The urinary tract system could be divided into 2 part including upper urinary tract system and lower urinary tract system. The abnormalities or diseases that occured in kidney and ureter of cats were called feline upper urinary tract diseases. In the same way, the abnormalities or diseases that occured in UB, urethra and also prostate gland in male cats were called feline lower urinary tract disease (FLUTD). In 1984, Osborne et al. (1984) proposed that cats presenting with the signs of hematuria, dysuria, pollakiuria were diagnosed FLUTD or formerly called Feline urologic syndrome (FUS). FLUTD could be classified into 2 group including; obstructive FLUTD and non-obstructive FLUTD. The causes of non-obstructive FLUTD were idiopathic 65.0%, uroliths 15.0%, anatomical defect or neoplasia 10.0%, behavioural problems <10.0% and bacterial infection <2.0%. The causes of obstructive FLUTD were urethral plug 59.0%, idiopathic 29.0%, uroliths 10.0%, and bacterial infection <2.0%. Idiopathic cause seem to affect the cats for 29.0-65.0% (Gunn-Moore, 2003). The clinical signs of FLUTD were hematuria, stranguria, dysuria, periuria and pollakiuria (Buffington et al., 2014). Seventy six percents of FLUTD cats presenting to the hospital often showed the sign of severe stranguria and dysuria resulting in urethral obstruction condition (Segev et al., 2011). The degrees of systemic signs and the complications correlated with the severity of obstruction. Twelve percents of cats with severe urethral obstruction had multiple acid-base imbalanced due to complete obstruction and fall into the life-threatening metabolic derangement (Lee and Drobatz, 2003). The prolonged obstruction can cause the increasing bladder pressure lead to submucosal hemorrhage, decreasing glomerular infiltration, progressive azotemia and hyperkalemia. The darker red urine was more frequently found in cats with severe obstructive condition (Brabson et al., 2015). Post-obstructive

diuresis (POD) was determined as urine output >2 ml/kg/h and usually occur in cats treated for urethral obstruction (Frohlich et al., 2016).

2.2. Feline idiopathic cystitis (FIC)

Cats with FLUTD presenting chronic irritative voiding signs, lower urinary tract signs, sterile and cytologically negative urine and cannot find the real causes or etiologies were called cats with feline idiopathic cystitis (FIC) (Buffington et al., 2014). Several studies investigated the risk factors of cats with FIC. Overweight and nervous behavior seem to be the potential factors in the development of FIC (Defauw et al., 2011; Lund et al., 2015). Castrated males predispose to have a higher risk for lower urinary tract disease (Lekcharoensuk et al., 2001). Since FIC had many possible etiologies and the clinical signs seem to associate with comorbid disorders, Buffington et al. (2014) proposed the term Pandora syndrome instead of FLUTD. Inappropriate environmental status seem to affect the developing of FIC, The multimodal environmental modification (MEMO) as changing the cat's environmental was also postulated to reduce the lower urinary tract signs (LUTS) (Buffington et al., 2006).

2.3. Urinary bladder structure

The urinary bladder structure in healthy cats consisted of 3 major layers including muscular layer, urothelium layer and GAGs layer (Fig. 1) (Lavelle et al., 2000). The urothelial cells can be classified in to 3 cell types, umbrella cells, intermediate cells and basal cells.

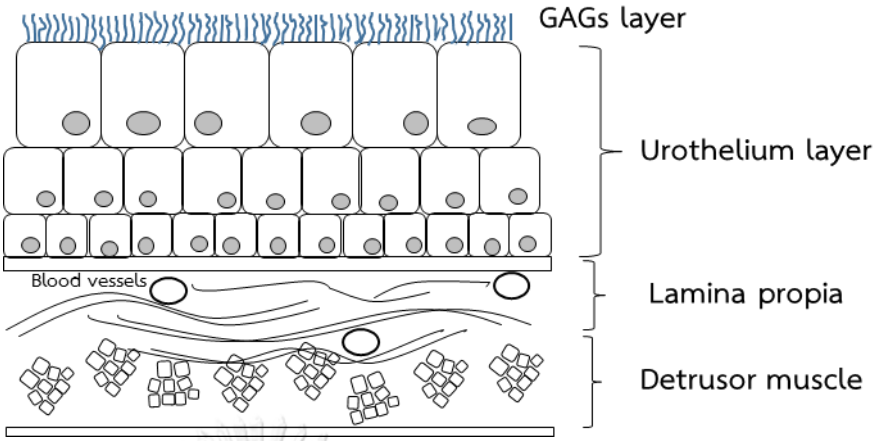


Fig. 1 The normal urinary bladder structure
(Adapted from Birder and Andersson, 2013) [24]

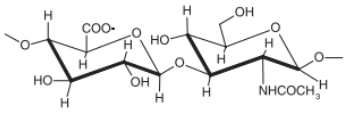
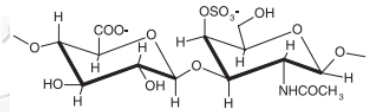
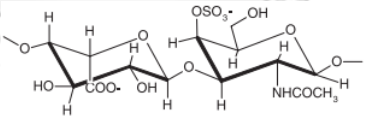
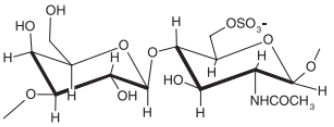
Urothelial cells could be activated by stimuli such as chemical, thermal and mechanical stimuli lead to releasing various mediators and neurotransmitters. The mediators and neurotransmitters could manipulate the nerve activity and bladder function (Birder and Andersson, 2013). The afferent innervation of UB consisted of small myelinated (A-delta) and unmyelinated (C-fiber). Pathological condition affected the afferent pathway by alter the chemical and electrical properties resulting in irritative voiding signs (de Groat and Yoshimura, 2009). The neural control of micturition, the C-fibres was the one of the afferent nerve innervated bladder which respond primarily to noxious substances (Fowler et al., 2008).

2.4. Glycosaminoglycans (GAGs)

The GAGs chains bounded to protein cores with covalent bonds to form a macromolecules called proteoglycans (PGs) (Kjellen and Lindahl, 1991). The GAGs molecules are the long unbranched polysaccharide which contained a repeating disaccharide unit and contributed to numerous functions in animal cells such as modulation of enzyme activities and control functions of extracellular matrix. Moreover, GAGs can effect on various cellular processes such as cell adhesion, motility and proliferation (Iozzo and Schaefer, 2015).

The main GAGs components comprise of a modified sugar group and either uronic acid or galactose unit in keratan sulfate. The modified sugar group can be classified as *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc). The uronic acid can be classified as D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) (Kjellen and Lindahl, 1991). There are many repeating disaccharide units of various GAGs such as hyaluronic acid, chondroitin sulfate, dermatan sulfate and keratan sulfate (Table. 1) (Gandhi and Mancera, 2008).

Table 1 Repeating disaccharide units of various glycosaminoglycans (Adapted from (Gandhi and Mancera, 2008))

Glycosaminoglycans	Disaccharide units	Origin
Hyaluronic acid (HA)	 D-GlcA- $\beta(1\rightarrow 4)$-D-GlcNAc-$\alpha(1\rightarrow 4)$	Synovial fluid, vitreous humour, extracellular matrix of loose CNT
Chondroitin sulfate (CS)	 D-GlcA-$\beta(1\rightarrow 3)$-D-GalNAc4S-$\beta(1\rightarrow 4)$	Cartilage, tendon, ligament, aorta
Dermatan sulfate (DS)	 L-IdoA-$\alpha(1\rightarrow 3)$-D-GalNAc4S-$\beta(1\rightarrow 4)$	Skin, blood vessels, heart valves
Keratan sulfate (KS)	 D-Gal-$\beta(1\rightarrow 4)$-D-GalNAc6S-$\beta(1\rightarrow 3)$	Cornea, cartilage

CNT = connective tissue

The GAGs are highly negative charged molecules and extremely hydrophilic due to sulfate substituents in various positions or also called sulfated GAGs (Iozzo and Schaefer, 2015). Because the sulfated GAGs have a high affinity for water, the water molecules would be trapped around the sulfated GAGs and play a role of the physical barrier as anti-adherence activity at the bladder surface by interposing between urine and cells (Parsons, 1993). There were many individual GAGs components of proteoglycan such as heparan sulfate and dermatan sulfate found in kidney and urinary tract, respectively. Moreover, chondroitin sulfate can be found in both plasma and urine of cats (Pereira et al., 2004). Chondroitin sulfate was the major component accounting for 81.0-84.0% of total GAGs in serum and plasma of domestic animal species such as dog, horse, donkey and rabbit (Ferlazzo et al., 1997). The GAGs layers lined inside the UB wall to protect other layers from noxious substances (Buffington, 2011). In human, the damage of urothelial GAGs barrier layers were postulated to be the underlie of pathogenesis in chronic bladder pathologies (Bassi et al., 2011). The changes in urinary GAGs excretion might be a tool for detecting and monitoring the pathogenesis of bladder cancer (Hennessey et al., 1981) and Mucopolysaccharidoses (Tanyalcin, 2015a). As a result of defective GAGs layers, many researchers try to investigate the intravesical exogenous GAGs into the damaged bladder of mouse (Kyker et al., 2005) and also in human IC (Davis et al., 2008). In addition, there are some study using the intravesical exogenous GAGs for cats with obstructive FIC as well (Bradley and Lappin, 2014; Delille et al., 2016).

2.5. Pathophysiology

The pathophysiology of FIC are not understood. The effective treatment for cats with FIC was not available. Many researchers try to investigate the possible etiologies and pathophysiology of FIC. Rubio-Diaz et al. (2009) suggested that the concentration of tryptophan and its metabolites might play a role of serum candidate for cats with FIC. Buffington (2011) noted that the abnormalities identified in FIC and interstitial cystitis (IC) can be classified into

3 groups including; local external abnormalities, internal abnormalities and intrinsic abnormalities. The 3 possible abnormalities might be the pieces of concept that lead to reveal the real pathophysiology. The local external abnormalities were the abnormalities of the substance in the urinary bladder lumen or microbial agent. Lemberger et al. (2011b) concluded that cats with FIC tend to have decreased urine trefoil factor 2 (TTF2) and TTF2 might play a role of protective factor in UB structure. The microbial agents that can be isolated from UB of cats with FIC were feline caliciviruses (FCVs) but this isolation might be associated with infection of other tissues (Rice et al., 2002). The mineralized material such as struvite crystal were found in urinary bladder of cats with FIC and might be considered as the noxious substance in urine (Bell and Lulich, 2015). The internal abnormalities were the imbalance of neuroendocrine system including sympathetic nervous system and hypothalamic pituitary axis (HPA) (Westropp et al., 2006). Hague et al (2013) studied the acoustic startle reflex, a brain stem reflex that responds to unexpected loud stimuli. This study results revealed that the cats with FIC tend to be more sensitive to environment than healthy cats (Hague et al., 2013). Moreover, cats with FIC have a tendency to have about the small size of adrenal gland resulting in a decreased in HPA function (Westropp et al., 2003). In contrast, increased sympathetic nervous system can induce high plasma catecholamine (norepinephrine) level lead to altered bladder permeability (Buffington et al., 2002; Westropp et al., 2006). Roppolo et al. (2005) studied the bladder A delta afferent nerve activity and concluded that this afferent nerve in cats with FIC are more sensitive to pressure changes than the healthy cats. The intrinsic abnormalities were the abnormalities of UB wall layers structure including GAGs layers, urothelium layers and muscular layers. Hauser et al. (2015) demonstrated changing of urothelium layer in UB such as abnormal protein expression and chondroitin sulfate patterns in cats with FIC compared to healthy cats. Many study reported about overactive bladder (OAB) in human IC, OAB is the condition which detrusor muscle excessively active resulting in urinary leakage or incontinence (Seth et al., 2013). Conversely, no evidence of

OAB was identified in female cats with FIC (Wu et al., 2011). The cats with FIC tend to have incomplete GAGs layers structure resulting in decreased urine GAGs excretion (Buffington et al., 1996; Panchaphanpong et al., 2011). According to decreased urine GAGs excretion, Pereira et al. (2004) stated that the low GAGs levels indicate a damaged of bladder surface lead to absorption and/ or degradation of the endogenous urinary GAGs.

In conclusion, when cats faced stress stimuli in environment, the stress stimulated the stress response system and cannot be terminated by cortisol and other adrenal corticosteroids due to decreased HPA (Fig. 2). Excessive norepinephrine from enhanced sympathetic activity can be upregulated the inflammatory process by stimulating C-fiber to released neuropeptide substance P resulting in altered the bladder permeability.

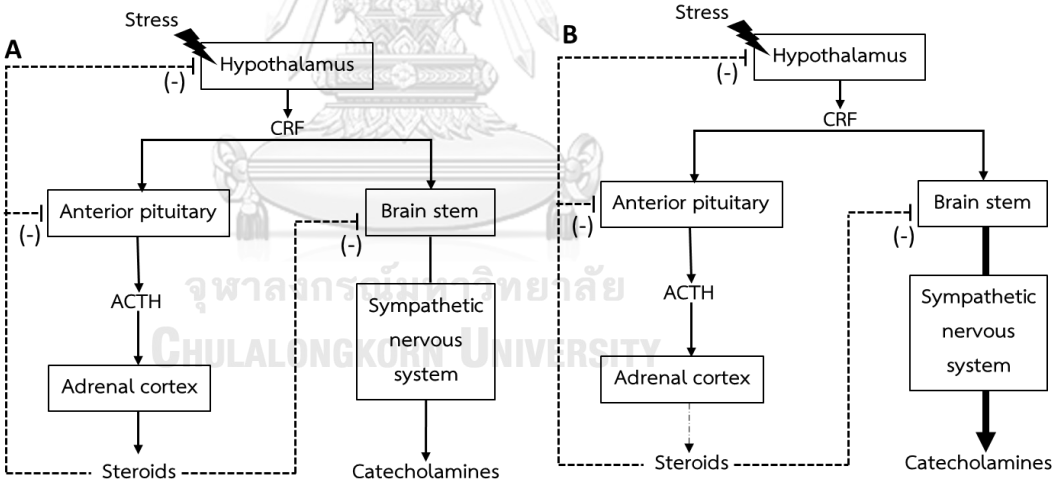


Fig. 2 The neuroendocrine system imbalances in cats with FIC

A, The normal stress response; B, Abnormal stress response

(Adapted from Buffington et al., 2014)

The neuropeptide substance P was an inflammatory cytokines which caused pain, vasodilation, mast cells degranulation, and submucosa edema and altered GAGs layers lead to damaged UB wall structure. The incomplete UB wall structure and/or defective GAGs layers can allow the noxious substance in bladder lumen stimulate back to C-fiber caused neurogenic inflammation. This process postulated to be the important pathophysiology of FIC (Buffington, 2011).

2.6. The candidate biomarker for FIC "N-acetyl-β-D-glucosaminidase (NAG)"

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme secreted from epithelial cells of the proximal convoluted tubule which can be classified into 2 types; isoenzyme A (NAG A) and isoenzyme B (NAG B) (Bourbouze et al., 1984). In healthy human, the urine contained small amount of NAG with NAG A: NAG B ratio 4:1 to 10:1. The NAG has a high molecular weight of 130,000-140,000 dalton and cannot filtrate through the glomerular basal membrane (Skalova, 2005). In pathological condition of tubular and interstitial renal impairment, the total NAG activity was elevated particularly NAG B lead to change the NAG A: NAG B ratio (Price, 1992). In human medicine, NAG can be an early biomarker for proximal tubular damaged particular in diabetic patients (Bouvet et al., 2014; Sheira et al., 2015). Moreover, urinary NAG could be a biomarker for children with upper urinary tract infection (Ali et al., 2014). In veterinary field, measurement of urinary NAG seem to yield benefit as well. The NAG can be an early biomarker for renal tubular damaged in cats with renal disease (Sato et al., 2002), cats with chronic kidney disease (CKD) (Jepson et al., 2010) and cats with hyperthyroidism (Lapointe et al., 2008). Jepson et al. (2009) reported about urinary NAG which positively correlated with urine protein to creatinine ratio in azotemic cats. The recent study investigated the role of urinary NAG in cats with FIC and found that urinary NAG might be associated with proteinuria condition (Panboon et al., 2017).

CHAPTER III

MATERIALS AND METHODS

3.1. Study population

The study population consisted of client-owned cats from Bangkok and surrounding areas presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The cases were recruited from a group of 19 cats diagnosed with FLUTD in the period May 2016- May 2017. The clinically normal cats were the cats coming to the veterinary hospitals for vaccination or neutering. The number of cats would be calculated by the formulae for determining needed sample sizes (Fig. 3).

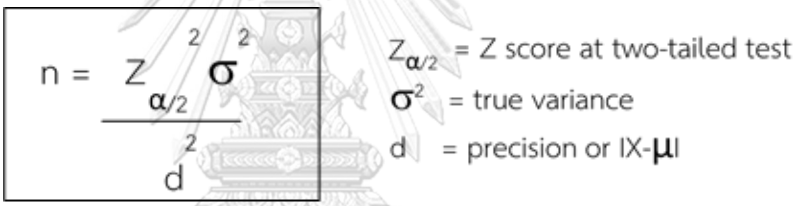


Fig. 3 The formulae for determining needed sample sizes

All 19 clinically normal cats with normal physical examination, hematology, serum chemistry and urinalysis was enrolled to this study. An equal number of cats with FIC group were adults cats aged 7 months or older presenting the typical clinical signs associated with FLUTD (hematuria, dysuria, stranguria, periuria and pollakiuria). A final diagnosis consistent with FIC made by excluding other causes of FLUTD and considering the results of physical examination, complete blood count (CBC), serum chemistry, urinalysis, urine bacteriologic culture, abdominal radiography and/or ultrasonography (Hague et al., 2013). Cats with neurologic problems, urethral plug, uroliths, CKD or other systemic diseases, anatomical defect, neoplasia and bacterial infection were excluded. Exclusion criteria included any treatment that can interfere the

diagnosis including any treatment such as antibiotics, hormones and medication altering blood pressure and urine production and glucosamine supplement. Moreover, the concurrent disease that can affect the urinalysis such as CKD, diabetes mellitus (DM) or hyperthyroidism were excluded (Lund et al., 2015). The cats with FIC and the control groups would be matched for gender and age. The criteria of cats in this study were shown in Fig. 4.

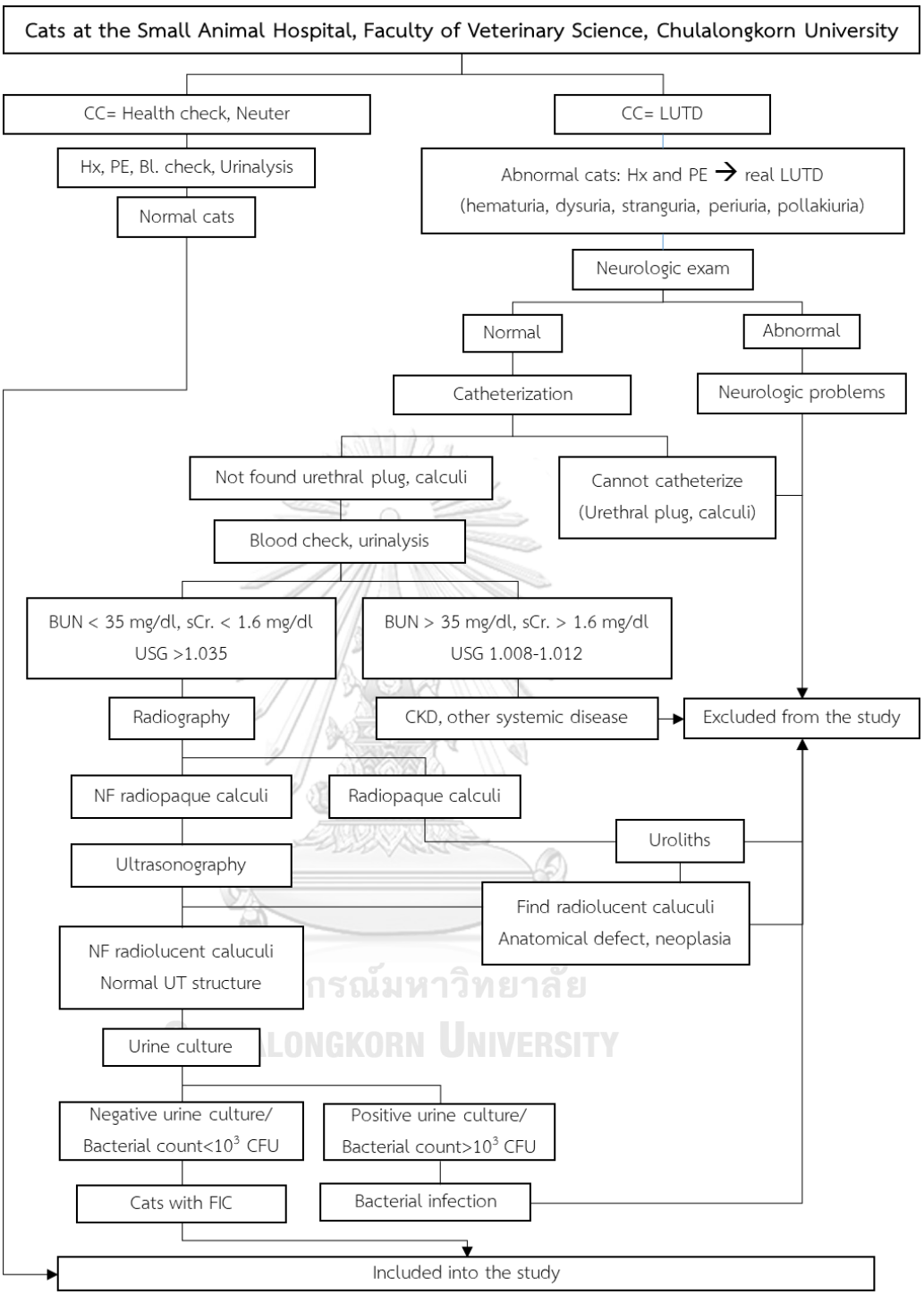


Fig. 4 Criteria of the cats in the study

CC = chief complaint, LUTD = lower urinary tract disease, Hx = history taking, PE = physical examination, UT = urinary tract

3.2. Study design

The thirty eight cats would be allocated into two groups consisting of 19 clinically normal cats and 19 cats with FIC. Blood (3 ml) and urine samples (5-10 ml) were collected at the time of initial examination. The urine samples were collected only one time due to factors such as inability to obtain client consent to hospitalize cats more than one day. Concentration of NAG, protein and creatinine were measured from urine samples. Only concentration of GAGs were measured from both urine and plasma sample. NAG index could be calculated by dividing NAG concentration into urine creatinine concentration ratio and UPC could be calculated by dividing urinary protein concentration into urine creatinine ratio. Urine GAGs-to-creatinine ratio could be calculated by dividing GAGs concentration into urine creatinine concentration ratio. (Fig. 5)

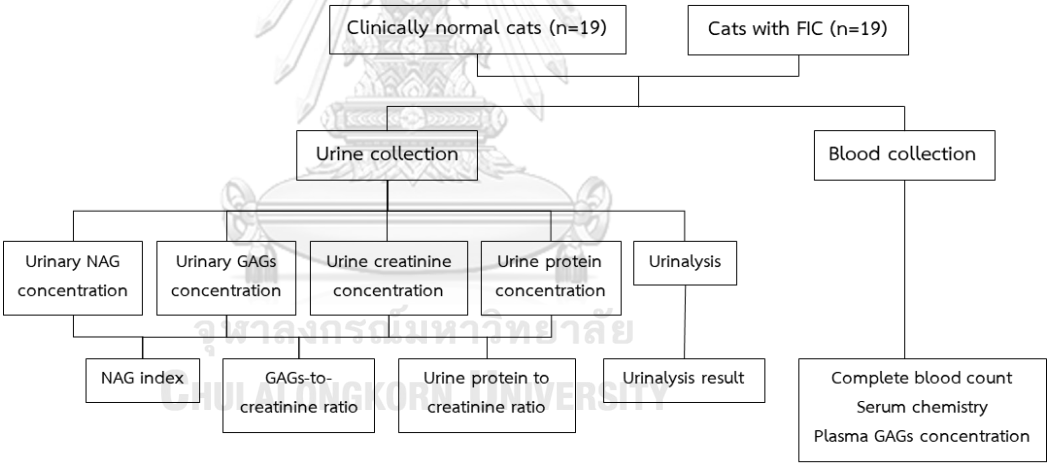


Fig. 5 Study designs

3.3. Samples collection

3.3.1. Blood samples collection

Blood samples were obtained by saphenous or cephalic venipuncture (3 ml) and collected into anticoagulant (ethylenediaminetetraacetic acid; EDTA and lithium heparin) including 2 aliquots of EDTA-containing tubes and 1 aliquot of lithium heparin-containing tubes for determining CBC and serum chemistry measurement. The remaining of aliquot blood sample in EDTA tube were centrifuged at 700 x g for 10 minutes for plasma obtained and stored at -80 °C for GAGs concentration analysis (Jepson et al., 2010).

3.3.2. Urine sample collection

The 5-10 ml of urine samples were obtained by sterile urinary catheterization from each clinically normal cats and cats with FIC. Urine samples were centrifuged at RCF 1500 x g for 5 minutes and separated the supernatant. The 4 aliquots of supernatants were stored at -80 °C for further analysis including urine protein quantification, urine creatinine quantification, NAG and GAGs analysis.

3.4. Clinical examination

The blood samples were transported to the Pathology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University for complete blood count (CBC) and serum chemistry measurement. The CBC were measured by automated blood count (Cell-Dyn® 3700) from plasma in EDTA-containing tube. The creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) alkaline phosphatase (ALP), total protein and albumin were measured by automated clinical analyzer (ILab650).

Standard urinalysis was performed immediately in all cases by commercial urine dipstick analysis (Combur9® test) for analysis pH, protein, glucose, ketone, bilirubin, leukocyte and erythrocyte. The urine specific gravity was measured by using a refractometer (Heska®) and microscopic examination of the sediment (native samples and samples stained with methylene blue). The 1 ml of urine samples were kept in 3 ml sterile syringe and submitted to

the Pathology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University to determine the quantitative urine bacteriology (Minimum Inhibitory Concentration; MIC). The cut point for positive urine bacterial culture (catheterized urine) in cats was > 10^3 CFU/ml (Ettinger and Feldman, 2009).

3.5. Laboratory examination

3.5.1. Plasma GAGs concentration

3.5.1.1. Extraction and purification of plasma GAGs

The extraction and purification of plasma GAGs were performed modified by Pereira et al. (2004). Blood samples (1 ml) were collected and kept in EDTA-containing tube. The plasma was obtained from centrifugation and hold in alkaline condition.

1. An equal volume of 0.5M NaOH was added into each sample and kept at 37°C for 12 hours for cleave the O-linkage between protein and carbohydrate to release the GAGs chains from proteoglycans.
2. The GAGs chains in sulfated form were isolated by using ion exchange chromatography on Q Sepharose Fast Flow in chloride form.
3. The column was washed with 5-10 ml of 0.3M NaCl, and the GAGs chains were eluted by 1M NaCl (five 1 ml fractions).
4. The 5 ml of elution fractions were kept in polystylene tube for analyzed the plasma GAGs quantification (Pereira et al., 2004).

3.5.1.2. Plasma GAGs quantification

The plasma GAGs concentrations were quantified by spectrophotometric with DMB reported by Farndale et al. (1986). The method was wide accepted as a quick and simple method for measuring the sulfated GAGs in tissue and fluid sample.

1. The color reagent prepared by dissolving 1.6 mg dimethylene blue in 100 ml distilled water containing 0.304 g glycine, 0.237 g NaCl and 9.5 ml 0.1M HCl.
2. The 1 ml color reagent mixed with 42 µl of each plasma in eppendorf tube.
3. The absorbance measured after 5 minutes of mixing at wavelength 525 nm by using spectrophotometer with semimicro cuvette.

The assay was calibrated by using reagent blanks and the standard curve was prepared by using chondroitin 4-sulphate sodium salt derived from bovine tracheas (calibration interval, 0 to 100 mg/L). The results were reported as plasma GAGs concentration in µg/ml (Farndale et al., 1986).

3.5.2. Urinary GAGs concentration

3.5.2.1 Extraction and purification of urinary GAGs

The extraction and purification of urinary GAGs was performed, according to the method reported by Panchaphanpong et al. (2011)

1. Urine samples are diluted with distilled water in ratio 1:1. 1M HCl was added to each sample for adjusted pH of 4.0 to 4.5.
2. An equal volume of Cetyltrimethylammonium bromide was added to each sample and incubate at 4°C for 24 hours.
3. Each of the samples was centrifuged and collected the precipitate. Washing precipitate 2 times with ethanol, drying at 37 °C and dissolving in 0.1M NaOH 0.5 ml.

3.5.2.2 Urinary GAGs quantification

The urinary GAGs concentrations were quantified by spectrophotometric with DMB reported by Panin et al. (1986). This method was simple, rapid, precise, and sensitive method for measuring urinary glycosaminoglycan sulfate excretion.

1. The color reagent prepared by dissolving 1.6 mg dimethylene blue in 150 ml distilled water containing 0.2 g sodium formate, 0.5 ml 95% ethanol and 0.2 ml formic acid.
2. One ml of color reagent mixed with urine 40 µl and added distilled water 160 µl to adjust a total volume of 1.2 ml.
3. The absorbance measured after 5 minutes of mixing at wavelength 525 nm by using spectrophotometer with semimicro cuvette.

The standard curve was prepared by using chondroitin 4-sulphate sodium salt derived from bovine tracheas (calibration interval 0 to 100 mg/L). The results were corrected with the amount of creatinine and express as both urinary GAGs concentration (µg/ml) and the GAGs-to-creatinine ratio ($x10^{-3}$)

3.5.3. Urinary NAG quantification

Urinary NAG activity was measured by using commercially calorimetric assay, according to Yakata et al. (1983). The substrate, 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminidase, was hydrolyzed by NAG to produce 3-cresol-sulfonphthalein (3-cresol purple) and N-acetyl-glucosamine. The assay was performed in accordance with the manufacturer's instructions for the microassay by use of half volumes. The urine samples that had not previously been subjected to a freeze-thaw cycle were used for assay validation.

1. Put 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminidase 500 µl in test tube at 37 °C for 5 minutes for incubation.

2. Urine samples 25 µl were added in the same test tube and incubated at 37°C for 15 minutes.
3. Sodium carbonate as alkaline stopping buffer was add in each test tube for stop reaction.
4. The absorbance measured after mixing and leaving for 10 minutes at wavelength 580 nm.

The urinary NAG concentrations were calculated by using reference value. The standard curve was prepared with lyophilized NAG enzyme. The results would be reported as urinary NAG activity (U/L) and NAG index (U/g). The NAG index was the ratio of urinary NAG concentration to grams of urine creatinine.

3.5.4. Urine protein quantitation

Urinary protein was measured by the Coomassie blue method, according to Bradford method (colorimetric method). The changing of Coomassie Brilliant Blue G-250 dye depended on the concentration protein in urine sample at acidic condition.

1. Mixed the Coomassie blue dye with distilled water at the ratio 1:4. The diluted dye reagent was filtrated by filter paper (Whatmann no.1).
2. The five dilutions of Bovine Serum Albumin (BSA) standard were prepared and test for the linear range standard.
3. Added the distilled water into the urine sample to make diluted urine sample at the ratio 1:20.
4. The diluted dye reagent 1,000 µl was added in test tube. Put the diluted urine sample 20 µl in each test tube and mixed with vortex.
5. The absorbance was measured after mixing 5 minutes at wavelength 595 nm.

The urine protein concentrations were calculated by using the slope of protein standard (mg/ml) and the equation which shown in Fig. 6 Finally, the results were corrected with the amount of creatinine and expressed as urine protein to creatinine ratio; UPC (Fig. 7) (Bradford, 1976).

$$\text{Urine protein concentration (mg/ml)} = \frac{OD_{sample}}{\text{Slope of protein standard}} \times \text{Dilution factor}$$

OD_{sample} = Optical density of sample

Fig. 6 The equation for calculation the urine protein concentration

$$UPC = \frac{\text{Urinary protein concentration}}{\text{Urinary creatinine concentration}} \times 100$$

Fig. 7 The equation for calculation the urine protein to creatinine ratio; UPC

3.5.5. Urinary creatinine quantitation

Urinary creatinine was measured by the Alkaline picrate method (colorimetric method) using the HUMAN Diagnostic kit, according to Jaffé (1886). The changing of orange-red colour depended on the complex of picric acid and creatinine in urine sample in alkaline solution. The absorbance of this complex was proportional to the creatinine concentration in the sample.

1. To prepare the working reagent, the distilled water was added to NaOH in the ratio 4:1 (distilled water : NaOH 4:1)
2. An equal volume of picric acid was added to the diluted NaOH (picric acid: diluted NaOH 1:1) to make the working reagent.

3. Put 1,000 µl of working reagent in each diluted urine sample (urine: distilled water = 1: 49) and creatinine standard and vortex.
4. After 30 second, read the absorbance at wavelength 490 nm as A_1, leave 2 minute and read the absorbance at the same wavelength as A_2 .
5. The creatinine concentration could be calculated with the formula shown in Fig. 8 and expressed in urine creatinine (mg/dl).

$$\text{Urine creatinine concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times 100$$

$\Delta A = A_2 - A_1$

A_1 = Absorbance after 30 second

A_2 = Absorbance after 2 minute

Fig. 8 The equation for calculation the urine creatinine concentration

3.6. Statistical analysis

The descriptive statistics was used for the signalment and urinalysis results. The results were presented as frequencies of occurrence, reported in percentages for categorical variables and mean ± standard error of mean (SEM) for continuous variables. Chi-square test was used to determine the significant association between factors and the development of FIC. Fisher's exact test was used when expected value was small frequency. Odds ratio (OR) and 95% confidence intervals (95% CI) was used to measure the association of risk factors and developing of FIC.

To measure the quantitative data including urine protein, urine protein to creatinine ratio, urine creatinine, plasma GAGs concentration, urinary GAGs concentration, GAGs-to-creatinine ratio, urinary NAG concentration and NAG index, normality of the distribution of data and homogeneity of variances was

assessed by using Shapiro-Wilk test and Levene test respectively. *P* values of quantitative data between cats with FIC and the clinically normal cats were calculated by using the Paired-t test for normally distributed and the Wilcoxon Signed Rank test for non-normally distributed. The relationship of the variables between the clinically normal cats and cats with FIC was performed by using the Pearson's correlation. Univariable analyses were used to compare the possible risk factor such as the cats' characteristic, the cats' environment, type of food and management of the cats' litter box between clinically normal cats and cats with FIC.

For statistical analysis, all data were analyzed by SPSS Statistics version 16.0 program. Differences were considered significant when $p < 0.05$.

CHAPTER IV
RESULTS

4.1. Study population and signalment

A total of thirty eight cats met the inclusion criteria for this study. There were randomly allocated into 2 groups consisted of 19 cats with FIC and 19 clinically normal cats. Both cats with FIC and the control groups were matched for gender and age. Odds ratio (OR), 95% confidence interval (CI) and chi-square of signalment were listed (Table 2). Mean±SEM age of clinically normal cats group and cats with FIC group were 4.6 ± 0.6 (median, 4.0 years; range, 11.0 years). Domestic shorthair (78.9%; 30/38) was the most prevalence breed in this study. There were 15.8% of Persian (6/38) and 5.3% (2/38) of other breeds (Scottish fold and mixed breed) (Fig. 9). However, the Persian breed was not represented in the control group (Fig. 10). Reproductive status of these cats were 36.8% (14/38) intact male, 47.4% (18/38) castrated male, 5.3% (2/38) intact female and 10.5% (4/38) sterile female (Fig. 11). Castrated male were mostly found with idiopathic cystitis (Fig. 12). Cats weighing more than four kilograms tend to had higher risk of developing FIC (OR= 2.98, 95% CI 0.789-11.248) than cats weighing one to four kilograms (Table 2). Most of cats in this study having BCS ≤ 3/5 (76.3%; 29/38) (Fig. 13). Cats having BCS more than 3/5 tend to had higher risk of developing FIC (OR = 4.96, 95% CI 0.873-28.152) than cats having lower BCS. The BCS of 3/5 and lower seem to be the protective factor for FIC (OR = 0.20, 95% CI 0.360-1.145) (Table 2). In addition, there were six longhaired-cats (31.6%) in FIC group (Fig. 15). Longhaired-cats tend to have higher risk for developing FIC (OR= 8.31, 95% CI 0.890-77.568) than the shorthaired-cats (OR= 0.12; 95% CI 0.013-1.124) (Table 2). There were eighteen shorthaired-cats (94.7.0%) in normal group (Fig. 15). Domestic shorthair cats (OR= 0.09; 95% CI 0.010-0.876) were protective factors for FIC (Table 2).

The nineteen clinically normal cats consist of 18 domestic shorthair cats (94.7%) and 1 Scottish fold cat (5.3%) (Fig. 10). Mean±SEM weight was 4.22 ±

0.15 kg (median, 4.00 kg; range, 2.60 kg). Seventeen cats had BCS ≤ 3 (89.5%) (Fig. 14). There were 9 sexually intact males (47.4%), 2 sexually intact females (10.5%), 7 castrated males (36.8%) and 1 spayed female (5.2%) (Fig. 12). The physical examination, hematology and urinalysis result of these cats were all normal and the hematologic values were within reference ranges.

Nineteen cats with FIC consist of 12 domestic shorthair cats (63.2%), 6 Persian cats (31.6%) and 1 mixed breed cat (5.3%) (Fig. 10). Mean ± SEM weight was 4.89±0.27 kg (median, 4.50 kg; range, 4.10 kg). Seven cats had BCS > 3 (36.8 %) (Fig. 14). There were 5 sexually intact males (26.3%), 11 castrated males (57.9%) and 3 spayed females (15.8%). The majority of cats with FIC were male castrated (Fig. 12).

Table 2 Odds ratio (OR), 95% confidence interval (CI) and chi-square of weight, body condition score, breed, reproductive status and coat length in clinically normal cats and cats with FIC

Characteristic	No. of clinically normal cat n/N (%)	No. of FIC cats n/N (%)	OR	95% CI	Chi-square	*P* value
Weight						
1-4 kg	11/19 (57.9%)	6/19 (31.6%)	0.34	0.089-1.267	2.661	0.103
>4 kg	8/19 (42.1%)	13/19 (69.4%)	2.98	0.789-11.248	2.661	0.103
Body condition score						
BCS ≤ 3/5*	17/19 (89.5%)	12/19 (63.15%)	0.20	0.360-1.145	3.640	0.062
BCS > 3/5*	2/19 (10.5%)	7/19 (36.85%)	4.96	0.873-28.152	3.640	0.062
Breed						
DSH*	18/19 (94.7%)	12/19 (63.2%)	0.09	0.010-0.876	5.700	0.021
Persian*	0/19 (0.0%)	6/19 (31.5%)	ND	ND	ND	ND
Other breeds*	1/19 (5.3%)	1/19 (5.3%)	1.00	0.058-17.249	0.000	1.000
Reproductive status						
Intact male	9/19 (47.4%)	5/19 (26.3%)	0.40	0.102-1.548	1.810	0.179
Castrated male	7/19 (36.8%)	11/19 (57.9%)	2.36	0.640-8.667	1.689	0.194
Intact female*	2/19 (10.5%)	0/19 (0.0%)	ND	ND	ND	ND
Sterile female*	1/19 (5.3%)	3/19 (15.8%)	3.38	0.318-35.789	1.118	0.604
Coat length						
Short hair*	18/19 (94.7%)	13/19 (68.4%)	0.12	0.013-1.124	4.378	0.045
Long hair*	1/19 (5.3%)	6/19 (31.6%)	8.31	0.890-77.568	4.378	0.045

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

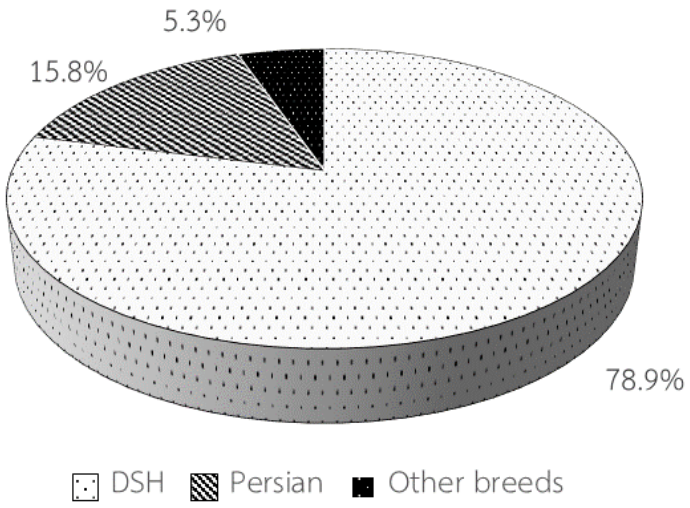


Fig. 9 Percentage of cats breed (total number of cats = 38)

DSH = domestic shorthair, other breed = American shorthair or Scottish fold

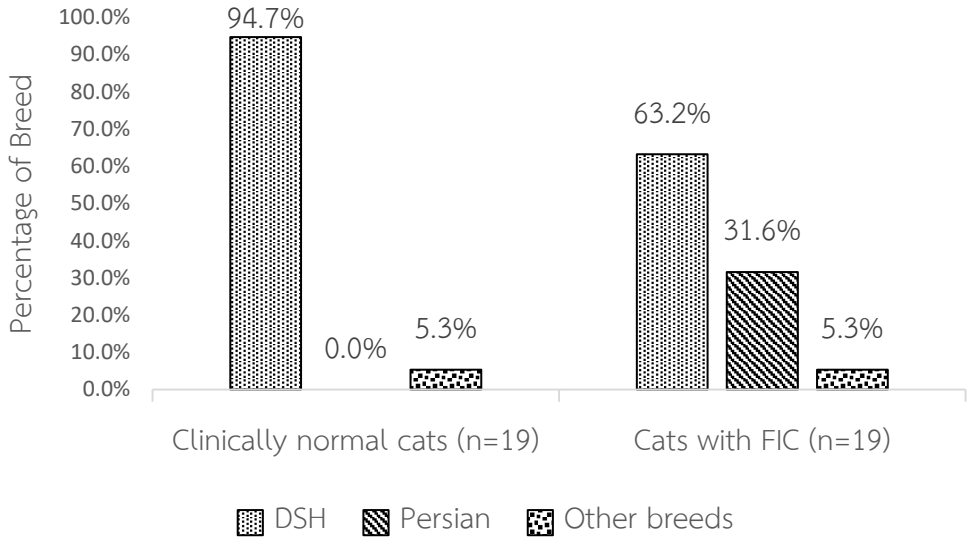


Fig. 10 Percentage of cats breed according to different groups

DSH = domestic shorthair, other breed = mixed breed or Scottish fold

n = number of cats in each group

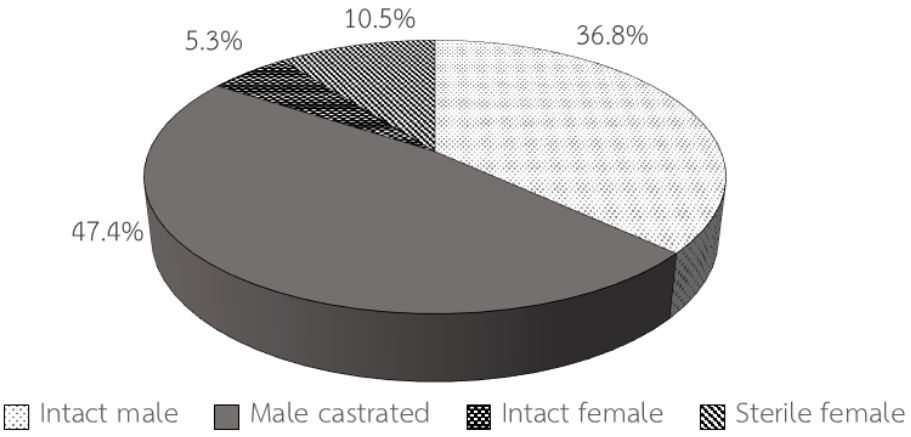


Fig. 11 Percentage of reproductive status (total number of cats = 38)

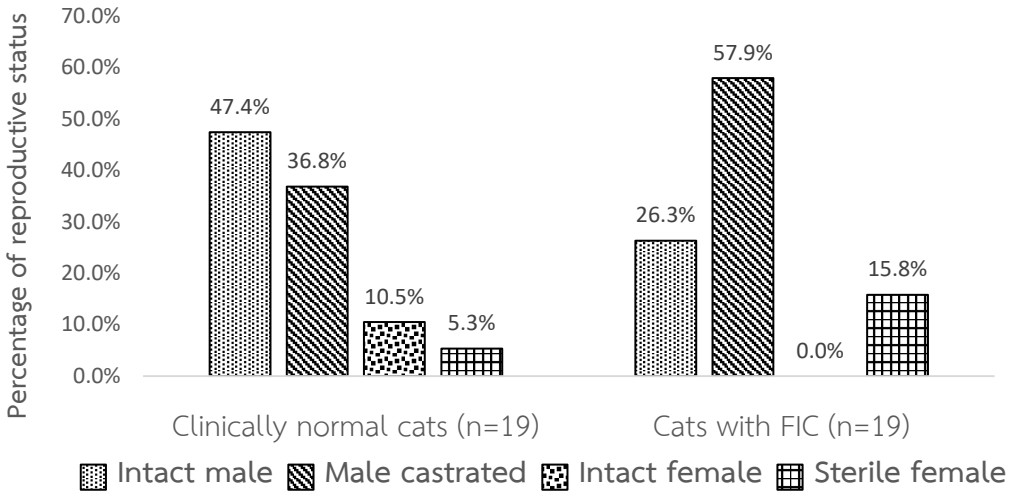


Fig. 12 Percentage of reproductive status of cats according to different groups
n = number of cats in each group

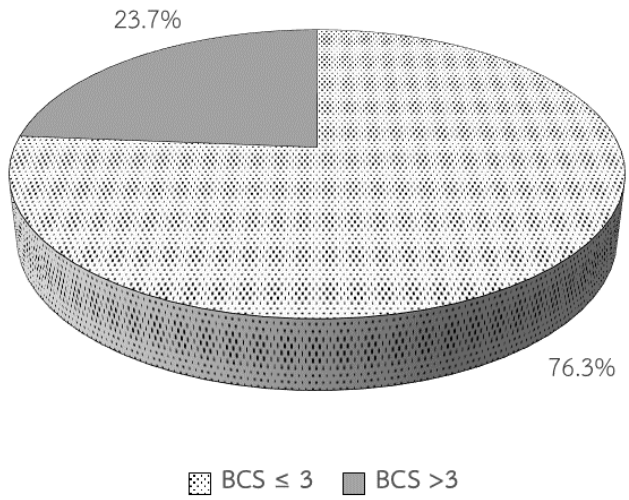


Fig. 13 Percentage of body condition score (total number of cats = 38)

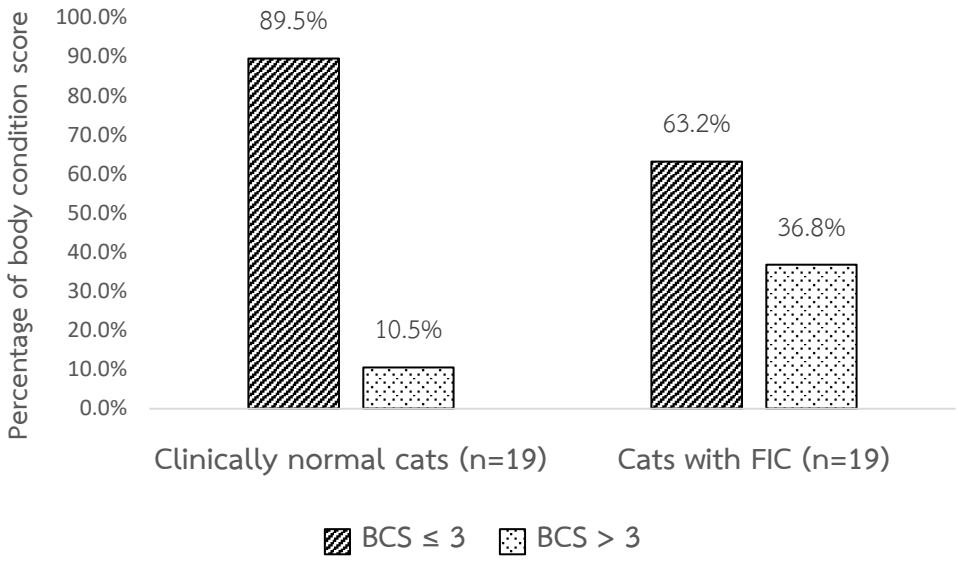


Fig. 14 Percentage of body condition score of cats according to different groups

n = number of cats in each group

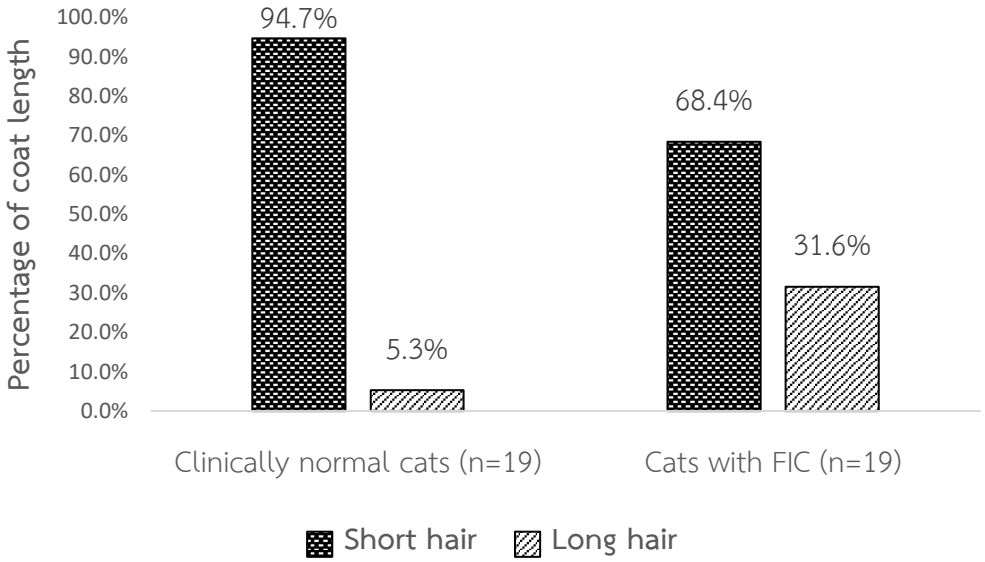


Fig. 15 Percentage of coat length of cats according to different groups

n = number of cats in each group

4.2. Clinical presentation of cats with FIC

The FIC group showed the abnormalities signs of lower urinary tract. Thirteeen cats (68.4%) suffered from stranguria, 5 cats (26.3%) displayed the sign of pollakiuria and 1 cat (5.3%) were reported to have urinate in appropriate places (periuria). The percentage of clinical presentations was displayed as Fig. 16. The clinically normal cats did not show any abnormalities in voiding urine.

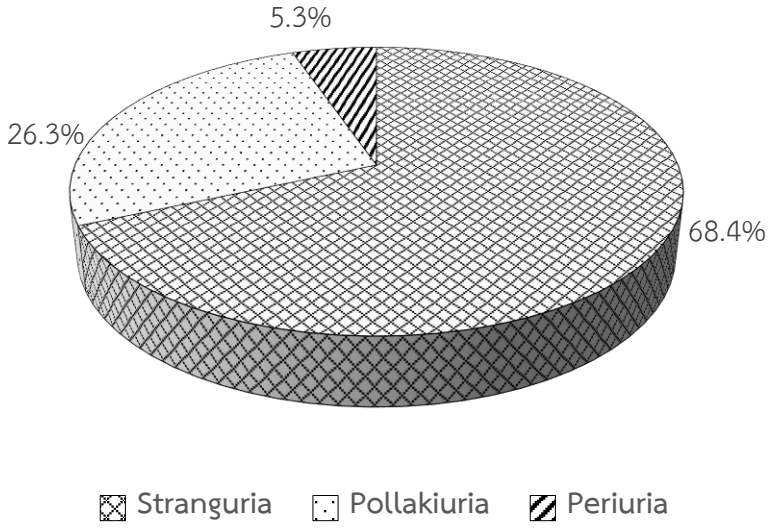


Fig. 16 Percentage of clinical signs in cats with FIC (total number of cats = 19)

4.3. Possible risk factors of cats with FIC

The overview of the univariable analyses results of the cats' characteristics were listed (Table 3). Six cats with FIC (31.6%) have a low playful activity while 8 cats (42.1%) and 5 cats (26.3%) with FIC have average (OR = 1.02; 95% CI 0.015-2.480) and high (OR = 3.04; 95% CI 0.509-18.108) playful activity respectively. Ten cats with FIC (52.6%) were described as having nervous and fearful behavior (OR = 3.11; 95% CI 0.797-12.140) and tend to have the recessive status (OR = 2.52; 95% CI 0.646-9.833) whereas in clinically normal cats have no conflict with other cats or dogs in the same household.

Table 3 Univariable analyses comparing between the clinically normal cats and cats with FIC. Part 1: the cats' characteristics

Characteristic	Alternative	No. of clinically normal cat n/N (%)	No. of FIC cats n/N (%)	OR	95% CI	*P* value
Degree of playful activity	Low	0/19 (0.0%)	6/19 (31.6%)	ND	ND	ND
	Average	17/19 (89.5%)	8/19 (42.1%)	1.02	0.015-2.480	0.090
	High	2/19 (10.5%)	5/19 (26.3%)	3.04	0.509-18.108	0.405
Aggressive behaviour	Yes	5/19 (26.3%)	3/19 (15.8%)	0.53	0.106-2.603	0.693
	No	14/19 (73.7%)	16/19 (84.2%)			
Nervous and fearful behaviour	Yes	5/19 (26.3%)	10/19 (52.6%)	3.11	0.797-12.140	0.092
	No	14/19 (73.7%)	9/19 (47.4%)			
Dominant status	Yes	5/19 (26.3%)	3/19 (15.8%)	0.53	0.106-2.603	0.693
	No	14/19 (73.70%)	16/19 (84.2%)			
Recessive status	Yes	5/19 (26.3%)	10/19 (52.6%)	2.52	0.646-9.833	0.313
	No	14/19 (73.7%)	9/19 (47.4%)			

n = number of cats in each group; N = total number of the clinically normal cats and cats with FIC

Seventeen cats with FIC (89.5%) live with other pets in the same household (OR = 4.25; 95% CI 0.729-24.769). Fifteen cats with FIC (78.9%) were more likely to live strictly indoors (OR = 2.19; 95% CI 0.516-9.271). None of the differences was detected between cats with FIC and the clinically normal cats when evaluating the cats' environment (Table 4).

Table 4 Univariable analyses comparing between the clinically normal cats and cats with FIC. Part 2: the cats' environment

Characteristic	Alternative	No. of clinically normal cat n/N (%)	No. of FIC cats n/N (%)	OR	95% CI	*P* value
Living style	Indoor	12/19 (63.2%)	15/19 (78.9%)	2.19	0.516-9.271	0.476
	Outdoor	1/19 (5.3%)	0/19 (0.0%)	ND	ND	ND
	Indoor and outdoor	6/19 (31.5%)	4/19 (21.1%)	0.58	0.133-2.505	0.715
Other animals in household	Single pet	6/19 (31.6%)	2/19 (10.5%)	0.26	0.044-1.475	0.232
	Live with other pet	13/19 (68.4%)	17/19 (89.5%)	4.25	0.729-24.769	0.124
Cat in the same household	Yes	12/19 (63.2%)	16/19 (84.2%)	3.11	0.663-14.596	0.269
	No	7/19 (36.8%)	3/19 (15.8%)			
Neighbouring cat can access	Yes	4/19 (21.1%)	4/19 (21.1%)	1.00	0.210-4.758	1.000
	No	15/19 (78.9%)	15/19 (78.9%)			
Fighting with neighbouring cat	Yes	2/19 (10.5%)	4/19 (21.1%)	2.27	0.362-14.185	0.660
	No	17/19 (89.5%)	15/19 (78.9%)			

n = number of cats in each group; N = total number of the clinically normal cats and cats with FIC

Seventeen cats with FIC (89.5%) were significantly more likely to use a litter box (OR = 14.57; 95% CI 2.566-82.732). In addition, ten cats with FIC (58.8%) did not receive the adequate litter box in their home (OR = 1.07; 95% CI 0.180-6.363). None of the differences was detected between cats with FIC and the clinically normal cats regarding type of litter substrate and size of the litter box. Eleven cats with FIC (57.9%) and 10 clinically normal cats (52.6%) were predominantly fed a commercial dry food (OR = 1.24; 95% CI 0.344-4.454) (Table 5).

Table 5 Univariable analyses comparing between the clinically normal cats and cats with FIC. Part 3: management of the cats' litter box and type of food

Characteristic	Alternative	No. of clinically normal cat n/N (%)	No. of FIC cats n/N (%)	OR	95% CI	*P* value
Use the litter box	Yes	7/19 (36.8%)	17/19 (89.5%)	14.57	2.566-82.732	0.001
	No	12/19 (63.2%)	2/19 (10.5%)			
Number of litter box	litter box = cat	3/7 (42.8%)	5/17 (29.4%)	0.56	0.090-3.445	0.647
	litter box > cat	0/7 (0.0%)	2/17 (11.8%)	ND	ND	ND
	litter box < cat	4/7 (57.2%)	10/17 (58.8%)	1.07	0.180-6.363	0.939
Type of litter substrate	Cat sand	6/7 (85.8%)	13/17 (76.5%)	0.54	0.049-5.943	0.612
	Litter pellet	0/7 (0.0%)	4/17 (23.5%)	ND	ND	ND
	Other	1/7 (14.2%)	0/17 (0.0%)	ND	ND	ND
Type of food	Commercial dry food	10/19 (52.6%)	11/19 (57.9%)	1.24	0.344-4.454	0.744
	Commercial canned food	1/19 (5.3%)	0/19 (0.0%)	ND	ND	ND
	Combination	7/19 (36.8%)	8/19 (42.1%)	1.00	0.276-3.625	1.000
	Homemade	1/19 (5.3%)	0/19 (0.0%)	ND	ND	ND

n = number of cats in each group characteristic; N = total number of clinically normal cats and cats with FIC

4.4. Blood analysis

The results of hematocrit, WBC count, BUN and serum creatinine values of the cats with FIC and the clinically normal cats were reported as mean±SEM (Table 6). All clinically normal cats had blood profile within the normal reference range. Cats with FIC had significantly higher serum creatinine (5.57±1.54 mg/dl) and blood urea nitrogen (63.73±14.76 mg/dl) than the clinically normal cats (1.49±0.07 and 26.61±0.82, respectively) ($p < 0.05$). Six cats with FIC had azotemia at the first presentation (Cr. > 1.6 md/dl and BUN > 35 mg/dl) which resolved after 3 days of unblocking the urethra and treating with intravenous crystalloid fluids (acetated Ringer's solution or saline 0.9%).

Table 6 Mean±SEM of blood profile in cats with FIC and the clinically normal cats.

Parameter	Normal value	Clinically normal cats		Cats with FIC	
		Value	n	Value	n
Hematocrit (%)	29.20-51.70	39.05 ± 1.06	19	39.32 ± 1.43	19
RBC count ($\times 10^6$ cell/µl)	5.24-10.89	8.74 ± 0.24	19	8.43 ± 0.31	19
WBC count ($\times 10^3$ cell/µl)	4.20-17.50	13.03 ± 0.98	19	16.58 ± 2.49	19
BUN (mg/dl)	15.00-35.00	26.61 ± 0.82	19	63.73 ± 14.76[a]	19
sCr. (mg/dl)	<1.6	1.49 ± 0.07	19	5.57 ± 1.54[a]	19

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. RBC = red blood cells; WBC = white blood cell; BUN = blood urea nitrogen; sCr = serum creatinine; n = number of cats in each group;

[a] $p < 0.05$ when compared with the clinically normal cats

4.5. Urinalysis

All clinically normal cats and thirteen cats with FIC (68.4%) had urine specific gravity more than 1.035. Other Six cats with FIC (31.6%) had urine specific gravity between 1.013-1.034 due to the post renal azotemia (Table 7). Most cats with FIC and the clinically normal cats had urine pH 6 accounting for 52.6% and 47.4% respectively (Fig. 17). Most cats with FIC had two plus (47.4%) and three plus (26.3%) level of dipstick protein reaction while all of the clinically normal cats had normal level (negative – 1+) of dipstick protein reaction in urine samples (Fig. 18). The WBC, RBC and amount and type of crystal were analyzed and counted by microscopic examination. Eleven clinically normal cats (57.9%) had no WBC in urine sample and other eight cats remaining (42.1%) had 1-5 WBC per high power field in urine sample. Eleven cats (57.9%), one cats (5.3%) and three cats (15.8%) with FIC were reported 1-5 WBC, 6-10 WBC and 20-30 WBC per high power field, respectively while four cats (21.1%) were reported no WBC in urine samples (Fig. 19). Most cats with FIC (42.1%) had too numerous to count RBC in the urine samples while most of the clinically normal cats (78.9%) had no RBC in urine samples (Fig. 20). Struvite crystals was predominantly found in cats with FIC and the clinically normal cats but the amount of crystals that found in the normal group (1+ to 2+) was less than FIC group (1+ to 4+) (Table 8).

Table 7 Urine specific gravity in the clinically normal cats and cats with FIC

Parameter	Clinically normal cats		Cats with FIC	
	Value	n/N (%)	Value	n/N (%)
Urine specific gravity	>1.050	12/19 (63.1%)	>1.050	7/19 (36.8%)
	1.048	4/19 (21.1%)	1.045	1/19 (5.3%)
	1.045	2/19 (10.5%)	1.040	1/19 (5.3%)
	1.040	1/19 (5.3%)	1.035	4/19 (21.0%)
			1.033	3/19 (15.7%)
			1.030	1/19 (5.3%)
			1.020	1/19 (5.3%)
			1.016	1/19 (5.3%)

n = number of cats in each group; N = total number of the clinically normal cats and cats with FIC

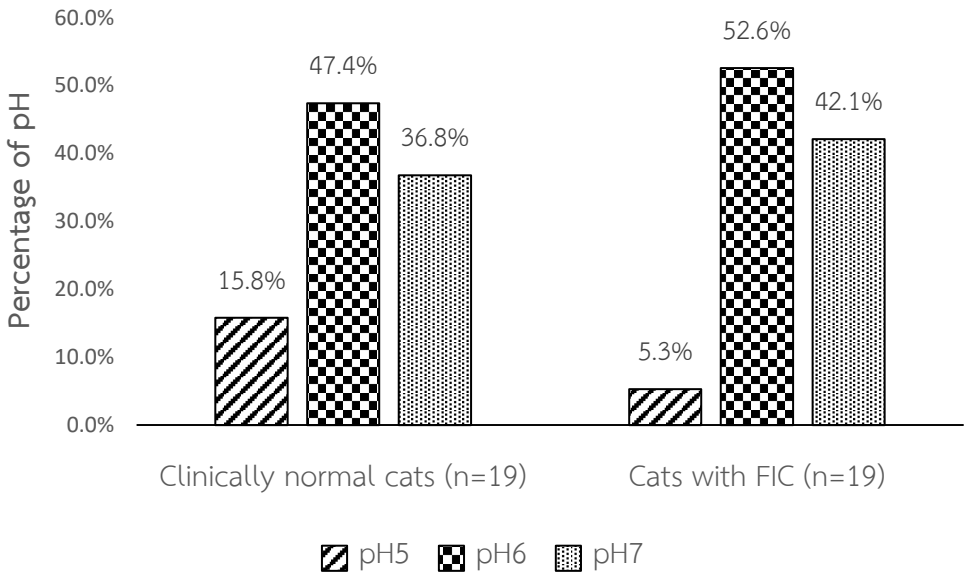


Fig. 17 Percentage of urine pH according to different groups

n = number of cats in each group

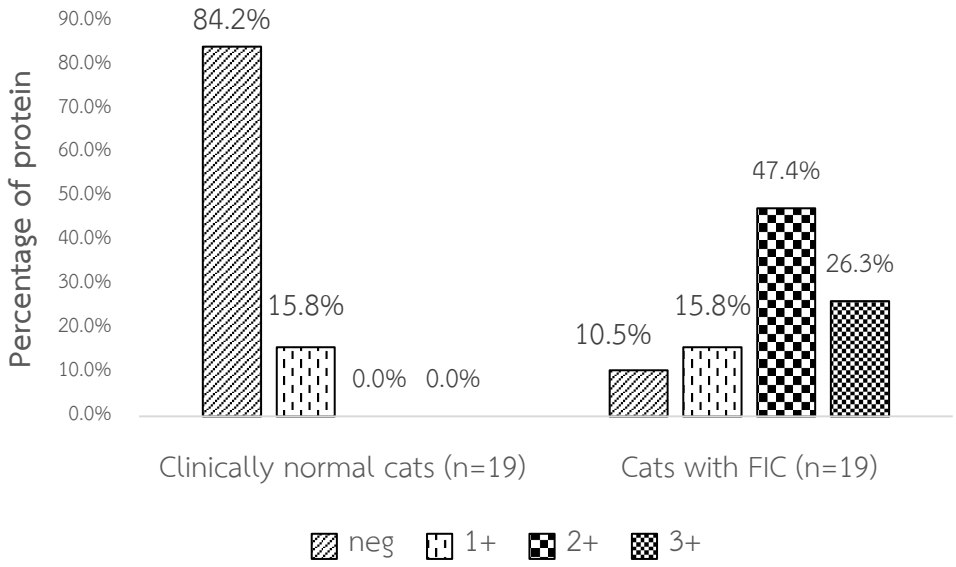


Fig. 18 Percentage of protein in urine samples using commercial strip test according to different groups

n = number of cats in each group

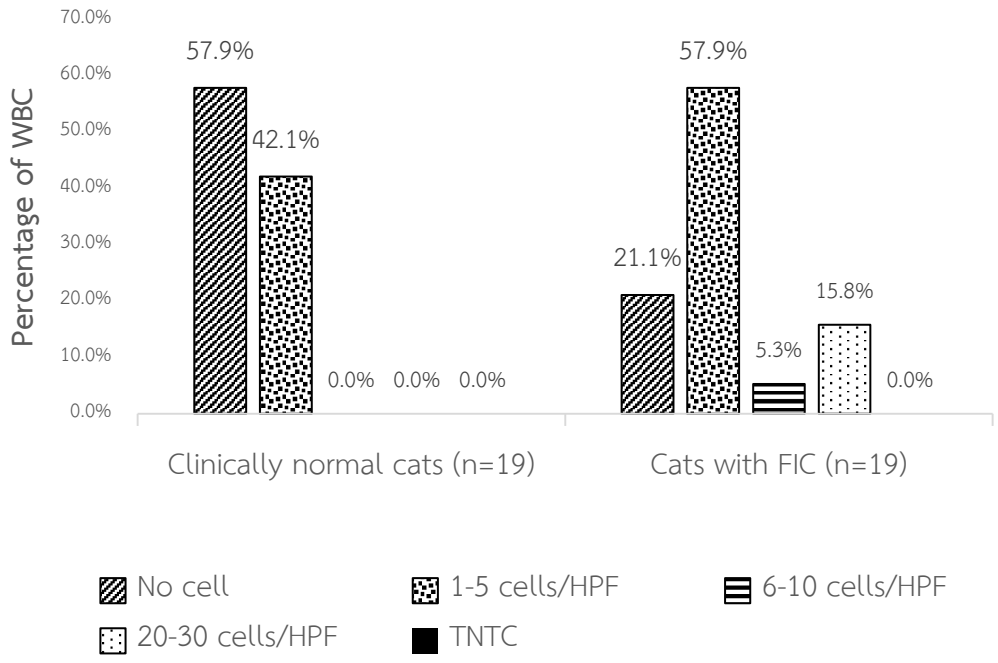


Fig. 19 Percentage of WBC in the urine samples using microscopic examination according to different groups

n = number of cats in each group; TNTC = Too numerous to count

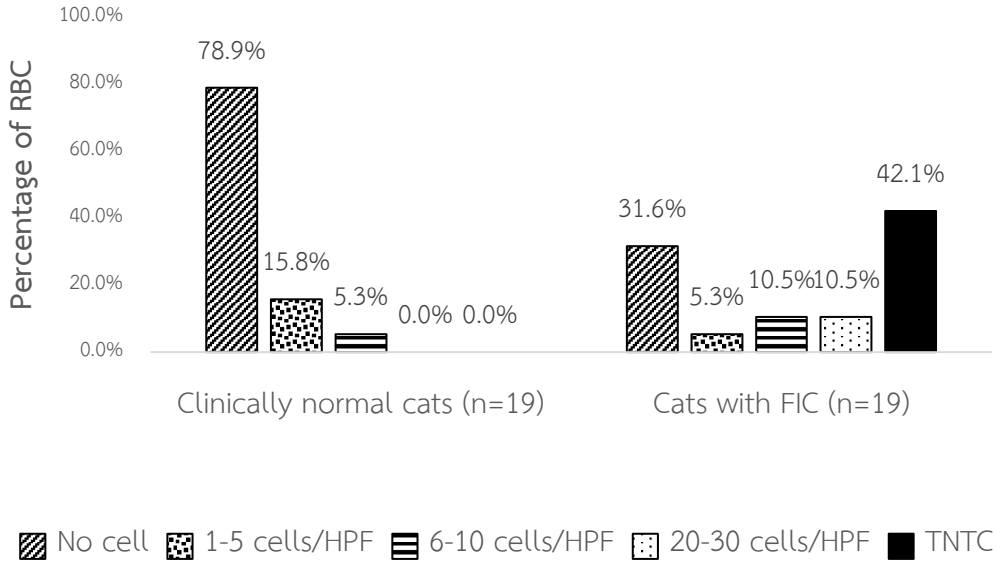


Fig. 20 Percentage of RBC in the urine samples using microscopic examination according to different groups

n = number of cats in each group; TNTC = Too numerous to count

Table 8 Amount and type of crystals in urine sediment in the clinically normal cats and cats with FIC

Parameter	Clinically normal cats		Cats with FIC	
	Value	**n/N (%)**	**Value**	**n/N (%)**
Amount and type of crystals in sediment	Struvite 2+	4/19 (21.1%)	Struvite 4+	1/19 (5.3%)
	Struvite 1+	2/19 (10.4%)	Struvite 3+	2/19 (10.5%)
	Calcium oxalate 2+	1/19 (5.3%)	Struvite 2+	1/19 (5.3%)
	Negative	12/19 (63.2%)	Struvite 1+	3/19 (15.7%)
			Calcium oxalate 1+	1/19 (5.3%)
			Negative	11/19 (57.9%)

n = number of cats in each group; N = total number of the clinically normal cats and cats with FIC

4.6. Urinary glycosaminoglycans and plasma glycosaminoglycans

The results of urinary GAGs and plasma GAGs level were listed (Table 9). Mean±SEM of urinary GAGs concentration in cats with FIC and the clinically normal cats were 10.59±1.12 μg/ml and 15.84±1.93 μg/ml respectively. Although the mean±SEM of urinary GAGs in cats with FIC was significantly lower than in the clinically normal cats ($p < 0.05$), the GAGs-to-creatinine ratio in cats with FIC (3.84±0.52) and clinically normal cats (4.52±0.76) were not different statistically significant. Moreover, Mean±SEM of plasma GAGs concentrations in the clinically normal cats (42.76±1.19 μg/ml) and cats with FIC (39.23±1.39 μg/ml) were not significantly different.

Table 9 Mean±SEM of Plasma GAGs, Urinary GAGs and GAGs-to-creatinine in the clinically normal cats and cats with FIC

Parameter	Clinically normal cats		Cats with FIC	
	Value	n	Value	n
Plasma GAGs (μg/ml)	42.76 ± 1.19	19	39.23 ± 1.39	19
Urinary GAGs (μg/ml)	15.84 ± 1.93	19	10.59 ± 1.12[a]	19
GAGs-to-creatinine ($x10^3$)	4.52 ± 0.76	19	3.84 ± 0.52	19

GAGs = Glycosaminoglycans; n = number of cats in each group

[a] $p < 0.05$ when compared with clinically normal cats

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

The result of UPC and NAG index were listed (Table 10). The urine protein of cats with FIC (405.81±87.33 mg/dl) was significantly higher than the clinically normal cats (91.84±13.85 mg/dl) ($p < 0.01$). The results of urine protein were concordantly with the UPC result, the UPC of cats with FIC (1.93±0.54) was statistically higher than the clinically normal cats (0.22±0.02) ($p < 0.01$). Mean±SEM of urinary NAG activity in cats with FIC (5.85±1.34 U/L) was higher than the clinically normal cats (3.48±0.67 U/L). Moreover, the NAG index of cats with FIC (2.36±0.69 U/g) was statistically higher than the clinically normal cats (1.00±0.21 U/g) ($p < 0.05$).

Table 10 Mean±SEM of urine protein, urine creatinine, UPC, urinary NAG and NAG index in the clinically normal cats and cats with FIC.

Parameter	Clinically normal cats		Cats with FIC	
	Value	n	Value	n
Urine protein (mg/dl)	91.84 ± 13.85	19	405.81 ± 87.33[b]	19
Urine creatinine (mg/dl)	394.22 ± 27.27	19	346.29 ± 45.39	19
UPC	0.22 ± 0.02	19	1.93 ± 0.54[b]	19
Urinary NAG Activity (U/L)	3.48 ± 0.67	19	5.85 ± 1.34	19
NAG index (U/g)	1.00 ± 0.21	19	2.36 ± 0.69[a]	19

UPC = Urine protein to creatinine ratio; NAG = N-acetyl-β-D-glucosaminidase; n = number of cats in each group

[a] $p < 0.05$ when compared with the clinically normal cats

[b] $p < 0.01$ when compared with the clinically normal cats

4.8. The relationship of the variables between the clinically normal cats and cats with FIC

The relationship between the UPC and NAG index, urinary NAG and urinary GAGs, NAG index and GAGs-to-creatinine ratio in the clinically normal cats and cats with FIC were listed (Table 11). The UPC and NAG index presented the significant moderate positive correlation ($r = 0.511$, $p < 0.05$) in FIC group (Fig. 21). None of the differences was detected between other variables.

Table 11 Relationship between UPC and NAG index, NAG and urinary GAGs, NAG index and GAGs to Cr. ratio in the clinically normal cats and cats with FIC

Group	Relationship	Pearson's Correlation	Significant (2-tailed)
Clinically normal cats	UPC and NAG index	0.276	0.252
	NAG and Urinary GAGs	-0.378	0.110
	NAG index and GAGs to Cr. ratio	0.094	0.701
Cats with FIC	UPC and NAG index	0.511	0.026*
	NAG and Urinary GAGs	0.123	0.615
	NAG index and GAGs to Cr. ratio	0.077	0.754

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

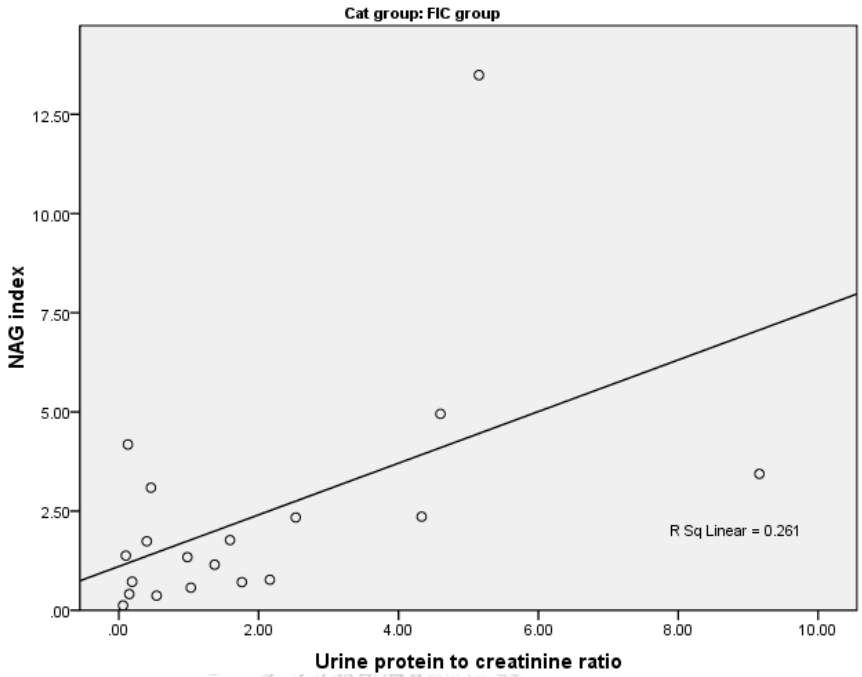


Fig. 21 Scatter plot of the Pearson's correlation between Urine protein to creatinine ratio (UPC) and NAG index in cats with FIC *(r = 0.512, p < 0.05)*

CHAPTER V

Discussion

5.1. Study population and signalment

Cats weighing more than four kilograms and having BCS > 3/5 tend to have higher risk for developing FIC in this study. It is possible that overweight condition may put the cats at risk of FIC as well as reported in the previous studies (Gerber et al., 2008; Defauw et al., 2011; Panchaphanpong et al., 2011; Lund et al., 2015; Panboon et al., 2017). Overweight cats might have the low daily activity and inappropriate voiding behaviour resulting in abnormalities of lower urinary tract.

Castrated male cats were the most prevalences gender in cats with FIC group who might play a role as a risk factor for developing FIC (OR = 2.36, 95% CI 0.640-8.667). Male gender or neutered status were reported to be the factors for developing urethral obstruction (Segev et al., 2011) and often lead to FLUTD (Lekcharoensuk et al., 2001) particularly FIC. However, when consider FIC group in this study, the values were not significantly more to provoke FIC (Pereira et al., 2004; Gerber et al., 2008; Defauw et al., 2011; Lemberger et al., 2011a; Panchaphanpong et al., 2011; Lund et al., 2015; Panboon et al., 2017). The recent study reported that castration affected the density of elastic fiber and collagen fiber in corpus spongiosum of penile extracellular matrix lead to decreased the compliance around the urethra region in domestic cats (Borges et al., 2017). Castration or testosterone deprivation seem to be associated with lower urinary tract signs in rat (Cheng and de Groat, 2016). In human, the painful bladder syndrome (PBS) or human IC has been widely reported in women particularly around the menstrual cycle (Bjorling and Wang, 2001) and estrogen might be the one factor predisposed PBS and human IC (Imamov et al., 2007). However, up to date there was no evidence about the effect of estrogen in cats with FLUTD or FIC. Therefore, the role of sex hormone and FIC remain to be investigated.

In this study, the predominant breed of FIC and the normal group were DSH. DSH breed was the most popular breed in Thailand. However, there were no Persian breed in the clinically normal cats group, therefore, odds ratio could

not be calculated. It's might be speculated that the main population cats brought to the clinic for vaccination or neutering were mostly DSH breed resulting in constituent the large proportion in normal group. Besides, DSH seem to be the most prevalent breed of cats with FIC in several study (Defauw et al., 2011; Panchaphanpong et al., 2011; Lund et al., 2015; Panboon et al., 2017). The previous study suggested that purebred was not a risk factor for FLUTD (Segev et al., 2011) and FIC (Defauw et al., 2011). Nevertheless, some study suggested that Persian cats were postulated to be the risk factor for FLUTD (Lekcharoensuk et al., 2001).

The longhaired-cats had significantly increased risk for developing FIC in this study (OR = 8.31; 95% CI 0.890-77.568). The main longhaired-cats breed in the FIC group were Persian. On the contrary, there was only one study reported about the coat-length in cats with FIC, the longhaired trait was not relate to pathogenesis of FIC (Defauw et al., 2011).

5.2. Clinical presentation of cats with FIC

The clinical presentation of cats with FIC in this study were stranguria, pollakiuria and periuria (Fig. 16). The most clinical sign of cats with FIC was reported to be stranguria (Defauw et al., 2011). Some cats with FIC in this study had complete urethral obstruction and severe stranguria resulting in systemic clinical sign consistent with metabolic derangement (Segev et al., 2011). Previous study also reported severe anemia condition associated with urinary bladder hemorrhage in cats with FIC (Beer and Drobatz, 2016). This severe anemia condition might occur in cats with chronic and recurrent FIC resulting in subtle urinary bladder wall and vascular fragile. On the contrary, there were no cats with severe anemia condition in this study. Most of cats with FIC have acute and/or the first episode of FIC. In addition, there was one study investigated about the post-obstructive diuresis (POD) condition in cats naturally occurring lower urinary tract obstruction. The POD could be defined as urine output > 2 ml/kg/hr and occurred in most FLUTD case after unblock the obstruction (Frohlich et al., 2016). In this study, the POD could not detected in cats with FIC.

5.3. Possible risk factors of cats with FIC

Several studies try to investigate the risk factors of cats with FIC. They postulated that nervous and fearful behavior were more likely to be the specific characteristics for FIC cats (Defauw et al., 2011; Lund et al., 2015). In this study, most cats with FIC tend to have nervous and fearful behavior. However, it was not statistically difference (p = 0.102) (Table 3). Cats with FIC were reported to have the neuroendocrine imbalance (Buffington and Pacak, 2001; Buffington et al., 2002; Westropp et al., 2006) due to mild decreased in adrenal gland size (Westropp et al., 2003) which resulting in more sensitive to stressful situation (Hague et al., 2013). Another factor, multimodal environment modification (MEMO) or decreasing stress environment were also reported to be the adjunctive therapy for cats with FIC (Buffington et al., 2006).

It was well recognized that litter box might be the potential factor for developing FIC. Cats with FIC were significantly more likely to use a litter box (p = 0.001) while the clinically normal cats were less likely to use a litter box (Table 5) concordant with the previous study (Defauw et al., 2011). Inappropriate litter box management seem to affect some cats with FIC which lead to abnormal voiding behavior such as infrequent urination.

5.4. Blood analysis

RBC, WBC and HCT in clinically normal cats and cats with FIC were within normal limit (Table 6). Serum creatinine and BUN in cats with FIC were significant higher than clinically normal cats at the time of first presentation (Table 6). Cats with FIC having severe stranguria might develop the post-renal azotemia condition. When the urethral obstruction occurred, the hydrostatic pressure within the urinary system will be increased from bladder and ascended to the Bowman's space resulting in decreased the GFR and accumulation the nitrogenous waste in blood circulation (Segev et al., 2011). Post-renal azotemina of cats with FIC were resolved after the relieved the urethral obstruction and given the intravenous fluid therapy.

5.5. Urinalysis

In this study, most cats with FIC had USG more than 1.035 (Table 7). The normal renal function or concentrated urine could be considered in these cats. The remaining cats with FIC had urine specific gravity lower than 1.035 (Table 7) which may be due to the concurrent with the post-renal azotemia condition (Table 6). On the contrary, all clinically normal cats had urine specific gravity higher than 1.035 (Table 7). Age and dietary moisture content could also affect the USG resulting low USG without abnormal urinary system (Rishniw and Bicalho, 2015). High USG from the lower water intake might be the potentially factor for developing FIC (Lund et al., 2013).

Many cats in FIC and normal group had mild acidic urine (pH ≤ 6) (Fig. 17) concordant with the previous study (Lund et al., 2013). Although the major results of urine WBC in FIC group were 1-5 cells/hpf (Fig. 19), all of cats with FIC had bacterial count < 10^3 CFU determined as negative urine culture. The presenting of WBC could not indicate the bacteriuria in cats (Swenson et al., 2011). Conversely, the higher pH with presenting of WBC in urine samples partly associated with bacteriuria lead to urinary tract infection in cats (Litster et al., 2009). In this study, the presenting of WBC with negative urine culture confirmed the idiopathic condition in cats with FIC.

Hematuria was the one of the clinical sign found mostly in cats with FLUTD. It was occurred due to the inflammation and high pressure in the bladder resulting in bladder hemorrhage (Segev et al., 2011). The interpretation of urine dipstick might incorrect, the darker urine color of cats with FIC could interfere the color of dipstick. Cats with FIC mainly found too numerous to count RBC presenting in urine as in the current study (Fig. 20). These finding might imply severity of urethral obstruction in FIC group. The recent study suggested that darker red urine color and severity of metabolic derangement were positive correlated (Brabson et al., 2015).

Interestingly, the results of protein in FIC group on urine dipstick mainly illustrated two plus and three plus level. These finding indicated that cats with FIC tend to have proteinuria condition (Fig. 18), the same trend as observed in previous studies (Panchaphanpong et al., 2011; Treutlein et al., 2013; Panboon et al., 2017) except only one study (Buffington et al., 1996). Hematuria might

be one of the cause for increasing urinary protein in cats with FLUTD (Pereira et al., 2004).

The presence of crystal in urine can be found in both cats with FIC and clinically normal cats group. Cats with FIC (3/19; 15.8%) tend to have a large amount of crystal (3+ to 4+) while the clinically normal cats (6/19; 31.5%) reported only a small amount of crystal (1+ to 2+) (Table 8). The presence of crystal in urine can be considered no clinical significant in clinically normal healthy cats but in cats with lower urinary tract signs (Archer, 2005). Conversely, one study reported about a large amount of crystals presumed to be struvite crystals and suggested that crystal may be associated in cats with FIC (Bell and Lulich, 2015).

5.6. Plasma glycosaminoglycans and urinary glycosaminoglycans

Plasma GAGs levels in cats with FIC was lower than the clinically normal cats but was not statistical significant (p = 0.09) (Table 9). It was possible that plasma GAGs and urinary GAGs were heterogeneous, most of urinary GAGs were unlikely to originate from serum (Bower et al., 1992) and some fraction of plasma GAGs can excrete directly into urine as urinary GAGs (Endo et al., 1979). The altered plasma GAGs were reported in juvenile patients with idiopathic arthritis (Winsz-Szczotka et al., 2015), adult patients with critically respiratory failure (Schmidt et al., 2014) and upper urinary tract diseases (Bower et al., 1992). However, the study of plasma GAGs in patients with lower urinary tract disease in other species was rarely published. To our knowledge, there had been only two studies investigated the plasma GAGs in cats with FLUTD (Pereira et al., 2004) and FIC (Panchaphanpong et al., 2011).

In cats with FIC group, urinary GAGs were statistical significant lowered than the clinically normal group. Although the GAG-to-creatinine ratio was lower in the cats with FIC compared to the clinically normal cats, this difference was not significant (p = 0.77). The GAG-to-creatinine results were consistent with urinary GAGs but not significant due to the lower value of urinary creatinine in cats with FIC than clinically normal cats (Table 9). The low urinary creatinine in cats with FIC was reported in several study (Buffington et al., 1996; Pereira et al., 2004; Panchaphanpong et al., 2011; Panboon et al., 2017). Similarly to the previous study, decreased GAG-to-creatinine ratio can be detected in cats with

FLUTD (Pereira et al., 2004) and FIC (Buffington et al., 1996; Panchaphanpong et al., 2011).

In human, urinary GAGs excretion was relatively high in childhood, decreased in adults and relatively increased again in old age (Manley et al., 1968). Several study suggested that urinary GAGs excretion can be a biomarker for screening many diseases such as Mucopolysaccharidoses (Tanyalcin, 2015b) and bladder carcinoma (Hennessey et al., 1981). As previously stated, The decreased urinary GAGs excretion has been demonstrated in patients with lower urinary tract diseases such as PBS/IC (Lucon et al., 2014), idiopathic detrusor overactivity (Siracusano et al., 2009) and also in cats with FIC (Buffington et al., 1996) but the mechanism was not precisely described (Lucon et al., 2014). The urinary GAGs might be the important factor reflecting the bladder urothelial damaged lead to the abnormalities micturition in human with PBS/IC and may occur in cats with FIC.

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

UPC in cats with FIC (1.93 ± 0.54) was statistically higher than the clinically normal cats (0.22 ± 0.02) ($p < 0.01$) (Table 10). The UPC above 0.4 was considered to be elevated and fall into proteinuria condition (Harley and Langston, 2012). Interestingly, this study was consistent with previous studies that cats with FIC tend to have the proteinuria condition (Panchaphanpong et al., 2011; Treutlein et al., 2013; Panboon et al., 2017) . Since the electrophoretic pattern in plasma proteins and urine proteins were not different, hematuria condition might be the cause of elevated urine protein in cats with FLUTD (Pereira et al., 2004). Increased bladder layer permeability was considered to be the important mechanism of plasma leakage during the inflammation process resulting in urethral plug formation (Westropp and Buffington, 2004). Likewise, the elevated UPC possibly detected simultaneously. Nevertheless, all of these findings could not indicate the mechanism of protein leakage into urine during the course of disease. These unknown mechanism might be the interesting part of pathophysiology part of FIC.

At the present time, there had several studies investigated the biomarker for urinary tract diseases. One protein which considered as interesting biomarker for upper urinary tract diseases was NAG. In healthy

human, low amount of urinary NAG excretion could be detected due to the physiological exocytosis in proximal tubular cells (Navarro et al., 2003). In pathological condition of human kidney, NAG index might rise due to increased protein presented to the proximal tubular cells lead to increased lysosomal turn over (Bosomworth et al., 1999) and proximal tubular cell damaged (Sato et al., 2002). NAG index was higher in cases of patients with renal tubular diseases (Ali et al., 2014) associated with diabetes mellitus or diabetic nephropathy (Ellis et al., 1983; Bouvet et al., 2014; Sheira et al., 2015). Moreover, it has been suggested that the increased NAG index associated in patient with proteinuria particularly albuminuria (Sheira et al., 2015).

In addition, there were several study of higher NAG index in cats with upper urinary tract diseases (Sato et al., 2002; Jepson et al., 2010). NAG index might be potentially biomarker for chronic kidney disease in cats with newly diagnosed hyperthyroidism (Lapointe et al., 2008). However, the study of NAG index in cats with FLUTD was scarce. To author's knowledge, there was only one study investigated the NAG index in cats with FIC (Panboon et al., 2017). Similarly to this study, NAG index in the urine of cats with FIC (2.36 ± 0.69 U/g) were statistically higher when compared to clinically normal cats (1.00 ± 0.21 U/g) ($p < 0.05$) (Table 10). Higher NAG index could be detected in cats with FIC particularly in cats having proteinuria condition. It was possibly that there had some mechanism related to the upper urinary tract occurred prior to the lower urinary tract abnormality which may be due to stress and pain. Conversely, the higher NAG index might happen during the post-renal azotemia as a result of acute proximal tubular cells damaged.

5.8. The relationship of the variables between clinically normal cats and cats with FIC

In this study, the increased of UPC and NAG index in cats with FIC demonstrated a significant moderate positive correlation (r = 0.511, $p < 0.05$) (Table 11; Fig. 21). These result indicated that the increased in NAG index might play a role of a biomarker for progressive idiopathic cystitis, particularly in cats with proteinuria condition, concordant with the recent study (Panboon et al., 2017). None of the correlation of other variables was detected (Table 11). The defective GAGs layer in cats with FIC resulting in decreased urinary

GAGs excretion and GAGs-to-creatinine ratio might relate to the increased lysosomal enzyme such as NAG from the kidney.

Although age and gender of cats were matched for exclusion the important confounder factors, the important limitation of the current study was the number of cats in FIC and normal group. A larger group of cat in future study may be need and to detect more statistically significant differences. Moreover, our study was not performed the bladder biopsy to evaluate the bladder histological lesions and the decreased urinary GAGs excretion could not be confirmed the damaged GAGs layer in the bladder structure in cats with FIC.

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APPENDICES

Appendix 1 Questionnaire1

<u>ส่วนที่1: สำหรับสัตวแพทย์</u>

ส่วนที่1 ข้อมูลเบื้องต้นของเจ้าของและสัตว์เลี้ยง

Hospital.....HN.....วันที่บันทึกข้อมูล.....

Breed ☐ DSH ☐ Persian ☐ Other .....

Coat length ☐ long hair ☐ short hair BCS...../5

ชื่อเจ้าของ..... เบอร์ติดต่อ.....

ชื่อสัตว์เลี้ยง..... น้ำหนัก.....Kg

วันเกิดสัตว์เลี้ยง..... อายุ.....ปี เพศ.....

ข้อมูลการทำหมัน ☐ทำหมันแล้ว ☐ ไม่ได้ทำหมัน

ประวัติวัคซีน ฉีดกระตุ้นเป็นประจำทุกปี ไม่ทราบแน่ชัด

ชนิดวัคซีน ☐ หัด-หวัด ☐พิษสุนัขบ้า ☐ มะเร็งเม็ดเลือดขาว ☐อื่นๆ.....

<u>ส่วนที่2-4 สำหรับเจ้าของสัตว์</u>

ส่วนที่2 ลักษณะนิสัยของสัตว์เลี้ยง

ระดับความอยากเล่นและทำกิจกรรม ☐ ต่ำ ☐ ปานกลาง ☐ สูง

มีพฤติกรรมดุร้ายและชอบขู่ตัวอื่น ☐ ใช่ ☐ ไม่ใช่

มีพฤติกรรมขี้กลัวและชอบหลบซ่อน ☐ ใช่ ☐ ไม่ใช่

มีพฤติกรรมเป็นแมวจ่าฝูง ☐ ใช่ ☐ ไม่ใช่

มีพฤติกรรมเป็นแมวที่โดนข่มขู่ ☐ ใช่ ☐ ไม่ใช่

Appendix 1 Questionnaire2

ส่วนที่3 สิ่งแวดล้อมของสัตว์และลักษณะการเลี้ยง

สัตว์เลี้ยงอื่นในบ้าน ☐ เลี้ยงตัวเดียว ☐ เลี้ยงรวมกับ ☐ สุนัข.....ตัว

☐ แมว.....ตัว

ลักษณะการเลี้ยง ☐ เลี้ยงในบ้าน ☐ เลี้ยงนอกบ้าน ☐ เลี้ยงใน+นอกบ้าน

แมวจรสามารถเข้ามาในบริเวณบ้านได้ ☐ ใช่ ☐ ไม่ใช่

มีการต่อสู้กับแมวจรนอกบ้านเสมอ ☐ ใช่ ☐ ไม่ใช่

ส่วนที่4 ชนิดของอาหารและสถานที่ขับถ่าย

ชนิดอาหาร ☐ เม็ด ☐ เปียก ☐ ผสมกัน (เม็ด+เปียก) ยี่ห้อ.....

☐ ปรุงเอง (Homemade)

สถานที่ขับถ่าย ☐ ไม่ใช้กระบะทราย ☐ ใช้กระบะทราย

<u>หากใช้กระบะทรายกรุณาตอบคำถามด้านล่างนี้</u>

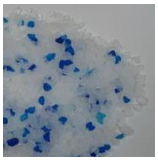
จำนวนกระบะทราย ☐ เทียบเท่าจำนวนแมวที่เลี้ยง

☐ มีมากกว่าจำนวนแมวที่เลี้ยง

☐ มีน้อยกว่าจำนวนแมวที่เลี้ยง

ชนิดของทรายแมว ☐ ทรายแมวทั่วไป ☐ ทรายแมวที่ย่อยสลายได้ ☐ อื่นๆ.....

(ขี้เลื่อย เปลือกไม้ ข้าวบาร์เลย์)



ขนาดของกระบะทราย ☐ เล็กกว่าตัวแมว ☐ ใหญ่กว่าตัวแมว

Appendix 2 Signalment of cats with FIC

FIC No.	Code	Name	Age (years)	Weight (Kg)	BCS (/5)	Breed	Sex	Coat length
1	F01	Brownie	7	4.5	3	DSH	Mc	short
2	F02	Nual	6	3.7	3	DSH	Fs	short
3	F03	Yam	12	5.6	5	DSH	Fs	short
4	F04	Davis	7	6.5	4	DSH	M	short
5	F05	Jingjok	6	4.6	3	Persian	M	long
6	F06	Butter	1.5	3.7	3	Persian	M	long
7	F07	City	3	3.2	2	Persian	Mc	long
8	F08	Nil	3	4.5	3	DSH	Mc	short
9	F09	Tubtim	6	5	3	DSH	Fs	short
10	F10	Junjao1	1.5	4.5	3	DSH	Mc	short
11	F11	Tiger	2	4.4	3	Mixed	Mc	short
12	F12	Nhon	3	5	4	DSH	Mc	short
13	F13	Latte	4	4	3	Persian	Mc	long
14	F14	Junjao2	4	7.3	5	Persian	Mc	long
15	F15	Mee-ngoen	1	3.8	3	Persian	M	long
16	F16	Jaokai	7	7	4	DSH	Mc	short
17	F17	Hero	2.5	5.6	4	DSH	M	short
18	F18	Fiao	3	4	3	DSH	Mc	short
19	F19	Taro	8	6	4	DSH	Mc	short

BCS = Body condition score, DSH = Domestic shorthair, M = Intact male, Mc = Castrated male, F = Intact female, Fs = Sterile female

Appendix 3 Signalment of clinically normal cats

Control No.	Code	Name	Age (years)	Weight (Kg)	BCS (/5)	Breed	Sex	Coat length
1	C01	Plamuek	7	5.3	3	DSH	Mc	short
2	C02	Kwangtung	6	4.3	3	DSH	F	short
3	C03	Kaotok	12	4	3	DSH	F	short
4	C04	Ngua	7	3.5	3	DSH	M	short
5	C05	Auan	6	4.8	4	DSH	M	short
6	C06	Kumsap	1.5	3.9	3	DSH	M	short
7	C07	Richie	3	4.7	3	DSH	Mc	short
8	C08	Million	3	6	4	DSH	Mc	short
9	C09	Nguea-sue	6	3.6	3	DSH	Fs	short
10	C10	Tiger	1.5	4.4	3	Scottish fold	M	short
11	C11	Moowan	2	3.9	3	DSH	M	short
12	C12	Imboon	3	3.8	3	DSH	Mc	short
13	C13	Khingkhing	4	3.5	3	DSH	Mc	short
14	C14	Tuaploo	4	4.8	3	DSH	Mc	short
15	C15	Nongtong	1	4	3	DSH	M	short
16	C16	Hengheng	7	4.8	3	DSH	M	short
17	C17	Numchoke	2.5	4	3	DSH	M	short
18	C18	Kwak-ngoen	3	3.6	3	DSH	M	short
19	C19	Jeeda	8	3.4	3	DSH	M	short

BCS = Body condition score, DSH = Domestic shorthair, M = Intact male, Mc = Castrated male, F = Intact female, Fs = Sterile female

Appendix 4 Complete blood count and serum chemistry values of cats with FIC

FIC No.	Code	RBC ($x10^6$ cell/µl)	Hct (%)	WBC ($x10^3$ cell/µl)	sCr (mg/dl)	BUN (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	F01	5.8	30	19.1	13.95	180	25	30
2	F02	9.85	41.1	7.17	1.8	26.9	49	31
3	F03	10.1	54.6	12	2.5	25.1	48	18
4	F04	8.29	43.5	13.3	1.6	19.4	62	26
5	F05	7.77	33.1	7.66	1.2	38.5	33	21
6	F06	8.86	36.7	13.2	10	138.8	77	23
7	F07	9.57	42.3	6.83	1.7	22	48	30
8	F08	9.34	43.3	15	1.8	25	75	29
9	F09	9.52	41.8	7.08	1.4	22	43	60
10	F10	8.26	43.6	17.6	1.7	31.5	63	40
11	F11	8.49	34.5	16.5	1.6	13.9	54	33
12	F12	7.53	30.7	38.5	14.4	135.1	20	13
13	F13	2.46	10.5	36.4	15.3	161.8	36	9
14	F14	9.35	44	5.35	2	23.3	51	22
15	F15	9.12	35.6	25.7	4.8	67.9	51	24
16	F16	8.3	40.4	39.18	24.6	206.7	36	11
17	F17	10.12	43.5	13.22	1.7	24.1	34	24
18	F18	6.51	34.8	6.96	2	25.9	57	11
19	F19	7.96	43.1	14.39	1.9	23.1	37	16

RBC = Red blood cell, Hct = Hematocrit, WBC = White blood cell, sCr = Serum creatinine, BUN = Blood urea nitrogen, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase

Appendix 5 Complete blood count and serum chemistry values of clinically normal cats

Control No.	Code	RBC ($x10^6$ cell/µl)	Hct (%)	WBC ($x10^3$ cell/µl)	sCr (mg/dl)	BUN (mg/dl)	ALT (IU/L)	ALP (IU/L)
1	C01	6.95	35.7	11.02	2.3	26.4	62	44
2	C02	10.02	49.2	8.3	1.7	27.3	52	28
3	C03	8.68	36.6	16.9	1.5	27.3	50	41
4	C04	8.54	37.1	9.72	1.8	31.5	59	19
5	C05	7.23	37.1	11.81	1.4	28.8	92	18
6	C06	8.42	37.4	20.3	1.2	22.7	102	94
7	C07	9.95	39.4	10.63	1.3	30.4	31	74
8	C08	8.94	39.4	11.07	1.7	29.7	41	36
9	C09	8.21	39.7	7.99	1.5	21.7	49	20
10	C10	8.72	34.3	17.79	1	25.7	37	17
11	C11	10.44	44.5	14.11	1.1	22.4	2	7
12	C12	9.12	37	9.55	1.5	21.3	43	133
13	C13	9.1	44.1	14.47	1.9	26.6	39	34
14	C14	9.25	42	21.11	1.4	27.7	41	61
15	C15	10.11	43.9	10.61	1.5	23	53	96
16	C16	8.2	35.4	8.91	1.8	35.2	45	49
17	C17	7.19	33.7	9.18	1.1	28.1	53	43
18	C18	9.89	48.6	19.75	1.2	24.8	87	38
19	C19	7.22	35.5	14.32	1.5	25.3	49	45

RBC = Red blood cell, Hct = Hematocrit, WBC = White blood cell, sCr = Serum creatinine, BUN = Blood urea nitrogen, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase

Appendix 6 Urinalysis values of cats with FIC

FIC No.	Code	uProtein	USG	pH	uWBC (cells/hpf)	uRBC (cells/hpf)	Crystal
1	F01	2+	1.035	6	6-10	TNTC	Neg
2	F02	Neg	1.040	6	1-5	Neg	Neg
3	F03	1+	1.016	6	Neg	1-5	CaOX 1+
4	F04	3+	1.050	6	20-30	6-10	Neg
5	F05	2+	1.050	7	1-5	TNTC	Neg
6	F06	2+	1.035	7	Neg	TNTC	Neg
7	F07	2+	1.050	6	1-5	20-30	Neg
8	F08	1+	1.050	5	Neg	Neg	Neg
9	F09	3+	1.050	7	1-5	Neg	Struvite1+
10	F10	2+	1.035	6	20-30	20-30	Struvite3+
11	F11	2+	1.035	6	1-5	Neg	Neg
12	F12	3+	1.033	6	Neg	TNTC	Neg
13	F13	2+	1.030	7	1-5	TNTC	Struvite 1+
14	F14	2+	1.050	7	1-5	TNTC	Struvite 1+
15	F15	2+	1.033	7	1-5	TNTC	Struvite 3+
16	F16	3+	1.020	6	20-30	TNTC	Neg
17	F17	1+	1.035	6	1-5	Neg	Neg
18	F18	Neg	1.050	7	1-5	Neg	Struvite 2+
19	F19	3+	1.045	7	1-5	6-10	Struvite 4+

uProtein = urine protein, USG = Urine specific gravity, uWBC = White blood cell in urine sample, Neg = Negative, uRBC = Red blood cell in urine sample, CaOX = Calcium oxalate

Appendix 7 Urinalysis values of clinically normal cats

Control No.	Code	uProtein	USG	pH	uWBC (cells/hpf)	uRBC (cells/hpf)	Crystal
1	C01	Neg	1.050	7	1-5	Neg	Neg
2	C02	Neg	1.050	6	Neg	Neg	Neg
3	C03	Neg	1.045	5	Neg	Neg	CaOX 3+
4	C04	Neg	1.050	6	Neg	Neg	Neg
5	C05	Neg	1.048	5	Neg	Neg	Neg
6	C06	Neg	1.050	6	1-5	Neg	Neg
7	C07	1+	1.050	6	1-5	Neg	Neg
8	C08	Neg	1.048	6	Neg	1-5	Neg
9	C09	Neg	1.045	6	1-5	1-5	Neg
10	C10	1+	1.050	6	1-5	Neg	Neg
11	C11	Neg	1.050	7	Neg	Neg	Struvite3+
12	C12	Neg	1.040	7	Neg	Neg	Struvite3+
13	C13	Neg	1.055	6	Neg	Neg	Neg
14	C14	1+	1.050	7	1-5	6-10	Struvite1+
15	C15	Neg	1.050	6	1-5	Neg	Neg
16	C16	Neg	1.048	5	Neg	1-5	Neg
17	C17	Neg	1.050	7	Neg	Neg	Struvite 3+
18	C18	Neg	1.050	7	Neg	Neg	Struvite 3+
19	C19	Neg	1.048	7	1-5	Neg	Struvite 1+

uProtein = urine protein, USG = Urine specific gravity, uWBC = White blood cell in urine sample, Neg = Negative, uRBC = Red blood cell in urine sample, CaOX = Calcium oxalate

Appendix 8 The cat's characteristics (FIC group)

FIC No.	Code	Activity	Aggressive	Nervous	Dominant	Recessive
1	F01	Average	No	Yes	No	Yes
2	F02	Low	Yes	No	Yes	No
3	F03	Low	No	Yes	No	Yes
4	F04	Average	No	No	No	No
5	F05	Average	No	No	No	No
6	F06	High	No	Yes	No	Yes
7	F07	High	No	Yes	No	Yes
8	F08	Average	No	Yes	No	Yes
9	F09	Low	No	Yes	No	Yes
10	F10	High	No	No	No	No
11	F11	Low	No	Yes	No	Yes
12	F12	Average	Yes	No	Yes	No
13	F13	Low	No	Yes	No	Yes
14	F14	Low	No	No	No	No
15	F15	Average	No	No	No	No
16	F16	High	No	No	No	No
17	F17	High	No	Yes	No	Yes
18	F18	Average	No	Yes	No	No
19	F19	Average	Yes	No	Yes	No

Activity = Degree of playful activity, Aggressive = Aggressive behavior, Nervous = Nervous behavior, Dominant = Dominant status, Recessive = Recessive status

Appendix 9 The cat's characteristics (Normal group)

Control No.	Code	Activity	Aggressive	Nervous	Dominant	Recessive
1	C01	Average	Yes	No	Yes	No
2	C02	Average	No	Yes	No	Yes
3	C03	Average	No	No	No	No
4	C04	High	Yes	No	Yes	No
5	C05	Average	No	No	No	No
6	C06	Average	No	No	No	No
7	C07	Average	Yes	Yes	Yes	Yes
8	C08	Average	Yes	Yes	Yes	Yes
9	C09	Average	No	No	No	No
10	C10	Average	No	No	No	No
11	C11	Average	No	Yes	No	Yes
12	C12	Average	No	No	No	No
13	C13	Average	No	No	No	No
14	C14	Average	Yes	Yes	Yes	Yes
15	C15	High	No	No	No	No
16	C16	Average	No	No	No	No
17	C17	Average	No	No	No	No
18	C18	Average	No	No	No	No
19	C19	Average	No	No	No	No

Activity = Degree of playful activity, Aggressive = Aggressive behavior, Nervous = Nervous behavior, Dominant = Dominant status, Recessive = Recessive status

Appendix 10 The cat's environment (FIC group)

FIC No.	Code	Other pet	Other cat	Living	Neighbouring	Fighting
1	F01	Yes	Yes	In&out	Yes	Yes
2	F02	Yes	Yes	Indoor	No	No
3	F03	Yes	Yes	Indoor	No	No
4	F04	Yes	Yes	Indoor	No	No
5	F05	Yes	Yes	Indoor	No	No
6	F06	No	No	Indoor	No	No
7	F07	Yes	Yes	Indoor	No	No
8	F08	Yes	Yes	In&out	No	No
9	F09	Yes	Yes	Indoor	No	No
10	F10	Yes	Yes	In&out	Yes	Yes
11	F11	Yes	Yes	Indoor	No	No
12	F12	Yes	Yes	Indoor	No	No
13	F13	Yes	Yes	Indoor	No	No
14	F14	Yes	No	Indoor	No	No
15	F15	No	No	Indoor	No	No
16	F16	Yes	Yes	Indoor	No	No
17	F17	Yes	Yes	Indoor	Yes	Yes
18	F18	Yes	Yes	Indoor	No	No
19	F19	Yes	Yes	In&out	Yes	Yes

Other pet = Other animal in household, Other cat = Other cat in the household, Living = Living type, Neighboring = Neighboring cat can access, Fighting = Fighting with neighboring cat, In&out = indoor and outdoor

Appendix 11 The cat's environment (Control group)

Control No.	Code	Other pet	Other cat	Living	Neighbouring	Fighting
1	C01	Yes	Yes	Indoor	Yes	No
2	C02	Yes	Yes	In&out	No	No
3	C03	No	No	Indoor	No	No
4	C04	No	No	In&out	Yes	Yes
5	C05	Yes	Yes	Indoor	No	No
6	C06	No	No	Indoor	No	No
7	C07	Yes	Yes	Indoor	No	No
8	C08	Yes	Yes	Indoor	No	No
9	C09	Yes	Yes	Outdoor	Yes	No
10	C10	Yes	No	Indoor	No	No
11	C11	No	No	In&out	Yes	Yes
12	C12	Yes	Yes	In&out	No	No
13	C13	No	No	Indoor	No	No
14	C14	Yes	Yes	Indoor	No	No
15	C15	Yes	Yes	Indoor	No	No
16	C16	No	No	Indoor	No	No
17	C17	Yes	Yes	in&out	No	No
18	C18	Yes	Yes	in&out	No	No
19	C19	Yes	Yes	Indoor	No	No

Other pet = Other animal in household, Other cat = Other cat in the household, Living = Living type, Neighboring = Neighboring cat can access, Fighting = Fighting with neighboring cat, In&out = indoor and outdoor

Appendix 12 Type of food and management of the cat's litter box (FIC group)

FIC No.	Code	Food	Litter box	Number	Type	Size
1	F01	Comb	Yes	Less	Sand	Long
2	F02	Comb	Yes	Less	Sand	Long
3	F03	Dry	Yes	Less	Sand	Long
4	F04	Dry	Yes	Same	Sand	Long
5	F05	Comb	Yes	Same	Sand	Long
6	F06	Dry	Yes	Same	Sand	Long
7	F07	Comb	Yes	More	Pellet	Long
8	F08	Dry	Yes	Less	Sand	Long
9	F09	Dry	Yes	Less	Pellet	Long
10	F10	Dry	Yes	Less	Sand	Long
11	F11	Dry	Yes	Less	Sand	Long
12	F12	Dry	Yes	Less	Sand	Long
13	F13	Comb	Yes	Less	Sand	Long
14	F14	Dry	Yes	More	Pellet	Long
15	F15	Dry	Yes	Same	Sand	Long
16	F16	Comb	No	ND	ND	ND
17	F17	Comb	Yes	Same	Pellet	Long
18	F18	Dry	Yes	Less	Sand	Long
19	F19	Comb	No	ND	ND	ND

Food = Type of food, Dry = Commercial dry food, Can = Commercial canned food, Comb = Combination (Dry and canned), Homemade = Homemade food, Litter box = Use the litter box, Number = Number of litter box, Same = Same as number of cat, More = More than number of cat, Less = Less than number of cat, Type = Type of litter substrate, Sand = Cat sand, Pellet = Litter pellet, Size = Size of litter box, Long = Longer than the cat, ND = Not determined

Appendix 13 Type of food and management of the cat's litter box (Control group)

Control No.	Code	Food	Litter box	Number	Type	Size
1	C01	Comb	No	ND	ND	ND
2	C02	Home	No	ND	ND	ND
3	C03	Dry	Yes	Same	Sand	Long
4	C04	Comb	No	ND	ND	ND
5	C05	Comb	No	ND	ND	ND
6	C06	Dry	Yes	Same	Sand	Long
7	C07	Dry	Yes	Less	Sand	Long
8	C08	Dry	Yes	Less	Sand	Long
9	C09	Can	No	ND	ND	ND
10	C10	Dry	Yes	Same	Sand	Long
11	C11	Dry	No	ND	ND	ND
12	C12	Comb	No	ND	ND	ND
13	C13	Dry	No	ND	ND	ND
14	C14	Dry	Yes	Less	Sand	Long
15	C15	Dry	Yes	Less	Sand	Long
16	C16	Dry	No	ND	ND	ND
17	C17	Comb	No	ND	ND	ND
18	C18	Comb	No	ND	ND	ND
19	C19	Comb	No	ND	ND	ND

Food = Type of food, Dry = Commercial dry food, Can = Commercial canned food, Comb = Combination (Dry and canned), Homemade = Homemade food, Litter box = Use the litter box, Number = Number of litter box, Same = Same as number of cat, More = More than number of cat, Less = Less than number of cat, Type = Type of litter substrate, Sand = Cat sand, Pellet = Litter pellet, Size = Size of litter box, Long = Longer than the cat, ND = Not determined

VITA

Miss Jeeranan Benjasiriwan was born on August 17, 1988 in Bangkok, Thailand. She finished high school from Triamudom Suksa School in 2006. She was graduated from Faculty of Veterinary Science, Chulalongkorn University (D.V.M.) in 2013. She studies for Master's degree in Veterinary Science Chulalongkorn University in 2015 and she also work as a clinician in private small animal hospital.