ผลของการให้เอ็น-อะซีติล-ดี-กลูโคซามีนต่อระดับไกลโคซามิโนไกลแคนในพลาสมาและปัสสาวะแมวป่วยด้วยโรคกระเพาะปัสสาวะอักเสบที่ไม่ทราบสาเหตุ

นางสาว จิณณพัต ปัญจพันธ์พงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ภาควิชาอายุรศาสตร์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

THE EFFECTS OF ORAL N-ACETYL-D-GLUCOSAMINE ADMINISTRATION ON PLASMA AND URINE GLYCOSAMINOGLYCANS LEVELS IN CATS WITH IDIOPATHIC CYSTITIS

Miss Jinnapat Panchaphanpong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2009

Thesis Title	THE EFFECTS OF ORAL N-ACETYL-D-GLUCOSAMINE ADMINISTRATION ON PLASMA AND URINE GLYCOSAMINOGLYCANS LEVELS IN CATS WITH IDIOPATHIC CYSTITIS
By	Jinnapat Panchaphanpong
Field of Study	Veterinary Medicine
Thesis Advisor	Associate Professor Rosama Pusoonthornthum, D.V.M., Ph.D.
Thesis Co-Advisor	Assistant Professor Tanong Asawakarn, D.V.M., Ph.D.

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

M. Techahu ..... Dean of the Faculty of Veterinary Science

(Professor Mongkol Techakamphu, Doctoratde 3e cycle.)

THESIS COMMITTEE

..... Chairman

(Associate Professor Ratanaporn Brahmasa, D.V.M.)

..... Thesis Advisor

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

..... Thesis Co-Advisor

(Assistant Professor Tanong Asawakarn, D.V.M., Ph.D.)

..... Examiner

(Associate Professor Achara Tawatsin)

..... External Examiner

(Assistant Professor Chalermpol Lekcharoensuk, D.V.M., Ph.D.)

จิณณพัต ปัญจพันธ์พงศ์ : ผลของการให้เอ็น-อะซีติล-ดี-กลูโคซามีนต่อระดับไกลโคซามิโนไกลแคนในพลาสมาและปัสสาวะแมวป่วยด้วยโรคกระเพาะปัสสาวะอักเสบที่ไม่ทราบสาเหตุ. (The Effects of Oral N-acetyl-D-glucosamine Administration on Plasma and Urine Glycosaminoglycans Levels in Cats With Idiopathic Cystitis) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.สพ.ญ.ดร. รสมา ภู่สุนทรธรรม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.น.สพ.ดร.ทนง อัศวกาญจน์. 95 หน้า.

ทำการศึกษาในรูปแบบการศึกษาแบบสุ่มปกปิดชนิดการรักษาด้วย N-acetyl-D-glucosamine และยาหลอกในแมวที่ป่วยด้วยอาการของโรคในระบบขับถ่ายปัสสาวะส่วนล่างแบบไม่ทราบสาเหตุ โดยมีวัตถุประสงค์เพื่อศึกษาผลของเอ็น-อะซีติล-ดี-กลูโคซามีนต่ออาการทางคลินิก ระดับของไกลโคซามิโนไกลแคนในพลาสมาและปัสสาวะของแมวที่ป่วยด้วยโรคกระเพาะปัสสาวะอักเสบที่ไม่ทราบสาเหตุจำนวน 19 ตัว ที่เข้ารับการรักษาที่โรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ทำการสุ่มแมวแบบไม่ปกปิดออกเป็นสองกลุ่ม คือ กลุ่มที่ได้รับเอ็น-อะซีติล-ดี-กลูโคซามีนชนิดกินวันละ 250 มิลลิกรัมต่อตัวต่อวัน จำนวน 12 ตัว และกลุ่มที่ได้รับยาหลอกจำนวน 9 ตัว ติดต่อกันเป็นเวลา 28 วัน จากนั้นจะติดตามผลการรักษาหลังหยุด NAG และยาหลอกต่อเป็นเวลาอีก 28 วัน จนถึงวันที่ 56 ของการศึกษา กำหนดให้เจ้าของเป็นผู้ประเมินความเจ็บปวดและประเมินอาการผิดปกติจากการปัสสาวะของแมวโดยใช้ visual analogue scale แมวจะได้รับการติดตามอาการทางคลินิกโดยการประเมินของเจ้าของโดยแบบประเมิน VAS เป็นเวลา 28 วัน และเก็บตัวอย่างพลาสมาและปัสสาวะในวันที่ 0, 7, 14, 21, 28 และ 56 เพื่อวิเคราะห์ปริมาณของไกลโคซามิโนไกลแคน ผลการทดลองพบว่าค่าเฉลี่ยระดับไกลโคซามิโนไกลแคนในปัสสาวะกลุ่มปกติ (44.26±6.16 µg/ml) สูงกว่าแมวกลุ่มป่วย (7.97±1.20µg/ml) อย่างมีนัยสำคัญทางสถิติ (P<0.05) ส่วนในพลาสมาแมวกลุ่มที่ได้รับยาหลอก (24.20±3.35 µg/ml) และกลุ่มที่ได้รับเอ็น-อะซีติล-ดี-กลูโคซามีน (39.96±5.34 µg/ml) ในวันที่ 21 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (P<0.05) ค่าเฉลี่ยระดับไกลโคซามิโนไกลแคนในพลาสมาของกลุ่มที่ได้รับเอ็น-อะซีติล-ดี-กลูโคซามีนเมื่อเปรียบเทียบระหว่างวันที่ 0 (27.46±3.9 µg/ml) กับวันที่ 21 (39.96±5.34 µg/ml) และวันที่ 28 (39.91±6.74 µg/ml) พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (P<0.05) ค่าเฉลี่ยระดับไกลโคซามิโนไกลแคนในปัสสาวะแมวกลุ่มที่ได้รับยาหลอก (4.89±1.08 µg/ml) มีค่าน้อยกว่า เมื่อเทียบกับกลุ่มที่ได้รับเอ็น-อะซีติล-ดี-กลูโคซามีน (9.15±1.39 µg/ml) ในวันที่ 14 อย่างมีนัยสำคัญทางสถิติ (P<0.05) จากผลการทดลองในครั้งนี้สรุปได้ว่าแมวป่วยด้วยโรคกระเพาะปัสสาวะอักเสบแบบไม่ทราบสาเหตุมีระดับของไกลโคซามิโนไกลแคนในปัสสาวะต่ำกว่าแมวปกติ แมวป่วยมีระดับไกลโคซามิโนไกลแคนในพลาสมาสูงอย่างมีนัยสำคัญในวันที่ 21 ของการศึกษา และมีระดับไกลโคซามิโนไกลแคนในปัสสาวะแตกต่างกับกลุ่มที่ใช้ยาหลอกในวันที่ 14 ของการศึกษา การใช้เอ็น-อะซีติล-ดี-กลูโคซามีนร่วมกับการเปลี่ยนแปลงสิ่งแวดล้อมเพื่อลดความเครียดของแมวในบ้านมีผลทำให้แมวป่วยด้วยโรคกระเพาะปัสสาวะอักเสบที่ไม่ทราบสาเหตุมีอาการทางคลินิกดีขึ้น

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์สัตวแพทย์ ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2552 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5075554131 : MAJOR VETERINARY MEDICINE
KEYWORDS : N-ACETYL-D-GLUCOSAMINE / IDIOPATHIC CYSTITIS / CATS
JINNAPAT PANCHAPHANPONG : THE EFFECTS OF ORAL N-ACETYL-D-GLUCOSAMINE ADMINISTRATION ON PLASMA AND URINE GLYCOSAMINOGLYCANS LEVELS IN CATS WITH IDIOPATHIC CYSTITIS. THESIS ADVISOR : ASSOC. PROF. ROSAMA PUSOONTHORNTHUM, Ph.D., THESIS CO-ADVISOR : ASST. PROF. TANONG ASAWAKARN, Ph.D., 95 pp.

Double-blind placebo-controlled study was performed to determine the effects of N-acetyl-D-glucosamine (NAG) on the clinical signs, glycosaminoglycan (GAGs) alteration in plasma and urine of cats with idiopathic cystitis (FIC). Nineteen cats with FIC were diagnosed by the veterinarian at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The FIC cats were randomly divided into two groups; treatment (n=12) and placebo (n=7). The treatment group received 250 mg of oral NAG once daily for 28 days while placebo group received placebo daily for the same period. Both groups were follow-up for twenty-eight more days after stop receiving NAG or placebo. Pain when urination from each cat was recorded by its owner using visual analogue scale. Both groups were monitored for changes in clinical sign on day 0, 7, 14, 21, 28 and 56. Plasma and urine GAG levels of the FIC cats of both groups were measured on day 0, 7, 14, 21, 28 and 56. The results from this study demonstrated that the mean urinary GAG level for normal cats (44.26$\pm$6.16 µg/ml) was significantly higher than the FIC group (7.97$\pm$1.20 µg/ml) (P<0.05). Both groups had improved clinical signs when received the treatments or placebo along with the recommendation on the changes in the environment. Mean plasma GAG level of the placebo group (24.20$\pm$3.35 µg/ml) was significantly lowered than the NAG treatment group (39.96$\pm$5.34 µg/ml) on day 21 of the study (P<0.05). There were significantly differences of plasma GAG levels in NAG treatment group on day 0 (27.46$\pm$3.9 µg/ml) compared with day 21 (39.96$\pm$5.34 µg/ml) and day 28 (39.91$\pm$6.74 µg/ml) (P<0.05). FIC cats received placebo also had mean urinary GAG level (4.89$\pm$1.08 µg/ml) significantly lowered than the treatment group received NAG (9.15$\pm$1.39 µg/ml) on day 14 of the study (P<0.05). In conclusion, cats with FIC have lower urinary GAG levels than normal cats. FIC cats received NAG had significantly increased plasma GAG levels on day 21 of the treatment and its urinary GAG levels increased significantly on day 14 of the study when compared with placebo. The clinical signs improved significantly in FIC cats received NAG and changed its environment to lower the stress in the household.

Department : Veterinary Medicine Student's Signature .....
Field of Study : Veterinary Science Advisor's Signature .....
Academic Year : 2009 Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

My gratefulness is to Associate Professor Rosama Pusoonthornthum, D.V.M., Ph.D., thesis advisor and Assistant Professor Tanong Asawakarn, D.V.M., Ph.D., thesis co-advisor for their valuable suggestion, intensive review and excellent clinical and laboratory advices.

My gratitude is to Associate Professor Achara Tawatsin and Associate Professor Chaweewon Boonshuyar, Department of Biostatistics, Faculty of Public Health, Mahidol University for the statistical suggestions.

My gratitude is also to Associate Professor Ratanaporn Brahmasa, D.V.M., the thesis proposal and the thesis committee chairman.

I would like to thank the assistance of friends, scientists and staffs of Companion Animal Medicine Unit, Department of Veterinary Medicine, and Biochemistry Unit, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University for their help on experimental works and laboratory technique.

I would like to thank the clinicians and veterinary nurses of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University for their assistance in collecting the FIC cases.

CONTENTS

Page

Page

LIST OF TABLES

Page

Page

LIST OF FIGURES

Page

Page

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APF	antiproliferative factor
ATP	adenosine triphosphate
BUN	blood urea nitrogen
CaOX	calcium oxalate
CBC	complete blood count
CFU	colony forming units
Cr	creatinine
CTAB	cetyltrimethylammonium bromide
C-2	carbon two
DMB	1,9-dimethyl-methylene blue
DSH	domestic short hair
EDTA	ethylenediaminetetraacetic acid
E-cadherin	epithelial calcium dependent adhesion molecules
FIC	feline idiopathic cystitis
FLUTD	feline lower urinary tract disease
FUS	feline urologic syndrome
g	gram
GAGs	glycosaminoglycans
GI tract	gastrointestinal tract
GAG/Cr	glycosaminoglycan per creatinine
GP-51	glycoprotein with a molecular weight of 51kDa

G6-P	glucosamine 6-phosphate
HCl	hydrochloric acid
Hct	hematocrit
HPAA	hypothalamic-pituitary-adrenal axis
HPF, hpf	high-power field
IC	interstitial cystitis
IU	international units
kg	kilogram
L, l	litre
LUTD	lower urinary tract disease
M	molar
MAP	magnesium
MEMO	multimodal environmental modification
mg	milligram
min	minute
ml	millilitre
mm	millimetre
NAG	N-acetyl-D-glucosamine
NaOH	sodium hydroxide
nm	nanometre
NO	nitric oxide
pH	power of Hydrogen ion
PK	pharmacokinetic
PMNs	polymorphonuclear cells
PPS	pentosan polysulphate sodium
RBC	red blood cell

rpm	round per minute
SD	standard deviation
SD_D	standard deviation different
SEM	standard error of the mean
SEM_D	standard error of the mean different
Sig.	significant
SP	substance P
Sp.Gr.	urine specific gravity
SQC	squamous epithelial cell
tNAG	terminal N-acetyl-D-glucosamine
TNTC	too numerous too count
TSC	transitional epithelial cell
UTI	urinary tract infection
UV	ultraviolet
VAS	visual analogue scales
WBC	white blood cell
ZO-1	tight junction protein 1
µg	microgram
µl	microlitre

Chapter I
Introduction

Importance and Rationale

Feline lower urinary tract disease (FLUTD) is common feline urological syndromes. Cystitis or formerly called feline urologic syndrome (FUS) is common disease found in most unhealthy cats (Lund et al., 1999). In the United States, FLUTD was found in 4.6% of cats presented to private practices (Dru Forrester and Roudebush, 2007). The incidence of FLUTD in British cats is believed to be 1% per year (Gunn-Moore, 2003). The signs of lower urinary tract diseases occur during 1 to 10 years of age in most cats. FLUTD is uncommon in cats less than 1 year old or greater than 10 years old. The recurrence rates of male cats are 39-45% with/without obstructive uropathy (Bovee et al., 1979).

FUS and FLUTD are technical terms that have been used to describe clinical signs related to irritative urination, such as hematuria, pollakiuria and urination in inappropriate places. Study indicated that major causes of FLUTD were feline interstitial or idiopathic cystitis, urolithiasis, urethral plugs, urinary tract infections, bladder neoplasm and congenital deformity of urinary tract (Osborne et al., 1996a). Other causes of FLUTD are metabolic disorders (including nutrition), inflammatory disorders, trauma, iatrogenic and anatomic abnormalities (Osborne et al., 1996a). Most cases with non-obstructive FLUTD usually have clinical sign resolve spontaneously within 4–10 days (Gunn-Moore, 2003; Pereira et al., 2004).

Forty-five to seventy percent of cats with LUTD were caused by feline idiopathic or interstitial cystitis (FIC) with non-obstructive urinary tract disease (Kruger et al., 1991; Buffington et al., 1997; Hostutler et al., 2005) and 29% are obstructive idiopathic cystitis (Gunn-Moore, 2003). Other causes of LUTD in cats may be due to behavioral disorders and neurologic problems (Kruger et al., 1991; Buffington et al., 1997). Several factors are suggested to be changed for the improvement of clinical signs of FIC such as changes in cat environment, reduce stress, certain type of diet, water, and giving pheromone to cats (Dru Forrester and Roudebush, 2007). Studies in women with IC and cats with FIC showed decreased amounts of excreted urinary glycosaminoglycans (GAGs) or GP-51(Buffington et al., 1996a; Pereira et al., 2004). The exogenous GAGs may be useful for the treatment of FIC in cats.

The clinical signs of FIC are dysuria, stranguria, hematuria (macroscopic and microscopic), pollakiuria and periuria. Similar clinical signs are also found in woman as interstitial cystitis (idiopathic pelvic pain syndrome) (Clasper, 1990); characterized by bladder pain, frequent urinations, urinary urgency and nocturia without a diagnosable cause, but not lethally. Clinical signs of FIC may result from multiple abnormalities of the bladder, central nervous system, and hypothalamic-pituitary-adrenal axis (Westropp and Buffington, 2004). There are many studies on etiologies and risk factors of idiopathic interstitial cystitis in cats but none of those studies can indicate the actual causes of FIC. Several hypotheses regarding the causes of FIC that has been proposed are for example: compromised GAGs lining on transitional epithelium of bladder which lead to the clinical manifestations (Gao et al., 1994), gene expression associated with interstitial cystitis or antiproliferative factor that inhibits cell proliferation in urothelium (Keay et al., 2003a), stress from surrounding indoor environments and behavior induced signs of FIC (Buffington et al., 2006a). There are several studies indicated that cats with FIC when fed with canned food compared with dry food had decreased recurrent clinical signs (Markwell et al., 1999; Gunn-Moore and Shenoy, 2004; Bartges and Kirk, 2006). Those studies suggested that water is also an important factor to control FIC in cats.

Study of interstitial idiopathic cystitis revealed that there is specific GAGs called GP-51 lining on normal bladder urothelium. GAGs are believed to prevent bacterial and crystal adhesion and protect the urothelium from noxious or toxic urine substances (Parsons et al., 1980). Urinary bladder urothelium extend from the renal pelvis to the urethra and composes of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial apical layer (Lewis, 2000; Apodaca, 2004). The sensory neurons, called unmyelinated pain fibers (C-fibers), are located in submucosa of the bladder wall and this C-fiber increased in both cats with FIC and human with IC (Buffington et al., 1996b). When these sensory fibers are stimulated by various causes such as inflammation or trauma, the action potentials will transmit the sensation of pain to the brain (local axon reflexes) leading to the release of neuropeptides (neurotransmitter; chemical mediators), such as substance P (SP) and others resulted in intramural blood vessels dilatation, increased vascular and bladder wall permeability, submucosa edema, smooth muscle contraction, pain, and mast cell degranulation leading to release of various inflammatory mediators (e.g., histamine, heparin, serotonin, cytokines and prostaglandins) (Gunn-Moore, 2003; Hostutler et al., 2005).

Urinary urothelial and suburothelial signaling and mediators (urothelial and suburothelial plexus) from urothelium such as capsaicin, ATP (adenosine triphosphate), NO (nitric oxide), tachykinins and prostanoids are also released when lower urinary tract

is damaged. ATP plays an important role in response to inflammatory process and injury by trigger purinergic (P2X) receptors on sensory fiber (Birder, 2005). When GAGs layer or urothelium is compromised, constituents of the urine or compounds within the urine such as acid pH, potassium, magnesium, and calcium ions pass the urothelium and exacerbate the sensory neurons and cause pain for FIC cats.

Glycosaminoglycans are mucopolysaccharide chains consisting of long unbranched polysaccharide link to proteins, called proteoglycans. There are many components of proteoglycans such as chondroitin sulfate, dermatan sulfate, heparan sulfate, keratin sulfate, and heparin which are different in structure and pattern (Pereira et al., 2004). GAGs can be found in animal tissues constituting mainly in intracellular substance (Thorne and Resnick, 1984; Akcay and Konukoglu, 1999), extracellular matrix (most chondroitin sulfate and dermatan sulfate) and cell surface (Pereira et al., 2004). There were studies found that chondroitin sulfate was the most highest amount of GAGs in plasma and urine when compared with dermatan sulfate and heparan sulfate in normal and FIC cats (Pereira et al., 2004).

GAGs replacement is used to replace urothelium. In human, there was some successful outcome of using pentosan polysulphate sodium (PPS), as exogenous form of GAGs, given to patient with IC by oral route with/without intravesical heparin. Administration of exogenous form of GAGs, oral medication, such as heparin, hyaluronic acid and PPS has been shown to be useful in human with IC. The treatment helps to increase urothelial GAGs and reduce transitional cell injury. However, there is no information on appropriated and safety dose of GAGs treatment in cat. N-acetyl-D-glucosamine, exogenous form of GAGs, has also been used as one of the popular administration in cats to increase GAGs lining despite no previous clinical study. There was significantly improvement of clinical signs in cats with FIC that received N-acetyl-D-glucosamine (NAG) but there was no report on GAGs levels in plasma or urine of cats with FIC in that study (Gunn-Moore, 2003).

Objectives of Study

1. To study the effects of N-acetyl-D-glucosamine on plasma and urine glycosaminoglycans levels in cat with idiopathic interstitial cystitis.
2. To study the side effects of N-acetyl-D-glucosamine in cat with idiopathic interstitial cystitis.
3. To evaluate the effect of N-acetyl-D-glucosamine on clinical signs in cat with idiopathic interstitial cystitis.

Keywords: N-acetyl-D-glucosamine, idiopathic cystitis, cats

Research Questions

Can N-acetyl-D-glucosamine increase plasma and urine glycosaminoglycans levels in cat with idiopathic cystitis?

Research Hypothesis

N-acetyl-D-glucosamine administration can increase plasma and urine glycosaminoglycans levels in cat with idiopathic cystitis

Advantages of Study

1. To determine the therapeutic effects of N-acetyl-D-glucosamine on clinical signs and recurrence rate in cats with idiopathic interstitial cystitis.
2. Understanding the role of N-acetyl-D-glucosamine on plasma and urine levels of glycosaminoglycans in cat with FIC.
3. To test weather spectrophotometric quantitation of GAGs levels in plasma and urine can be used as an initial screening test for cats with FIC in the future.

Chapter II
Literature Review

2.1 Brief Literature Review

FLUTD is one of the most common lower urinary tract abnormalities found in cats. The main cause of FLUTD is FIC with or without obstructive urinary tract disease (Kruger et al., 1996; Lekcharoensuk et al., 2001). This abnormality is commonly found in castrated or spayed cats (Hostutler et al., 2005). Most cats with FIC are middle age (1-10 years) (Hostutler et al., 2005). The clinical signs of cats with FIC are dysuria, stranguria, hematuria (macroscopic and microscopic), pollakiuria and/or periuria (urination in inappropriate places). The same clinical signs have been observed in woman with interstitial cystitis.

Several hypotheses have been postulated for the cause of this abnormality. One of the hypotheses is the glycosaminoglycans layer alteration in urinary bladder. Glycosaminoglycans layer which cover transitional epithelium of the bladder wall has function to prevent microbes and crystals attachment on the urothelium bladder. It also helps to limit transepithelial movement for urine proteins and other toxic substances across the urinary bladder wall (Parsons et al., 1980). Quantitative and qualitative changing of GAGs layer increased urothelial permeability in both the woman with IC and cat with FIC. The changing in glycosaminoglycan layer may be one of the leading causes of FIC.

N-acetylglucosamine or N-acetyl-D-glucosamine is the exogenous form of GAGs that has been commonly used for the treatment of recurrent/nonrecurrent idiopathic cystitis to increase GAGs lining in cats and human. There are no clinical studies on the effect of NAG on GAGs alteration of the urinary bladder in cats with idiopatic cystitis. The purpose of this study is to determine the effect of NAG on clinical signs, GAGs alteration in plasma and urine of cats with idiopathic interstitial cystitis.

2.2 Feline Lower Urinary Tract Disease (FLUTD)

FUS and FLUTD are technical terms that have been used to describe clinical signs related to irritative urination, such as hematuria, pollakiuria and periuria (urination in inappropriate places) (Osborne et al., 1996a). FLUTD is common disease found in most unhealthy cats (Lund et al., 1999). In the United States, FLUTD was found in 4.6% of cats presented to private practices (Dru Forrester and Roudebush, 2007). The incidence of FLUTD in British cats is 1% per year (Gunn-Moore, 2003). The signs of lower urinary tract occur during 1 to 10 years of age in most cats (Dru Forrester and Roudebush, 2007). The recurrence rates of male cats are 39-45% with/without obstructive uropathy (Bovee et al., 1979).

Studies indicated that major causes of FLUTD in cats less than 10 years of age is feline interstitial or idiopathic cystitis with non-obstructive urinary tract disease (45%-70%) (Kruger et al., 1991; Buffington et al., 1997; Hostutler et al., 2005) and obstructive idiopathic cystitis (29%) (Gunn-Moore, 2003). Other causes include urolithiasis (15%-21%), urethral plugs (10%-21%), anatomic defects (10%), behavioral disorders (9%), neoplasia (1%-2%), and urinary tract infections (UTI; 1%-8%) (Kruger et al., 1991; Kruger et al., 1996; Osborne et al., 1996a; Buffington et al., 1997; Lekcharoensuk et al., 2001; Dru Forrester and Roudebush, 2007). Dru Forrester (2007) concluded causes of FLUTD as shown from four studies **(Table 1)**. UTI is more common in older cats, cats with chronic kidney disease or cats that have had urinary tract procedures such as urethral catheterization, perineal urethrostomy) (Dru Forrester and Roudebush, 2007). Most cases with non-obstructive FLUTD usually have clinical sign resolve spontaneously within 4–10 days (Gunn-Moore, 2003; Pereira et al., 2004; Kruger et al., 2009).

Table 1 Causes of FLUTD from Four Studies (Dru Forrester, 2007)

Diagnosis*	**Prevalence**			
Idiopathic (FIC)	63%	55%	64%	57%
Urethral obstruction	19%	21%†	NA	58%‡
Urethral plugs	NR	21%	NA	10%
Uroliths	19%	23%	15%	22%
Behavioral disorder	NR	NR	9%	0%
Incontinence	4%	0%	0%	0%
Bacterial UTI	3%	3%	1%	8%
Anatomic anomaly	0.3%	NR	11%	0%
Neoplasia	0.3%	0%	2%	0%
Unknown	0%	0%	0%	3%
Study Characteristics				
Type	Retrospective	Prospective	Prospective	Prospective
Population	All clinical presentations	All clinical presentations	Nonobstructed clinical presentations	All clinical presentations
Collection period	1980-1997	1982-1985	1993-1995	2000-2002
Number of cases	22,908	141	109	77

Note: *Some cats had multiple disorders.
†All cats had urethral obstruction associated with urethral plugs.
‡Included 24 cats with FIC, 13 cats with uroliths, and 8 cats with urethral plugs.
FIC = feline idiopathic cystitis; NA = not applicable; NR = not reported; UTI = urinary tract infection

2.3 Feline Idiopathic Cystitis (FIC)

One of the main causes of FLUTD is FIC. Most cats with FIC are middle age (1-10 years) (Hostutler et al., 2005). The clinical signs of cats with FIC are dysuria, stranguria, hematuria (macroscopic and microscopic), pollakiuria and/or periuria (urination in inappropriate places). The same clinical signs have been observed in woman with interstitial cystitis.

2.3.1 Clinical sign & Recurrent

The most common clinical sign observed in cats with nonobstructive idiopathic cystitis are periuria, pollakiuria, stranguria, and gross hematuria and may precede the obstructive form of the disorder. These clinical signs subside within 5-7 days without therapy in up to 92% of cats with acute nonobstructive idiopathic cystitis (Kruger and Osborne, 1995; Osborne et al., 1996b; Kruger et al., 2003; Kruger et al., 2009). About 39% to 65% of cats with acute FIC had recurrences of clinical signs within 1 to 2 years after the initial episode (Markwell et al., 1999; Kruger et al., 2003; Gunn-Moore and Shenoy, 2004). There was report that increased in the number of prior episodes of lower urinary tract signs was related with a significantly higher risk of recurrence of clinical signs, while increased age was related with a significantly lower risk of recurrence of clinical signs (Kruger et al., 2003). There was a recurrent episode approximately 65% in a 2-years period after control age, number of prior episodes and moist food, suggest that recurrent episodes of acute FIC decrease in frequency and severity as the cats become older (Kruger and Osborne, 1995; Kruger et al., 2003). Recurrent clinical signs in cats with FIC may be a recurrent episode of the original disease or result of a delayed manifestation of the original disease or a different FLUTD which related with similar clinical signs (Kruger et al., 2009). Less than 15% of cats that initially presented with acute FIC develop this form of disease. Some cats with chronic or frequently recurrent forms of FIC had spontaneous remission of their clinical signs (Kruger et al., 2009).

2.3.2 Etiology & Hypothesis

There are many studies on etiologies and risk factors for idiopathic interstitial cystitis in cats but none of those studies can indicate the actual causes of FIC since no single model explains all the biological variability observed in cats with FIC. Several hypotheses regarding the causes of FIC that has been proposed are for example: compromised GAGs lining on transitional epithelium of bladder which lead to the clinical manifestations (Gao et al., 1994), antiproliferative factor that inhibits cell proliferation in urothelium (Keay et al., 2003a), stress from surrounding indoor environments and behavior induced signs of FIC (Buffington et al., 2006a).

Dysfunctional Urothelial Barrier

Urothelial barrier dysfunction has been studied as one of the cause of interstitial cystitis in human (Elbadawi, 1997; Hurst et al., 2007; Parsons, 2007). Several studies in

cats supported this concept which involved in pathogenesis of idiopathic cystitis (Gao et al., 1994; Buffington et al., 1996a; Lavelle et al., 2000). Bladder urothelium is an important host defense mechanism by serving as a barrier to prevent the entry of uropathogens into deeper structures of the bladder. Urothelium also selectively controls passage of water, ions, macromolecules and other solutes across the mucosal surface into underlying tissues (Apodaca, 2004; Hurst et al., 2007). Urothelial barrier function largely depends on: specialized high-resistance tight junctions between apical membranes of adjacent urothelial cells; the unique lipid and protein composition of urothelial cell membranes; a layer of GAGs located on the luminal surface of urothelial cells (Lewis, 2000; Apodaca, 2004). In contrast, there were reports that disagreement to the relative contributions of the GAGs layer and urothelium as barrier to urine ions, macromolecules, toxins and microorganisms (Lewis, 2000; Hurst et al., 2007). Those reports suggested that any disease process that: directly injures urothelium; alters structural or functional characteristics of urothelial GAGs or apical tight junctions; and/or disrupts active transport mechanisms may lead to loss barrier function (Lewis, 2000; Lavelle et al., 2002; Birder, 2005). These changes may allow translocation of toxic substances into underlying tissues, leading to submucosal edema, hemorrhage, neovascularization, mastocytosis, mononuclear inflammatory cell infiltration, fibrosis, and clinical signs of frequency, urgency and pain (Clasper, 1990; Lavelle et al., 2000; Birder, 2005).

Defective glycosaminoglycan layer

Transitional epithelium of the urinary bladder is covered by a glycocalyx which composed of hydrated glycoconjugates including glycoproteins and glycosaminoglycans (Hurst et al., 2007). Urothelial GAGs reduce adherence of microorganisms and crystals to the bladder urothelium and also limit movement of urine proteins and other ionic and nonionic solutes from the bladder lumen into surrounding tissues (Parsons et al., 1990; Hurst et al., 2007; Parsons, 2007). GAGs are ubiquitous components of animal tissues, where they occur covalently linked to a protein core, forming proteoglycans (Kjellen and Lindahl, 1991). Chondroitin sulfate, dermatan sulfate, heparan sulfate, keratin sulfate and heparin are components of proteoglycans, and each has a unique tissue distribution pattern and structure (Dietrich et al., 1976; Cassaro and Dietrich, 1977). Bladder surface GAGs has been reported to contain predominantly chondroitin sulfate and heparan sulfate, whereas GAGs deeper in the bladder wall contains more hyaluronic acid, high-sulfated heparan sulfates and dermatan sulfates (Hurst et al., 1987). There were studies found that chondroitin sulfate was the most highest amount of GAGs in plasma and urine when compared with dermatan sulfate and heparan sulfate in normal and FIC cats (Pereira et al., 2004). Chondroitin 4-sulphate containing proteoglycan located on the

luminal surface of the bladder epithelium and in the lamina propria (Hurst et al., 1996). The proteoglycans from the bladder luminal surface are the products of uroepithelial cells (Kurth and Parsons, 2003).

Quantitative or qualitative defects in surface GAGs and subsequent increased urothelial permeability have been hypothesized to be a causative factor in the pathogenesis of FIC in cats and IC in human (Parsons et al., 1990; Buffington et al., 1996b; Parsons, 2007). Chronic exposure of bladder wall tissues to urine constituents could result in sensory afferent nerve stimulation mast cell activation, and/or induction of immune-mediated or neurogenic inflammatory responses (Elbadawi, 1997). Compared to normal cats, some cats with FIC appear to have increased urinary bladder permeability to salicylates, urea and fluorescein, decreased surface GAGs expression, and decreased total urinary GAGs excretion (Gao et al., 1994; Buffington et al., 1996a; Lavelle et al., 2000; Westropp et al., 2006) that have identified similar abnormalities in human with IC (Parsons et al., 1990; Parsons et al., 1991; Hurst et al., 1993).

The cause of the decrease in urine GAGs concentration in patients with IC is unknown. Most urinary GAGs is believed to come from GAGs sloughed from or excreted by the cells lining the urinary tract (Parsons and Hurst, 1990). Decreased urinary GAGs excretion in patients with IC could result from an abnormality in bladder surface GAGs synthesis or retention, inactivation by some substance in the urine, and/or loss due to increased permeability of the bladder. Chondroitin sulphate proteoglycans comprise roughly one third of the total proteoglycans on the bladder surface (Kurth and Parsons, 2003). Patients with IC have been reported to have a lower percentage of chondroitin sulfate and heparan sulfate, and a higher percentage of hyaluronic acid and dermatan sulfate in bladder surface GAGs, suggesting a loss of the surface GAGs and appearance of a pattern of GAGs associated with deeper layers of the bladder wall (Holm-Bentzen et al., 1986). GAGs layer may be inactivated in some IC patients by a protamine-like substance, which has a high affinity to bind with and inactivate GAGs by neutralizing its negative charge in urine (Kaufman et al., 1987; Parsons et al., 1988; Parsons et al., 1990) and increase bladder permeability and permit penetration of the bladder wall by small molecules (Lilly and Parsons, 1990). It has been suggested that increased permeability may result in damage underlying tissue leading to clinical signs of IC (Parsons, 1994).

However, decreased urinary GAGs excretion and increased urothelial permeability may be associated with other non-interstitial cystitis lower urinary tract disorders such as urolithiasis, chemical cystitis and bladder overdistension and also associated with other diseases such as spinal cord injury, diabetes mellitus and chronic

renal failure (Nikkila, 1989; Hurst et al., 1993; Elbadawi, 1997; Michelacci et al., 2001; Erturk et al., 2002; Ombra et al., 2003; de Lima et al., 2005).

Defective urothelial proliferation or differentiation

Urinary bladder urothelium extend from the renal pelvis to the urethra and composes of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial apical layer composed of large hexagonal cells (umbrella cells) (Lewis, 2000; Apodaca, 2004). The two deeper layers of urothelial cells serve as progenitors for the overlaying superficial layer. Superficial urothelial cells are responsible for maintaining the bladder permeability barrier and process features including: 1) specialized apical membrane proteins (uroplakins); 2) high resistance tight junctions between cells; 3) an active trafficking mechanism designed to insert cytoplasmic vesicles into the apical membrane to accommodate bladder filling (Lewis, 2000). Primary responses of urothelium to injury are desquamation, necrosis and/or apoptosis (Veranic and Jezernik, 2000; Lavelle et al., 2002; Jezernik et al., 2003). Rapid replacement of exfoliated or injured superficial cells and restoration of urothelial tight junction integrity prevents long-term urothelial barrier malfunction in healthy animals (Veranic and Jezernik, 2001). However, urothelial injury in any disease process that alters normal urothelial proliferation and differentiation could affect urothelial barrier function.

Urothelial ulceration, erosion and thinning are common abnormalities identified in human with IC (Johansson and Fall, 1990; Rosamilia et al., 2003; Slobodov et al., 2004) and chronic FIC (Clasper, 1990; Lavelle et al., 2000). These studies made researchers to hypothesize that impaired urothelial proliferation may be involved in the pathogenesis of human interstitial cystitis (Keay and Warren, 1998). Identification of abnormal expression of protein biomarkers associated with urothelial growth and differentiation such as uroplakins, E-cadherin, ZO-1, glycoproteins, GAGs and keratins (Moskowitz et al., 1994; Slobodov et al., 2004; Laguna et al., 2006; Hurst et al., 2007; Hauser et al., 2008). Antiproliferative factor (APF), which was identified in urine human with IC, inhibited proliferation of normal bladder urothelial cells, increased transcellular permeability, inhibited tight junction protein expression and reduced growth factor production (Keay et al., 2003a; Keay et al., 2003b; Zhang et al., 2005). Despite histopathologic similarities to human interstitial cystitis, there were relatively few studies evaluating protein biomarkers of urothelial growth and urothelial differentiation in cats with acute or chronic idiopathic cystitis. Urothelial cells underlying areas of mucosal erosion expressed abundant levels of the superficial urothelial cell differentiation marker

AE-31 that distribution of its was altered with staining localized to the perinuclear region of cells rather than at its normal apical membrane position (Lavelle et al., 2000). There was study that immuno-histochemical staining for uroplakin was significantly less in cats with chronic FIC compared with normal cats but not significantly different compared with cats with urolithiasis (Kruger et al., 2009). Nowadays, it is unclear that alterations of urothelial markers will be primary dysfunction of urothelium or secondary response to urothelial injury.

Systemic Psychoneuroendocrine Disease

The association of idiopathic cystitis with environmental, psychologic and pathologic stressors and the identification of multiple abnormalities of the nervous and endocrine systems in affected cats have led to hypothesis that systemic psychoneuroendocrine factors may be causative factor in the pathogenesis of FIC (Buffington and Pacak, 2001; Westropp et al., 2003; Buffington et al., 2006b; Westropp et al., 2006). Stressful events such as earthquakes, seasonal weather changes, moves to a new home and diet changes have been associated with recurrent episodes of lower urinary tract signs in cats (Jones et al., 1997; Buffington et al., 2006b). In addition, living in multicat households and inter-cat conflicts have also been related with an increased risk of disorders of the lower urinary tract (Jones et al., 1997; Buffington, 2002; Cameron et al., 2004). Increased tyrosine hydroxylase (enzyme catalyze norepinephrine biosynthesis) immunoreactivity (Reche Junior and Buffington, 1998) and plasma norepinephrine concentrations (Buffington and Pacak, 2001; Westropp et al., 2006), and decreased functional sensitivity of alpha-2-adrenoceptors (Westropp et al., 2007) in cats with chronic FIC are consistent with increased sympathetic drive in affected cats. Previous studies found that significant abnormalities of the hypothalamic-pituitary-adrenal axis (HPAA) were identified in affected cats, despite having significantly increased plasma norepinephrine concentrations (Buffington and Pacak, 2001; Westropp et al., 2006) while there was significantly decreased plasma cortisol responses to exogenous ACTH and reduction in size of adrenal glands in cats with chronic idiopathic cystitis (Westropp et al., 2003). They were indicated dissociation between responses of sympathetic nervous system and hypothalamic-pituitary-adrenal axis to stress in cats with chronic FIC (Westropp et al., 2003; Buffington, 2004; Westropp et al., 2006). Specific mechanisms by which systemic psychoneuroendocrine disorders in cats may precipitate clinical signs referable to lower urinary tract apparently have not been identified (Kruger et al., 2009).

Alternatively, it has been hypothesized that physiologic responses to stressors may directly affect urothelial integrity such as loss of urothelial tight junction integrity, increased paracellular permeability, activation of mast cells and desquamation of superficial urothelial cells from studies in rodent models (Veranic and Jezernik, 2000; Veranic and Jezernik, 2001) while as no report in cats. However, it is possible that activation of the sympathetic nervous system and subsequent increases in norepinephrine could increases urothelial permeability by reducing tight junction integrity so permit urine substances greater contact with bladder sensory afferent neurons (Buffington et al., 2006a; Westropp et al., 2006)

2.3.3 Overall of Pathophysiology

Study of interstitial idiopathic cystitis revealed that there is specific GAGs called GP-51 lining on normal bladder urothelium. GAGs are believed to prevent bacterial and crystal adhesion and protect the urothelium from noxious or toxic urine substances as describe above.

Neurogenic inflammation process is initiated by excitation of small C-fibers sensory afferent neurons and mediated by neuropeptides (neurotransmitter; chemical mediators) released from stimulated nerves (Elbadawi, 1997). C-fibers located in submucosa of the bladder wall and this C-fiber increased in both cats with FIC and human with IC (Buffington et al., 1996b). Structural or functional defects in urothelial barrier could permit hydrogen, calcium, and potassium ions or other urine constituents to come into contact with sensory afferent neurons innervating the urothelium. When these sensory fibers are stimulated by various causes such as inflammation or trauma, the action potentials will transmit the sensation of pain to the brain (local axon reflexes) leading to the release of neuropeptides such as substance P, neurokinin and calcitonin gene-related peptide. Alternatively, injury-induced alterations in urothelial release of chemical signaling molecules (e.g., ATP, nitric oxide, acetylcholine, substance P, tachykinins and prostanoids) may activate sensory afferent neurons and mast cells when lower urinary tract is damaged (Elbadawi, 1997; Birder, 2005; Sant et al., 2007). Interaction of neuropeptides with tissue receptors resulted in intramural blood vessels dilatation, increased vascular and urothelial permeability, increased leukocyte migration, and mast cell activation and degranulation leading to wide range of biological effects in submucosa edema, smooth muscle contraction, pain, inflammation, tissue injury and fibrosis (Elbadawi, 1997). Although, many studies found that urinary bladder

mastocytosis may not be specific for either human interstitial cystitis or FIC (Sant et al., 2007; Kruger et al., 2009), these events lead to hypothesis that neurogenic inflammation represents the common pathway in multifactorial pathogenesis in human with IC (Elbadawi, 1997; Sant et al., 2007). Increased numbers of substance P-containing sensory afferent neurons and high affinity substance P receptors have been observed in bladder submucosa of cats with FIC (Buffington and Wolfe, 1998) that suggest associated with inflammation of feline urinary bladders (Buffington et al., 1996b). Hypothesis for neurogenic inflammation in FIC was concluded as diagram **(Figure 1)** (Buffington et al., 1996b; Gunn-Moore, 2003)

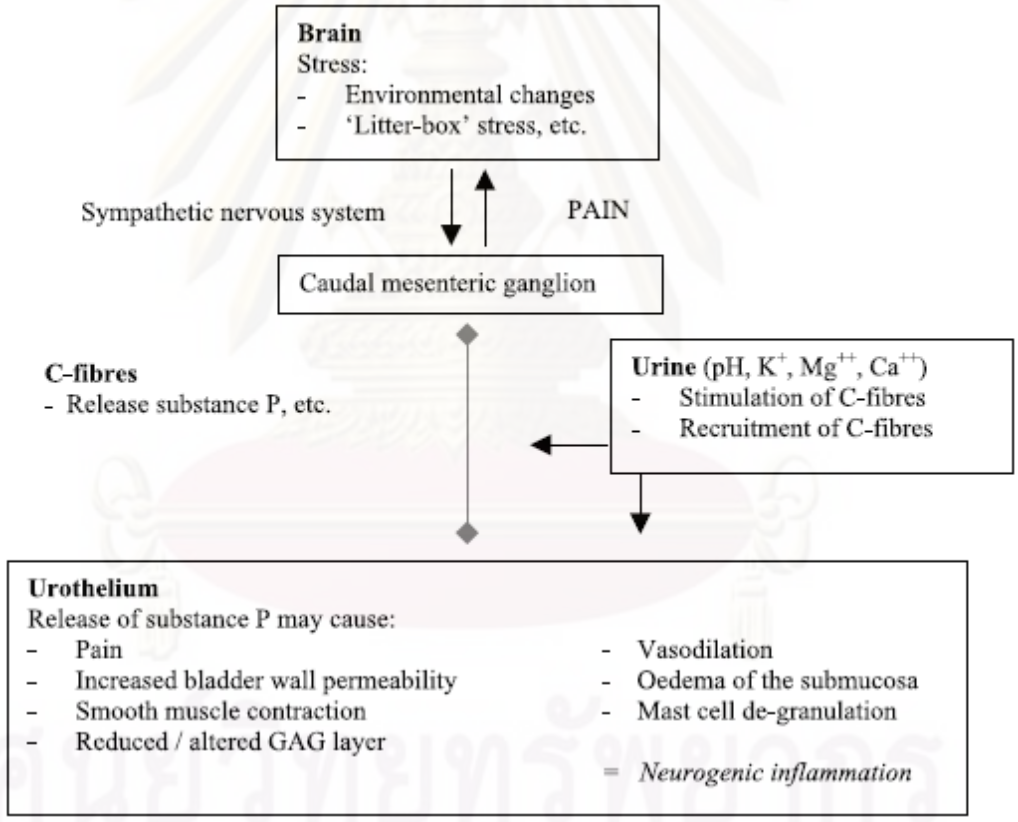


Figure 1 Hypothesis for neurogenic inflammation in FIC. (Gunn-Moore, 2003; Adapted from Buffington et al., 1996b)

2.3.4 Diagnosis

The diagnosis of FIC can be done using various methods as the followings:

Signalment & Clinical Signs

Clinical signs of hematuria, dysuria, and pollakiuria may result from fundamentally different causes. No clinical signs, which are distinguishable from other causes of FLUTD. There is no single specific diagnostic test or marker that can be relied upon to detect idiopathic cystitis (Kruger et al., 2009). Marking a diagnosis involves integrating findings from the signalment, history, physical examination, clinical signs, time course of the disease, urinalysis with sediment evaluation, urine culture and sensitivity testing, and urinary tract imaging. The modality of imaging chosen may include a combination of plain abdominal radiography, ultrasonography of the urogenital system, contrast radiography, and uroendoscopy (including urethroscopy and cystoscopy) (Hostutler et al., 2005). Lulich (2007) concluded typical signalment, physical examination, urinalysis, and radiographic findings for common lower urinary tract diseases in cats (**Table 2**).

Periuria is the most common clinical sign reported by owners of cats with LUTD, and these cats often are suspected by veterinarians to have a behavioral disorder. Approximately half of the cats with inappropriate urination as the only client-reported clinical sign have been reported to have interstitial cystitis diagnosed by uroendoscopy (Buffington et al., 1997). The time course of the clinical signs also may be helpful in arriving at a diagnosis. Initial duration of interstitial cystitis generally resolve within 7 days with or without treatment. Other diseases, such as urolithiasis and bacterial UTI, often result in clinical signs that are present for longer periods and may be progressive in severity unless adequate therapy is instituted (Hostutler et al., 2005).

Table 2 Typical signalment, physical examination, urinalysis, and radiographic findings for common lower urinary tract diseases in cats (Lulich, 2007)

	FIC	Urethral Obstruction	Struvite Urocystolith	CaOx Urocystolith	Behavioral Periuria	Bacterial UTI	Urinary Incontinence
Breed predisposition	None	None	None	Persian, Himalayan, Burmese, but also many breeds	None	None	Manx
Gender predisposition	None	M	F > M	M > F	None	F > M	None
Common age (yr)	2 – 6	3 – 7	7 ± 3.5	7.3 ± 3.4	Variable	> 8 – 10	> 1
Neurologic abnormalities	Absent	Absent	Absent	Absent	Absent	Absent	Common
Bladder size	Small	Large	Variable	Variable	Unremarkable	Variable	Variable
Urine pH	Acidic	Acidic	> 6.5	6.3 – 6.7	Unremarkable	Variable	Variable
Hematuria	Very frequent	frequent	frequent	frequent	Variable	frequent	Variable
Pyuria	Rare	Infrequent	frequent	frequent	Rare	frequent	Absent
Crystals	Sometimes	MAP	MAP	CaOx	Rare	None	Variable
Bacteria	Absent	Absent	Variable	Absent	Absent	Variable	Variable
Survey radiography results	Small bladder	Large bladder, possible radiodense urethral plug or urolith	Moderately dense round uroliths	Very dense smooth or irregular small uroliths	Unremarkable	Unremarkable	Unremarkable to large bladder

Note: CaOx = calcium oxalate, FIC = feline idiopathic cystitis, F = female, M = male, MAP = magnesium ammonium phosphate, UTI = urinary tract infection

Urinalysis & Urine Culture

Urinalysis with sediment evaluation should be performed if there is recurrence of clinical signs, evidence of underlying chronic renal failure, or previous urinary catheterization or if a perineal urethrostomy is present. Study found that mean urine specific gravity values ranged from 1.032 to 1.041 in cats eating moist food and from 1.051 to 1.052 in cats eating dry food (Dru Forrester and Roudebush, 2007). Urine dipstick pads that detect white blood cell (WBC) esterase often are positive in the absence of pyuria in cats (i.e., they frequently yield false-positive results). When evaluating feline urine sediment, care must be taken not to overinterpret the presence of bacteria (Hostutler et al., 2005). Up to 40% of unstained feline urine sediments classified as positive for bacteriuria by light microscopy were found to be false positives when

compared with results of quantitative culture of urine for bacteria (Kruger et al., 2009). Microscopic examination of unstained urine sediment was associated with only an 11% positive predictive value. Staining of urine sediment with a modified Wright-stain procedure (Diff Quik®) reduced the frequency of false positives to approximately 2% (Swenson et al., 2004). False-positive bacterial culture results associated with improper urine collection techniques, storage equipment and time of storage. Cellular debris may exhibit Brownian motion and be misinterpreted as bacteria. Dilute urine in the face of pyuria or significant pyuria (>5 WBCs per high-power field [hpf]) regardless of urine specific gravity warrents urine culture and sensitivity testing on urine collected by cystocentesis. The presence of crystals in urine sediment may have no clinical importance in cats without a stone or urethral plug, because crystals do not damage healthy urothelium (Hostutler et al., 2005). Urine that has been refrigerated or stored for hours often contains crystals in the urinary sediment, and this phenomenon is exaggerated in urine that is highly concentrated (Sturgess et al., 2001).

Radiographs

Plain abdominal radiographs that include the pelvic and penile urethra can be helpful in identifying radiopaque calculi (e.g., struvite, oxalate) of more than 3 mm in diameter (Hostutler et al., 2005). Contrast radiography, including cystography, urethrography, and urethrocystography is indicated in cats with recurrent clinical signs. Contrast cystography often is normal in FIC or may present various combinations of thickened urinary bladder wall or irregular urinary bladder mucosa, but the technique may be helpful in detecting small calculi, radiolucent calculi, urachal diverticula, urethral narrowing and neoplasia as well as in determining bladder wall thickness (Kruger et al., 1996). Occasionally, contrast material may be seen permeating through the bladder wall in severe cystitis. Contrast evaluation of the urethra generally is normal but may be helpful in diagnosing urethral strictures in male cats and stones in the urethra (Scrivani et al., 1997; Scrivani et al., 1998).

Abdominal Ultrasonography

It is useful to evaluate the bladder be using abdominal ultrasonography but is unrewarding for evaluation of the entire length of the urethra. Abdominal ultrasonography may detect small calculi, radiolucent calculi, and bladder masses like polyps and neoplasia and may aid in assessing bladder wall thickness if the bladder is sufficiently distended (Hostutler et al., 2005).

Uroendoscopy

This is a valuable tool in the evaluation of cats that have recurrent or persistent clinical signs associated with the lower urinary tract. Uroendoscopy allows visualization of the urethral and bladder mucosa, detection of small calculi not seen on abdominal ultrasonography, evaluation for urachal remnants, and direct visualization of masses that may be present. Uroendoscopy of female cats is performed using a rigid pediatric cystoscope, which affords much greater detail and manipulation than can be obtained with the flexible fiberoptic ureteroscope that is used in male cats (Hostutler et al., 2005).

2.3.5 Treatment

Identification of safe and effective treatment and prevention strategies of FIC will likely vary, depending on the underlying causes. The currently recommendation for cats with FIC includes environmental enrichment, stress reduction and feeding moist food. Additional treatments such as analgesics may help to minimize clinical signs and pain during acute episodes. For cats with severe recurrent episodes of FIC, administration of GAGs and amitriptyline may be considered in addition to standard treatment (Dru Forrester and Roudebush, 2007).

Gold standard of treatment in cats with FIC is to decrease the severity of clinical signs and increase the interval between episodes of FLUTD (Hostutler et al., 2005; Dru Forrester and Roudebush, 2007). Owners should be educated and understood about known factors involved in pathogenesis of FIC.

Environmental enrichment and stress reduction

Environmental enrichment is the process of improving or enhancing an animal's environment and care within the context of its behavioral biology and natural history; the goal is to increase behavioral choices and draw out species-appropriate behaviors (Laule, 2003). Based on psychoneuroendocrine abnormalities identified in cats with FIC, multimodal environmental modification (MEMO) is used to reduce stress response system for prevention and decreasing the severity and interval of FIC episodes before other treatment or drug therapy (Westropp and Buffington, 2004; Buffington et al., 2006a; Dru Forrester and Roudebush, 2007; Kruger et al., 2009). . For indoor-housed cats with FIC, this has been defined as providing all necessary resources, enhancing

interactions with owners, minimizing conflict, and making any changes gradually (Westropp and Buffington, 2004). Environmental modification includes as follow:

1. Changes in physical environment (space) for providing opportunities to play and rest (e.g., horizontal and vertical surfaces for scratching, hiding places, climbing platforms). Cats seem to prefer to monitor their surroundings from elevated vantage points. Playing a radio to habituate cats to sudden changes in sound and human voices also has been recommended, and videotapes to provide visual stimulation are available (Westropp and Buffington, 2004; Dru Forrester and Roudebush, 2007).

2. Increased owner-cat interaction, avoiding punishing. Cats also may enjoy playing with toys, particularly those that are small, move, and mimic prey characteristics (Westropp and Buffington, 2004; Dru Forrester and Roudebush, 2007).

3. Diet gradually change to canned food, increase water intake and manner food dishes and water bowls that may decrease competition for food and water and be quiet location for cats to eat alone. If a diet change is appropriate, offering the new diet in a separate adjacent container rather than removing the usual food and replacing it with new food permits the cats to express its preference. Natural cat feeding behavior also includes predatory activities, such as stalking and pouncing. These may be simulated by hiding small amounts of food around the house or by putting dry food in container from which the cat has to extract individual pieces or move something to release the food pieces if such interventions appeal to the cat. Cats also seem to have preferences for water that can be investigated. Water-related factors to consider include freshness, taste, movement and shape of container. As with foods, changes in water-related factors should be offered in such a way that permits the cat to express its preferences (Westropp and Buffington, 2004; Dru Forrester and Roudebush, 2007).

4. Enhanced litter box hygiene and management by providing in different locations throughout the house to the extent possible, particularly in multiple cat households (Horwitz, 1997). Placing litter boxes in quiet convenient locations that provide an escape route if necessary for the cat could help to improve condition for normal elimination behaviors. If different litters are offered, it may be preferable to test the cat’s preferences by providing them in separate boxes, because individual preferences for litter type have been documented. For cats with a history of urinary problems, unscented clumping litter should be considered. Litter boxes should be cleaned regularly and replaced; some cats seem quite sensitive to dirty litter boxes. Litter box size and

whether or not it is open or covered also may be important to some cats (Westropp and Buffington, 2004; Dru Forrester and Roudebush, 2007).

5. Efforts to reduce inter-cat conflict, which commonly is present when multiple cats are housed indoors together and health problem exist. Conflict among cats can develop because of threats to their perception of their overall status or rank in the home, from other animals in the home or from outside cats (Barry, 1999; Westropp and Buffington, 2004).

Multimodal environmental modification can significantly reduced lower urinary tract signs, fearfulness and nervousness after treatment for 10 months (Buffington et al., 2006a). Success of multimodal environmental modification, owners should understand how their efforts would contribute to the cat's recovery and remission of lower urinary tract system, available manage the cat's environment, and reduced the cat's perception of environmental threat whatever the potential sources and if available consult additional resources with helpful information (Buffington et al., 2006a).

Nutritional management

Nutritional management of cats with FIC has been recommended to dilute urine, which may decrease the concentration of substances in urine that irritate to urinary bladder mucosa. Feeding moist food related with increased water intake and urine volume in cats compared with feeding dry food. Although healthy cats drink more water when eating dry food compared with moist food, the total volume of water ingested is significantly greater when cats are fed moist food and more water is excreted in urine and feces. Feeding frequency affect water intake in cats in other words water intake increased significantly when cats were fed two or three meals compared with a single meal each day (Dru Forrester and Roudebush, 2007). There has been a report that cats fed a canned food had only an 11% recurrence of signs over a 1-year period, whereas cats fed the dry formulation of the same food had a 39% recurrence rate over the same period (Markwell et al., 1999). In another study, clinical improvement and decreased recurrence of clinical signs in cats with FIC related with feeding canned food by owners (Gunn-Moore and Shenoy, 2004). Therefore, it was recommended to gradually transition to moist food when FIC is diagnosed that require weeks to months in some cats. The owner should try to maintain urine specific gravity values less than 1.040 or lower based on clinical signs by using a refractometer. Finally, owner should consider feeding two to three meals per day instead of a single meal (Dru Forrester and Roudebush, 2007).

Synthetic formulation of feline facial pheromone

Synthetic feline facial pheromone therapy has been recommended to decrease signs of stress and anxiety-related behavior in cats, including urine marking and destructive scratching. In a double-blind placebo-controlled clinical study of 20 hospitalized cats, exposure to feline facial pheromone was associated with significant increase in grooming, interest in food, and food intake which these results suggested that feline facial pheromone had an anxiolytic effect in some cats (Griffith et al., 2000). There was a trend for cats exposed to facial pheromone to show fewer days with clinical signs of cystitis, reduced number of episodes and reduced negative behavioral traits, less aggression and fear, although there was no significant difference between placebo and feline facial pheromone for 2 months (Gunn-Moore and Cameron, 2004). Other reports show reduced urine marking during this pheromone treatment, which may be a consequence of reduced vigilance of the cats, because perception of their environment has been favorably altered (Mills and White, 2000). Feline facial pheromone may be justified in cats with FIC to reduce the impact of an activated symphathetic nervous system on the disease process and often is used in combination with environmental enrichment to decrease stress in cats with FIC (Hostutler et al., 2005).

Drug therapy

It may be indicated if environmental enrichment and modification in combination with dietary modification, enhanced water turnover, and feline facial pheromone use do not control clinical signs. Long-term drug use is reserved for the most severely affected cats that have persistent clinical signs or those that have multiple episodes of FIC.

Amitriptyline

Amitriptyline is a tricycle antidepressant with anticholinergic, antihistamine, sympatholytic, analgesia, and anti-inflammatory properties that has been used in cats with FIC and in women with interstitial cystitis (Chew et al., 1998; Kraijer et al., 2003; Kruger et al., 2003). In study of cats with severe recurrent FIC that failed to respond to other treatments administration of amitriptyline for 12 months was associated with decreased clinical signs during the last 6 months of treatment (Chew et al., 1998). Other study of amitriptyline treatment for 7 days presented no significant difference in the rate of recovery from pollakiuria or hematuria; overall, clinical signs recurred significantly faster and more frequently in cats treated with amitriptyline compared with control cats (Kruger et al., 2003). In similar study, amitriptyline combined with amoxicillin was no more effective than placebo and amoxicillin when given for 7 days to cats with FIC

(Kraijer et al., 2003). Thus, amitriptyline is considered not to be beneficial as a short-term treatment that therapeutic results depend on peripheral effects of the drug. Considering long-term treatment in cats with FIC continue severe or recurrent despite increased water intake and use environmental enrichment and reduce stress by amitriptyline (5-10 mg/cat oral once daily) (Dru Forrester and Roudebush, 2007)

Anti-inflammatory agents and analgesics

In acute episode, these drugs are recommended to manage discomfort in cats with FIC. No clinical trials evaluating these opioid analgesics (e.g., butorphanol, buprenorphine and fentanyl) or NSAID (e.g., meloxicam, piroxicam and ketoprofen) were studied. Prednisolone (1 mg/kg oral twice daily for 10 days) has no effect to reduce severity or duration of clinical signs in affected cats when compared with placebo (Osborne et al., 1996b).

Glucosamine

Glucosamine is the substrate for GAGs biosynthesis. Replacement of urothelial GAGs layer with oral or intravesicular administration of pentosan polysulfate sodium, exogenous semisynthetic GAGs analog) appears to significantly reduce the severity of symptoms in some human patients with IC (Bade et al., 1997; Davis et al., 2008). Other clinical trial found that heparin, hyaluronic acid, chondroitin sulphate and semisynthetic compound PPS are clinically available in human when instilled intravesically (Kurth and Parsons, 2003).

Theoretically, orally administered GAGs is excreted in the urine and attaches to defective urothelium, leading to decreased bladder permeability and less neurogenic inflammation. Treatment with GAGs (e.g., pentosan polysulfate sodium, glucosamine, chondroitin sulfate) has been suggested in cats with FIC, because defects in the GAGs layer covering the urinary bladder epithelium may play a role in the pathogenesis of disease like in human. Up to present, there is no evidence is available in veterinary medicine to indicate that such replacement decreases the severity or recurrence rate of FIC. There was only one study that determines whether administration of 125 mg glucosamine administered per mouth once daily for 6 months would reduce the severity or recurrence rate of clinical signs in cats with FIC compared with placebo. Owner assessments suggested that glucosamine-treated cats achieved a slightly greater improvement by the end of the study compared with the placebo group however, this difference was not statistically significant (Gunn-Moore and Shenoy, 2004).

NAG can be hydrolyzed to glucosamine both in vivo and vitro (Conchie and Hay, 1963; Talent and Gracy, 1996). Half-life of glucosamine in the blood is relatively short (Talent and Gracy, 1996). There was one previous study about the metabolism of glucosamine and NAG which found that NAG was less completely oxidized resulting in more excretion in the urine and also present concepts of the interrelationships between the metabolism of the amino sugars and D-glucose **(Figure 2)** (Kohn et al., 1962). In certain tissues, glucosamine has a higher affinity for glucose transporters than glucose itself and is incorporated into glycoproteins faster than glucose (Uldry et al., 2002). Moreover, there was also study about metabolism of N-acetyl-D-glucosamine 6- *O*[^{35}S]-sulphate in the rat by intraperitoneal injection (Lloyd, 1961).

Glucosamine (2-amino-2-deoxyalpha-D-glucose) is one of the two hexosamine sugars (six carbon amino sugars). Structurally, glucosamine is modified glucose, with an $–NH_3$ group replacing the –OH group found on carbon two (C-2). Glucosamine 6-phosphate (G6-P) is an amino monosaccharide (amino sugar) produced in the body by the combination of glutamine with fructose, through the enzymatic action of glucosamine synthetase (Kelly, 1998). About 90% of glucosamine administered orally as a glucosamine salt gets absorbed from the small intestine and from there it is transported, via the portal circulation, to the liver. It appears that a significant fraction of the ingested glucosamine is catabolized by first-pass metabolism in the liver (Setnikar and Rovati, 2001).

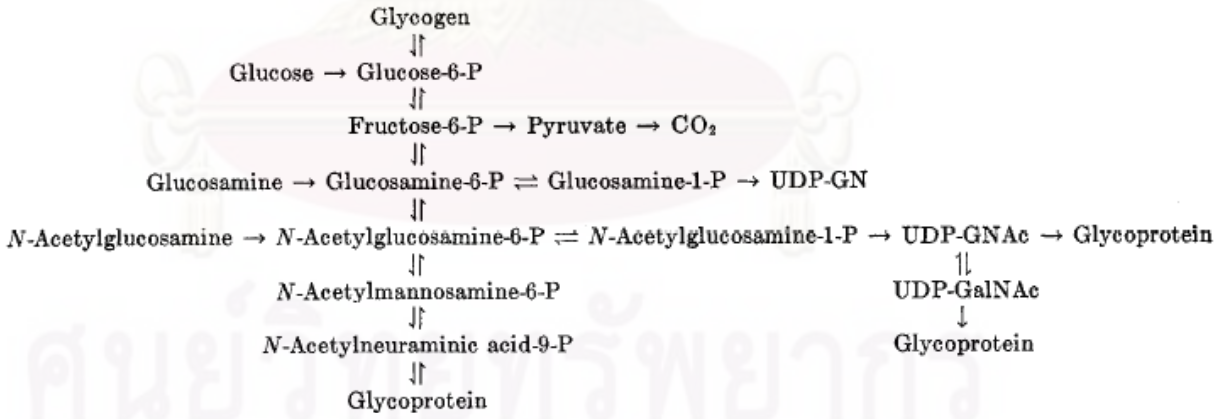


Figure 2 Interrelationships between the metabolism of the amino sugars and D-glucose (Kohn et al., 1962)

Note: = N-acetyl-D-glucosamine: GNAc (NAG), GalNAc = N-acetylgalactosamine.

The metabolic clearance of glycoproteins can be caused by specific receptors which recognize certain structural features on the glycan moieties (Ashwell and Harford, 1982). The two major receptors which have received extensive study are the asialoglycoprotein receptor (Stockert, 1995) and the mannose (Man) receptor (Stahl, 1992). The mannose receptor recognizes high-Man N-linked glycans and terminal N-acetyl-D-glucosamine (tNAG) residues and could also be referred to as the mannose/N-acetyl-D-glucosamine (Man/NAG) receptor. Animal studies of glycoprotein clearance have shown that alterations in the glycan structures can cause changes in their pharmacokinetic (PK) properties (Morell et al., 1971). In the rapid initial phase of clearance, glycans carrying tNAG were selectively cleared from the circulation when analysis of the acidic glycans in humans and cynomolgus monkeys that conclude the mannose receptor, which can also bind tNAG, causes the variable clearance of this molecule (Jones et al., 2007). However, there was not study about the receptors in cat.

Glucosamine is available commercially as a nutritional supplement in three forms: glucosamine HCl, glucosamine sulphate and N-acetyl-D-glucosamine. All three forms are water soluble, the salt acting as a delivery vehicle. At neutral and physiological pH, the amino group in glucosamine is protonated, resulting in a positive charge. Salt forms of glucosamine contain negative anions to neutralise the charge. In the case of glucosamine hydrochloride, the anion is chloride, and in glucosamine sulphate the anion is sulphate. NAG is a delivery form of glucosamine in which the amino group is acetylated, thus neutralising its charge (Thakral et al., 2007).

Orally administering NAG in rat no obvious toxicity at concentration 5% in the diet for 13 weeks (Lee et al., 2004). Oral administration of glucosamine at very large doses (5000–15,000 mg/kg body weight) is well tolerated without documented toxicity (Anderson et al., 2005). The most common symptoms reported with placebo and oral glucosamine are: mild gastrointestinal symptoms including constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension and abdominal cramps. Headache and skin rash or pruritis are also known to occur (Kelly, 1998).

Chapter III
Materials and Methods

3.1 Animals

Cats presented to Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University with clinical signs of dysuria, stranguria, hematuria, pollakiuria and/or urination in inappropriate places were included without breed, age, and gender preference. Cats with history of previous urolithiasis, urinary tract bacterial infection, bladder neoplasia or congenital deformity of urinary tract were excluded. The owners of each cat were asked to sign written consent statements to allow their cats to be studied. In addition, fifteen normal cats were also included to measure plasma and urinary GAG.

3.2 Study Design

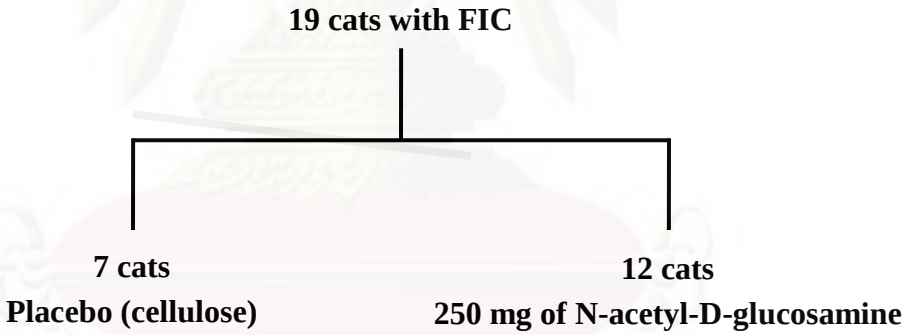


Figure 3 Diagram of study design in this study

Double-blind placebo-controlled study was performed. All cats presented with clinical signs suggesting FLUTD such as dysuria, pollakiuria, hematuria and/or inappropriate urination were included. The information of diets, water intake and environment were collected. Physical examination, survey abdominal radiography, double contrast cystography, ultrasonography and blood collection CBC, blood urea nitrogen and creatinine concentrations were performed. Urines were collected for urinalysis and/or urine culture. Cats with evidence of urolithiasis, bacterial infection, bladder neoplasia or congenital deformity were excluded.

Nineteen cats with FIC were randomly divided into two groups; treatment and placebo group. Twelve cats in the treatment group received 250 mg of oral N-acetyl-D-glucosamine (Cystaid®, VetPlus Ltd) daily for 28 days while seven cats in placebo group received placebo **(Figure3)**. Both groups were monitored for clinical improvement on day 0, 7, 14, 21, 28 and 56. Plasma and urine of the cats were collected for analyzing of GAGs levels on day 0, 7, 14, 21, 28 and 56. Owners were given client education on caring of their cats.

3.3 Clinical Examination

Cats of both groups were subjected to a complete history and physical examination. Blood samples were taken for complete blood count (CBC) and biochemical analysis. Urine was collected by catheterization or voiding of midstream urine sample and analyzed by chemical testing using commercial dipstick (pH, protein, glucose, ketone, blood and others). The urine specific gravity is measured by refractometer. Urine sediment was examined for microscopic findings of casts, RBC, WBC, and crystals. Urine were diluted in a transport medium and subjected to microbiological analysis for determine positive urine bacterial culture ($>10^5$ CFU/ml). Survey abdominal radiography, double contrast cystography and/or ultrasonography of the lower urinary tract were performed. Visual analogue scale (score 100 mm) which has been modified from Gunn-Moore (2003) was evaluated by owner of each cat for pain of urination (micturiction). The clinical signs and behaviors were adapted to 7 questions as follow:

1. increased frequency of urination
2. crying out while urinating
3. the presence of blood in the urine (macroscopic haematuria)
4. urination outside the litter box
5. increase grooming around the perineum
6. altered behavior
7. straining while urinating

The score for each question is 10 points. The 0 point means that the least pain and gradually increased until maximum score of 10 points as the most severe pain **(Appendix 2)**. Therefore, the more cats get points, the more cats are in pain.

Owners and clinicians were asked to assess result of treatment; 1 = complete cure, 2 = improved, 3 = no change and 4 = worsened. Cats were excluded from the study if signs of known urinary tract disease or any other serious conditions are found during examination such as severe anemia including protein >1 g/l from urinalysis without hematuria.

3.4 Laboratory Examination

3.4.1 Blood collection

1) Collect blood from cephalic or femoral vein
2) Keep blood in anticoagulant tubes (EDTA and heparin)
3) Analyze for complete blood count (CBC) by manual blood count
4) Plasma was taken by centrifugation (Heraeus Biofuge 22R) at 3,000 rpm for 10 min at 4^{o}C and measured for chemistry profiles including blood urea nitrogen (BUN) (Patton and Crouch, 1977), creatinine by the alkaline picrate method, alanine aminotransferase (ALT) (Reitman and Frankel, 1957), and alkaline phosphatase (Bessey et al., 1946).
5) Store the plasma at -80 ^{o}C for further GAGs analysis

3.4.2 Urine collection

1) Collect urine by cystocentesis and/or voiding during midstream
2) Centrifuge (IECCR-6000, Damon/IEC Division) collected urine at 3,000 rpm for 30 min at 4^{o}C
3) Keep the supernatant at -20 ^{o}C for further analysis

3.4.3 Plasma GAGs extraction and purification according to Pereira et al. (2004)

1) Hold 0.1 ml plasma in 0.1 ml of 0.5 M NaOH at 37 ^{o}C for 12 hour to cleave covalent O-linkages between protein and carbohydrate and to release the glycosaminoglycan chains from proteoglycans or peptidoglycans
2) Isolate the glycosaminoglycan chains by ion exchange chromatography on HiTrap Q Sepharose Fast Flow, 1 ml (GE Healthcare Bio-Science, USA) in chloride form

3.4.4 Urine GAGs extraction and purification according to de Jong et al. (1989)

1) Dilute the urine with an equal volume of distilled water and adjust to pH 4.0-4.5 with 1 M HCl.
2) Add cetyltrimethylammonium bromide (CTAB) solution at final concentration is 1g/L.
3) Incubate the solution at 4 ^{o}C for 24 hours and centrifuge
4) Wash the precipitate 2 times with ethanol, dry at 37^{o}C, and dissolve in 0.5 ml of 0.1 M NaOH.

3.4.5 Plasma GAGs quantitation

1) Quantify the plasma sulphated glycosaminoglycans by spectrophotometric method with 1,9-dimethylmethylene blue (DMB) which has been modified from Farndale et al. (1986) as follows:
    - 2.4 ml of 16 µg/ml of DMB solution (3.04 g glycine, 2.37 g NaCl and 95 ml 0.1 M HCl) was mixed with 0.1 ml of plasma solution.
2) Stand the mixture for 5 min and measure for an absorbance at 525 nm by Evolution 60 UV-Visible Spectrophotometer (Thermo Fisher Scientific, USA) with semi-micro cuvette (Brand, Germany).
3) Chondroitin 4-sulphate solutions were used for standard curve (the calibration interval is 0-100 mg/l).
4) The result were calculated as GAGs levels (µg/ml)

4.4.6 Urine GAGs quantitation

1) Quantify the urine sulphated glycosaminoglycans by spectrophotometric method with 1,9-dimethylmethylene blue (DMB) as discuss by Panin et al. (1986) as follow:
    - 2.5 ml of 10.67 µg/ml of DMB solution (5 ml of ethanol, 2 g of sodium formate and 2 ml of formic acid) was mixed with 0.1 ml of urine solution and adjusted to 3 ml of total volume with distilled water.
2) Stand the mixture for 5 min and measure for an absorbance at 525 nm by Evolution 60 UV-Visible Spectrophotometer (Thermo Fisher Scientific, USA) with semi-micro cuvette (Brand, Germany).
3) Chondroitin 4-sulphate solutions were used for standard curve (the calibration interval is 0-100 mg/l).

4) Corrects the urinary GAG values with regard to the amount of creatinine and measures by the alkaline picrate method.
5) The final results were presented in both GAG/creatinine ratio ($x10^{-3}$) and GAG levels (µg/ml).

3.5 Statistical Analysis

Signalments and urinalysis results were described as descriptive statistics. Complete blood counts and blood chemistry are presented as mean±SEM and were tested between placebo and treatment group by student t-test. Modified visual analogue scales were compared inter-groups by Mann-Whitney U test and intra-groups (pre and post-treatment) by Wilcoxon matched-pairs signed-ranks test. Mean total score of VAS from day 0 to day 28 in each question (Q1-Q7) in placebo and treatment group were compared by Mann-Whitney U test. Mean±SEM was calculated from plasma GAG levels, urinary GAG levels, urine creatinine and GAG/creatinine ratio in both groups. These values were compared using a Student's t-test between placebo and treatment group and between FIC and normal cats. Paired t-test was used for intra-group using SPSS Statistics 17.0 program. P-value of less than 0.05 is considered significant.

Chapter IV
Results

4.1 Signalments

The average age of 19 cats with FIC in the study is 4.41±3.92 years old. Most cats in this study are domestic short haired (DSH), which is 78.9% (15/19) of all cats **(Figure 4)**. In addition, there were pure breeds (21%; 4/19) such as Siamese (10.5%; 2/19) and Persian (10.5%; 2/19). The castrated male cat has the highest percent (57.9%; 11/19) when compared with male (31.6%; 6/19) and sterile female (10.5%; 2/19) **(Figure 5)**. The mean weight of FIC cats is 4.62±0.97 kg. There are 52.6% of cats living outdoor (10/19) and 47.4% of cats living indoor (9/19) **(Figure 6**). Approximately 90% of FIC cats in the study lived with other cats (17/19) in the same household and there are 10.5% of the FIC cats (2/19) which stay alone **(Figure 7)**. From family history of the cats with FIC, we found that 78.9% (15/19) of the owners never have other cats in the same house as FIC and/or FLUTD and 21.1% (4/19) of the cats have relation-cats which ever were FIC and/or FLUTD. The types of foods consumed by cats with FIC were dry (52.6%; 10/19), mixed (36.8%; 7/19) and homemade diet (10.5%; 2/19) **(Figure 8)**. In addition, all of FIC cats received water ad libitum.

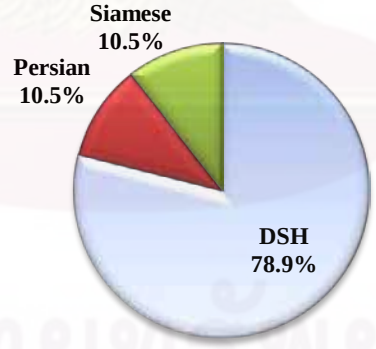


Figure 4 Percentage of FIC cats according to breed.

Note: DSH = domestic short hair

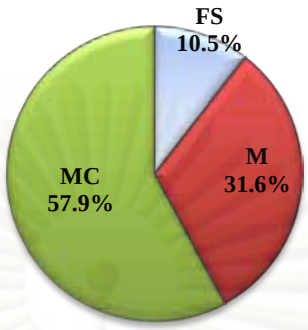


Figure 5 Percentage of FIC cats according to gender.

Note: FS = sterile female
M = male
MC = castrated male

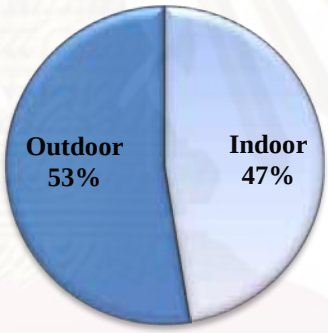


Figure 6 Percentage life style of FIC cats

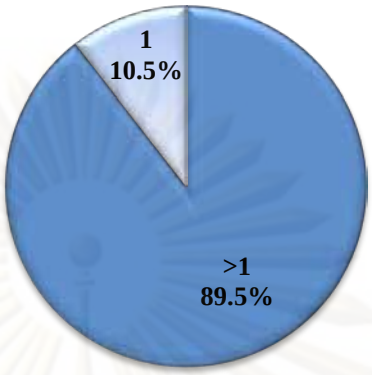


Figure 7 Number and percentage of other cats in same household as the FIC cats

Note: 1 = there was one cats in the same household with FIC cats.
>1 = there were more than one cats in the same household with FIC cats.

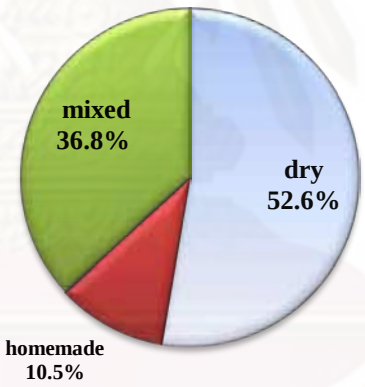


Figure 8 Percentage of type of food consumed by FIC cats

Note: Mixed = combined any type of food
Dry = dry food
Homemade = foods prepared by owners

4.2 The results of complete blood count and serum chemistry test in FIC cats

From the results of CBC and serum chemistry profiles, there were no significantly differences in CBC value except monocytes that there was higher in the treatment group (74±23 cells/µl) when compared to placebo (10±10 cells/µl) on day 56 **(Appendix 3, 4, 5 and 6)**. There were no significant differences in BUN and creatinine levels from day 0 to day 28 of the study **(Figure 9 and 10)**. However, ALT value was statistically significant difference between placebo and treatment group on day 0 **(Figure 11)**.

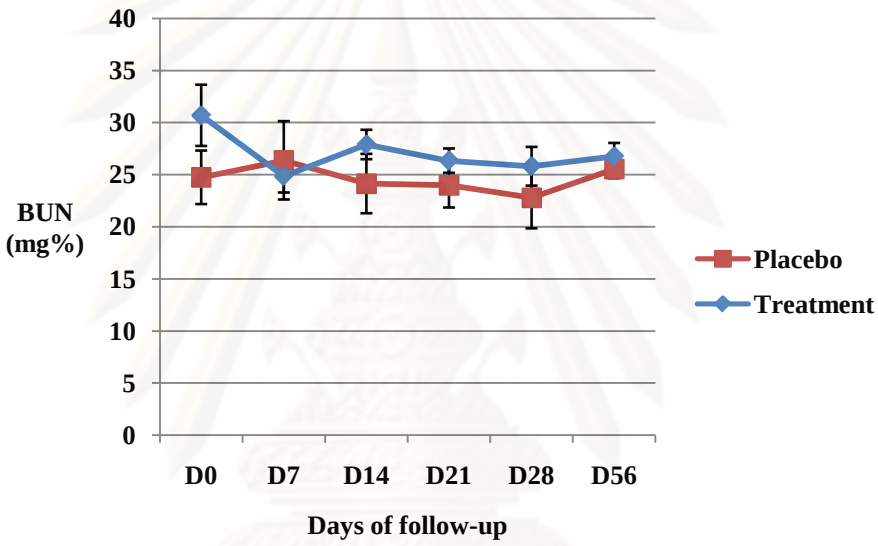


Figure 9 Mean±SEM of blood urea nitrogen (BUN) between placebo and treatment group

Note: Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

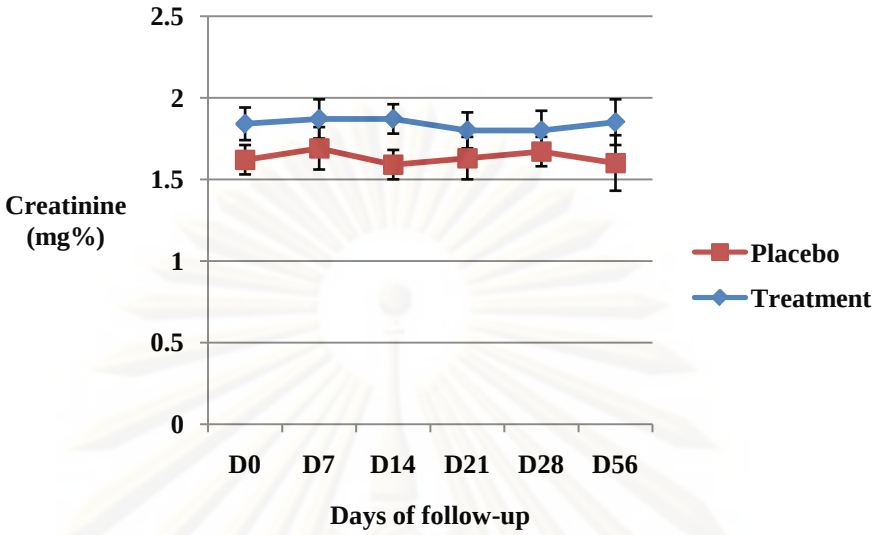


Figure 10 Mean±SEM of creatinine between placebo and treatment group

Note: Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

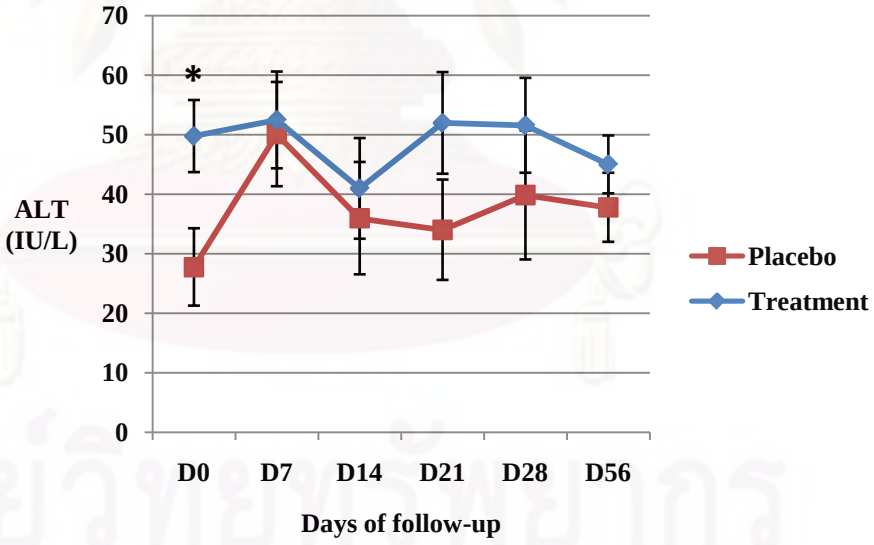


Figure 11 Mean±SEM of alanine aminotransferase (ALT) between placebo and treatment group

Note: *$P<0.05$ when compared between placebo and treatment group
Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

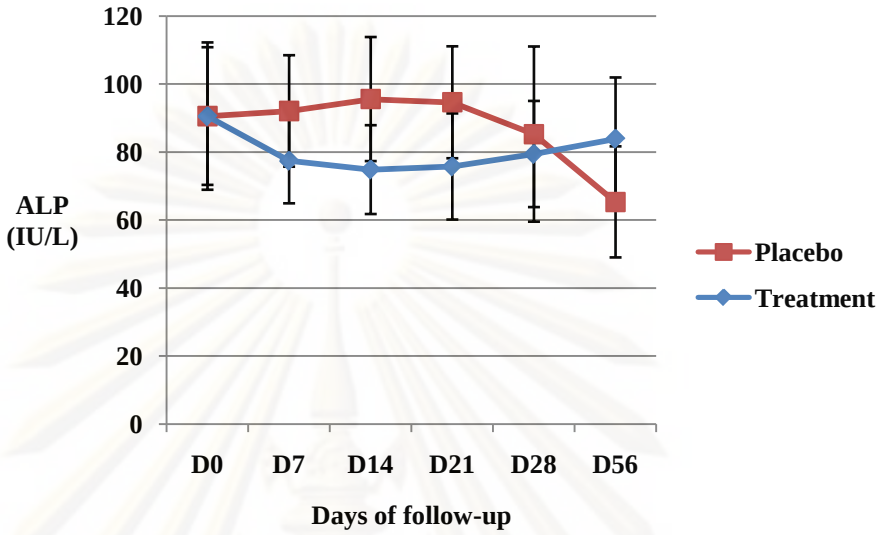


Figure 12 Mean±SEM of alkaline phosphatase (ALP) between placebo and treatment group

Note: Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

4.3 The results of urinalysis in FIC cats

Chemical Property

Urine specific gravity of FIC cats in this study varies from 1.014 to >1.050 in placebo and treatment group from day 0 to day 28. Urine pH also ranged from pH 5 to pH 9. Most of leukocyte values were 3+ (range from 0 to 4+). Protein values varied from 0 to 3+.

Cytology Results

Results from the urinalysis in this study demonstrated the presence of hematuria and proteinuria in the urine sample of FIC cats on the first day of diagnosis (day 0). At the end of the treatment (D28), it was found that 33% of FIC cats received NAG had decreased number of red blood cells in the urine sediment. The amount of RBC in urine sediment increased after we stop given NAG to FIC cats for twenty-eight days (D56).

Most of crystal in urine of cats with FIC in the study was struvite. There were other crystals such as Ca^{2+} oxalate and amorphorous crystal. No casts were found except rare waxy cast in only one cat. There were some rod motile bacteria, epithelial cells such as transitional and squamous cells, RBC clumps, WBC clumps, debris and fat droplet in urine.

Table 3 Urinalysis results of placebo and treatment groups on day 0 of the follow-up.

Parameters	**Normal Value#**	**Placebo**	**Treatment**
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (4/7) 1.040 (1/7) 1.035 (1/7) 1.028 (1/7)	>1.050 (3/12) 1.049 (2/12) 1.045 (1/12) 1.040 (1/12) 1.032 (1/12) 1.026 (2/12) 1.021 (1/12) 1.015 (1/12)
pH	6-7	9 (2/7) 7 (2/7) 6 (3/7)	9 (2/12) 8 (3/12) 7 (3/12) 6 (3/12) 5 (1/12)
Leukocyte	Slight or negative	3+ (7/7)	3+ (9/12) 2+ (2/12)
Protein	Slight or negative	3+ (3/7) 2+ (2/7) negative (2/7)	3+ (2/12) 2+ (1/12) 1+ (8/12) negative (1/12)
Blood	negative	4+ (5/7) 3+ (1/7) negative (1/7)	4+ (6/12) 3+ (1/12) negative (5/12)
Glucose	negative	negative (7/7)	2+ (1/12) negative (11/12)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 4 Urinalysis results (cytology) of placebo and treatment groups on day 0 of the follow-up.

Parameters	**Placebo**	**Treatment**
Cytology		
RBC	TNTC (2/7)	TNTC (2/12)
	100-200cells/HPF (1/7)	50-100cells/HPF (1/12)
	50-100cells/HPF (1/7)	20-30cells/HPF (1/12)
	10-20cells/HPF (1/7)	10-20cells/HPF (1/12)
	0-1cells/HPF (1/7)	5-10cells/HPF (1/12)
	negative (1/7)	negative (6/12)
WBC	TNTC (1/7)	TNTC (1/12)
	3-5cells/HPF (3/7)	20-30cells/HPF (1/12)
	1-2cells/HPF (1/7)	5-10cells/HPF (1/12)
	negative (2/7)	3-5cells/HPF (1/12)
		2-3cells/HPF (1/12)
		negative (7/12)
Crystals	struvite 3+ (1/7)	struvite 2+ (1/12)
	struvite 2+ (1/7)	Ca^{2+}oxalate1+ (1/12)
	struvite rare (2/7)	struvite rare (3/12)
	Ca^{2+}oxalate rare (1/7)	negative (8/12)
	negative (3/7)	
Casts	negative (7/7)	negative (12/12)
Others	TSC rare (2/7)	SQC rare (3/12)
	fat droplet (1/7)	RBC clumps (1/12)
	mucous (1/7)	WBC clumps (1/12)
	debris (1/7)	rod motile bacteria 3+ (1/12)
	negative (3/7)	negative(7/12)

Note: TNTC = Too numerous too count
TSC = Transitional epithelial cells
SQC = Squamous epithelial cells

Table 5 Urinalysis results of placebo and treatment groups on day 7 of the follow-up.

Parameters	**Normal Value**[#]	**Placebo**	**Treatment**
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (4/7) 1.042 (1/7) 1.040 (1/7) 1.029 (1/7)	>1.050 (6/12) 1.046 (1/12) 1.042 (1/12) 1.040 (1/12) 1.026 (1/12) 1.016 (1/12) 1.015 (1/12)
pH	6-7	9 (1/7) 8 (2/7) 7 (1/7) 6 (3/7)	9 (1/12) 8 (3/12) 7 (2/12) 6 (5/12) 5 (1/12)
Leukocyte	Slight or negative	3+ (7/7)	3+ (9/12) 2+ (3/12)
Protein	Slight or negative	3+ (1/7) 2+ (2/7) 1+ (4/7)	3+ (1/12) 2+ (2/12) 1+ (8/12) negative (1/12)
Blood	negative	4+ (3/7) 2+ (2/7) negative (2/7)	4+ (4/12) 3+ (1/12) 1+ (2/12) negative (5/12)
Glucose	negative	negative (7/7)	negative (12/12)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 6 Urinalysis results (cytology) of placebo and treatment groups on day 7 of the follow-up.

Parameters	Placebo	Treatment
Cytology		
RBC	TNTC (1/7) 10-20cells/HPF (1/7) 1-2cells/HPF (1/7) negative (4/7)	TNTC (3/12) 30-50cells/HPF (1/12) 5-10cells/HPF (1/12) 2-3cells/HPF (1/12) negative (6/12)
WBC	TNTC (1/7) 1-2cells/HPF (1/7) negative (5/7)	TNTC (2/12) 30-50 cells/HPF (1/12) 5-10 cells/HPF (2/12) 2-3cells/HPF (1/12) 1-2cells/HPF (1/12) negative (5/12)
Crystals	struvite3+ (2/7) struvite2+ (1/7) negative (4/7)	amorphous3+ (1/12) amorphous rare (1/12) struvite3+ (1/12) struvite1+ (1/12) struvite rare (1/12) negative (8/12)
Casts	negative (7/7)	negative (12/12)
Others	SQC rare (2/7) RBC clump (1/7) rod bacteria (1/7) negative (3/7)	fat droplet (1/12) SQC 10cells/HPF (1/12) rod bacteria (3/12) negative (6/12)

Note: TNTC = Too numerous too count
SQC = Squamous epithelial cells

Table 7 Urinalysis results of placebo and treatment groups on day 14 of the follow-up.

Parameters	**Normal Value**[#]	**Placebo**	**Treatment**
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (5/7) 1.042 (1/7) 1.010 (1/7)	>1.050 (4/11) 1.050 (1/11) 1.042 (1/11) 1.041 (1/11) 1.040 (1/11) 1.038 (1/11) 1.019 (1/11) 1.018 (1/11)
pH	6-7	9 (1/7) 8 (2/7) 7 (3/7) 6 (1/7)	9 (1/11) 8 (2/11) 7 (4/11) 6 (3/11) 5 (1/11)
Leukocyte	Slight or negative	3+ (6/7) 2+ (1/7)	4+ (1/11) 3+ (7/11) 2+ (1/11) 1+ (2/11)
Protein	Slight or negative	3+ (1/7) 2+ (2/7) 1+ (3/7) negative (1/7)	2+ (3/11) 1+ (7/11) negative (1/11)
Blood	negative	4+ (4/7) 2+ (1/7) 1+ (1/7) negative (1/7)	4+ (5/11) 2+ (1/11) 1+ (1/11) negative (4/11)
Glucose	negative	negative (7/7)	negative (11/11)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 8 Urinalysis results (cytology) of placebo and treatment groups on day 14 of the follow-up.

Parameters	Placebo	Treatment
Cytology		
RBC	10-20cells/HPF (2/7) negative (5/7)	TNTC (2/11) 1-2cells/HPF (1/11) 5-10cells/HPF (1/11) negative (7/11)
WBC	2-3 cells/HPF (1/7) 0-1cells/HPF (1/7) negative (5/7)	TNTC (1/11) 2-3cells/HPF (1/11) 1-2cells/HPF (2/11) negative (7/11)
Crystals	struvite 1+ (4/7) negative (3/7)	struvite 3+ (1/11) struvite 1+ (1/11) negative (9/11)
Casts	negative (7/7)	negative (11/11)
Others	TSC rare (1/7) SQC rare (1/7) fat droplet (1/7) negative (5/7)	TSC clump (1/11) renal tubular cell (1/11) amorphorous trace (1/11) rod motile bacteria (1/11) cocci rare (1/11) fat droplet (1/11) debris (1/11) negative (6/11)

Note: TNTC = Too numerous too count
TSC = Transitional epithelial cells
SQC = Squamous epithelial cells

Table 9 Urinalysis results of placebo and treatment groups on day 21 of the follow-up.

Parameters	Normal Value[#]	Placebo	Treatment
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (3/7) 1.050 (1/7) 1.048 (1/7) 1.038 (1/7) 1.018 (1/7)	>1.050 (5/10) 1.050 (1/10) 1.042 (1/10) 1.041 (1/10) 1.038 (1/10) 1.014 (1/10)
pH	6-7	9 (1/7) 8 (3/7) 7 (1/7) 6 (2/7)	7 (6/10) 6 (3/10) 5 (1/10)
Leukocyte	Slight or negative	3+ (5/7) 2+ (1/7) negative (1/7)	3+ (7/10) 2+ (1/10) 1+ (2/10)
Protein	Slight or negative	3+ (2/7) 2+ (1/7) 1+ (3/7) negative (1/7)	1+ (6/10) negative (4/10)
Blood	negative	4+ (2/7) 3+ (1/7) negative (4/7)	4+ (3/10) 3+ (2/10) 1+ (1/10) negative (4/10)
Glucose	negative	negative (7/7)	negative (10/10)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 10 Urinalysis results (cytology) of placebo and treatment groups on day 21 of the follow-up.

Parameters	Placebo	Treatment
Cytology		
RBC	TNTC (1/7) 30-50cells/HPF (1/7) 2-3cells/HPF (1/7) negative (4/7)	30-50cells/HPF (1/10) negative (9/10)
WBC	30-50cells/HPF (1/7) 10-20cells/HPF (1/7) negative (5/7)	1-2cells/HPF (1/10) negative (9/10)
Crystals	Hippuric acid 10-20/LPF (1/7) struvite rare (3/7) Ca^{2+} oxalate rare (1/7) negative (4/7)	struvite 3+ (1/10) amorphorous (1/10) negative (8/10)
Casts	negative (7/7)	negative (10/10)
Others	SQC rare (2/7) fat droplet (1/7) negative (4/7)	TSC (2/10) RBC clump (1/10) rod motile bacteria (numerous) (1/10) rod 2+ (1/10) debris (1/10) negative (6/10)

Note: TNTC = Too numerous too count
TSC = Transitional epithelial cells
SQC = Squamous epithelial cells

Table 11 Urinalysis results of placebo and treatment groups on day 28 of the follow-up.

Parameters	Normal Value[#]	Placebo	Treatment
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (6/7) 1.028 (1/7)	>1.050 (8/12) 1.049 (1/12) 1.039 (1/12) 1.024 (1/12) 1.020 (1/12)
pH	6-7	9 (1/7) 8 (2/7) 7 (2/7) 6 (1/7) 5 (1/7)	8 (1/12) 7 (5/12) 6 (4/12) 5 (2/12)
Leukocyte	Slight or negative	3+ (6/7) 2+ (1/7)	3+ (9/11) 2+ (1/11) negative (1/11)
Protein	Slight or negative	3+ (1/7) 2+ (3/7) 1+ (2/7) negative (1/7)	3+ (1/12) 2+ (2/12) 1+ (5/12) negative (4/12)
Blood	negative	4+ (2/7) 2+ (1/7) negative (4/7)	4+ (2/12) 2+ (1/12) negative (9/12)
Glucose	negative	1+ (1/7) negative (6/7)	4+ (1/12) negative (11/12)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 12 Urinalysis results (cytology) of placebo and treatment groups on day 28 of the follow-up.

Parameters	Placebo	Treatment
Cytology		
RBC	TNTC (2/7) negative (5/7)	TNTC (1/12) 3-5cells/HPF (1/12) negative (10/12)
WBC	30-50cells/HPF (1/7) 3-5cells/HPF (1/7) negative (5/7)	30-50 cells/HPF (1/12) 1-2cells/HPF (2/12) negative (9/12)
Crystals	struvite rare (2/7) MAT + CaOX (1/7) negative (4/7)	struvite2+ (1/12) struvite rare (2/12) amorphorous (1/12) negative (9/12)
Casts	negative (7/7)	waxy cast rare (1/12) negative (11/12)
Others	SQC rare (3/7) fat droplet (1/7) negative (3/7)	RBC clumps (1/12) SQC 1-2cells/HPF (1/12) rod motile bacteria (2/12) cocci bacteria (1/12) sperm (1/12) negative (7/12)

Note: MAT + CaOX = Magnesium ammonium phosphate and Calcium Oxalate
SQC = Squamous epithelial cells
TNTC = Too numerous too count

Table 13 Urinalysis results of placebo and treatment groups on day 56 of the follow-up.

Parameters	**Normal Value**[#]	**Placebo**	**Treatment**
Chemical			
Sp.Gr.	1.020-1.040	>1.050 (4/6) 1.050 (1/6) 1.028 (1/6)	>1.050 (3/9) 1.042 (1/9) 1.040 (1/9) 1.034 (1/9) 1.028 (1/9) 1.024 (1/9) 1.015 (1/9)
pH	6-7	8 (1/6) 7 (3/6) 6 (2/6)	9 (1/9) 8 (1/9) 7 (4/9) 6 (2/9) 5 (1/9)
Leukocyte	Slight or negative	3+ (5/6) negative (1/6)	3+ (8/9) 2+ (1/9)
Protein	Slight or negative	2+ (2/6) 1+ (2/6) negative (2/6)	3+ (1/9) 2+ (2/9) 1+ (4/9) negative (2/9)
Blood	negative	4+ (1/6) negative (5/6)	4+ (6/9) negative (3/9)
Glucose	negative	negative (6/6)	negative (9/9)

#Normal Reference Value from Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 72-85.

Table 14 Urinalysis results of placebo and treatment groups on day 56 of the follow-up.

Parameters	Placebo	Treatment
Cytology		
RBC	100-200/HPF (1/6) negative (5/6)	100-200/HPF (1/9) 100/HPF (1/9) 10-20/HPF (2/9) 3-5/HPF (1/9) 2-3/HPF (1/9) negative (3/9)
WBC	20-30/HPF (1/6) negative (5/6)	TNTC (1/9) 10-20/HPF (1/9) 5-10/HPF (1/9) 1-2/HPF (2/9) negative (4/9)
Crystals	negative (6/6)	struvite 3+ (1/9) struvite 1+ (2/9) negative (6/9)
Casts	negative (6/6)	negative (9/9)
Others	SQC rare (1/6) TSC rare (1/6) fat droplet (2/6) negative (3/6)	TSC 1-5/HPF (1/9) WBC clumping (1/9) rod motile bacteria (numerous) (2/9) cocci bacteria 3+ (1/9) negative (4/9)

Note: TNTC = Too numerous too count
TSC = Transitional epithelial cells
SQC = Squamous epithelial cells

4.4 Visual Analogue Scales Evaluation

Total VAS score of all questions between pre-treatment (day 0) and post-treatment (day 28) in placebo and treatment group were compared by Wilcoxon matched-pairs signed-ranks test. It was found that the mean of total VAS score on pre-treatment (D0) (15.86±3.25) and post-treatment (D28) (9.71±3.5) of the placebo group were not significant differences. Pre-treatment (D0) (18.8±2.27) and post-treatment (D28) (15.58±3.37) in treatment group were also not statistically different **(Figure 13)**. However, there was a decreased trend in the mean of total VAS score from day 0 to day 28 in both groups. The mean score in treatment group was higher than placebo group (**Figure 14**).

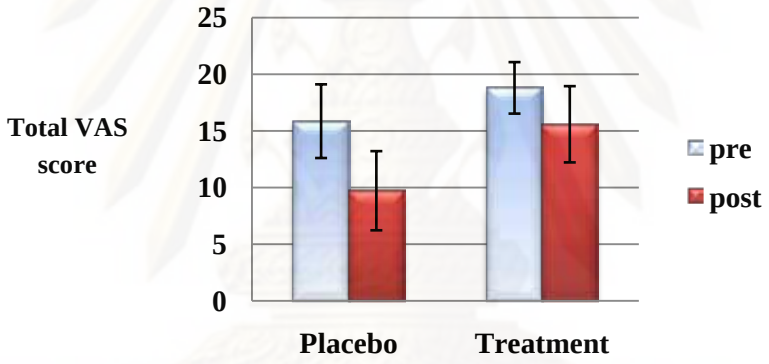


Figure 13 Mean±SEM of total VAS score in placebo and treatment group on pre-treatment (day 0) and post-treatment (day 28)

Note: pre = pre-treatment (D0)
post = post-treatment (D28)
placebo = cats received placebo
treatment = cats received N-acetyl-D-glucosamine

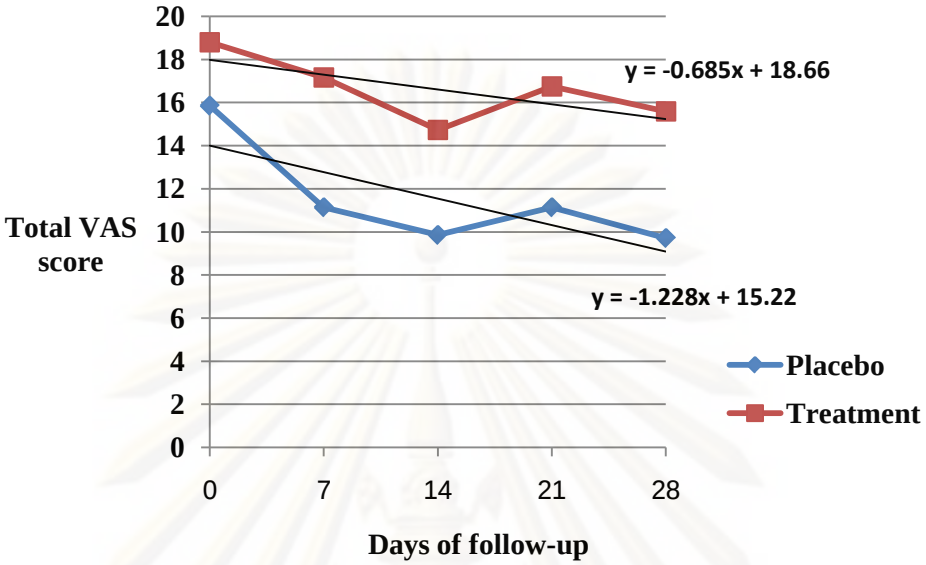


Figure 14 Mean of total VAS score from day 0 to day 28 in placebo and treatment group

Note: Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

Mean total VAS score of each question (Q1-Q7) compared from day 0 to day 28 in placebo and treatment group were compared by Mann-Whitney U test. There were no significantly differences between placebo and treatment group in all questions (**Table 15**). However, there were three questions that have the highest mean score such as question 1, 5 and 7 in placebo (120±24.48, 92.71±21.25 and 33.29±18.86) and treatment group (114.17±15.18, 100.92±16.29 and 74.08±17.45) **(Table 15 and Figure 15)**.

Table 15 Mean±SEM of total visual analogue scale (from day 0 to day 28) in each question (Q1-Q7) in placebo and treatment group

Group	Q1	Q2	Q3	Q4	Q5	Q6	Q7
Placebo	120±24.48	1.29±1.13	11.14±7.34	39.57±21.59	92.71±21.25	19.29±12.96	33.29±18.86
Treatment	114.17±15.18	18.92±11.31	30.83±13.11	69.67±16.40	100.92±16.29	45.33±15.00	74.08±17.45

Note: Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine

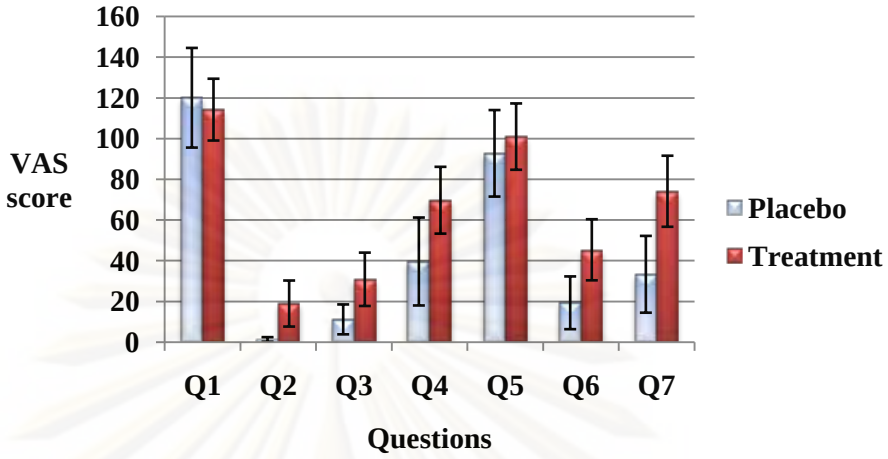


Figure 15 Mean±SEM of visual analogue scales for each question compared between placebo and treatment group

Owners were asked to assess results of treatment that classified as follow:

1 = complete cured
2 = improved
3 = no changed
4 = worsened

After completed the study, it was found that the owner of each cat in the placebo group think that their cat were complete cured (42.9%) and improved (42.9%). Owner of cat in the treatment group also reported to have complete cured (41.7%) and improved (41.7%) **(Table 16)**.

Table 16 Assessment of the outcome at the end of the study from cats' owner by personal interview (D28)

Group	Percent outcome of treatment		
	Complete cured	Improved	No changed
Placebo (n=7)	42.9% (3/7)	42.9% (3/7)	14.3% (1/7)
Treatment (n=12)	41.7% (5/12)	41.7% (5/12)	16.7% (2/12)

4.5 GAG levels in plasma and urine of FIC cats

The mean plasma GAG levels of normal cats were 26.09±2.94 µg/ml and cats with FIC were 27.29±3.10 µg/ml which was not statistically difference **(Table 17)**. In contrast, the mean urinary GAG level for normal cats (44.26±6.16 µg/ml) was significantly higher than that of the cats with FIC (7.97±1.20 µg/ml) (P<0.001) **(Table 17)**. In addition, the mean GAG/Creatinine ($x10^{-3}$) ratio from normal cats (14.23±3.47 $x10^{-3}$) was also significantly higher than that of the cats with FIC (3.11±0.62 µg/ml) (P<0.05) **(Table 17)**.

Table17 Mean±SEM of GAG levels in plasma and urine between normal cats and cats with FIC from day 0.

Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Creatinine (mg%)	GAG/Creatinine ($x10^{-3}$)
Normal cats	26.09±2.94 (10)	44.26±6.16**(10)	3.89±0.61 (9)	14.23±3.47* (9)
FIC cats	27.29±3.10 (18)	7.97±1.20 (18)	3.36±0.38 (18)	3.11±0.62 (18)

* P<0.05 when compared between normal cats and cats with FIC

** P<0.001 when compared between normal cats and cats with FIC

This study found that there was an increased in plasma GAG levels from day 0 to day 28 in the treatment group and a decreased on day 56 in treatment group 28 days after stop given NAG **(Figure 16)**. There was a slightly declined in plasma GAG level of placebo group on day 14 to 56 **(Figure 16)**.

There were significantly differences of GAG levels in plasma on day 0 (27.46±3.9 µg/ml) compared with day 21 (39.96±5.34 µg/ml) and day 28 (39.91±6.74 µg/ml) in treatment group (P<0.05) **(Table 18 and Figure 16)**. The mean plasma GAG levels for treatment group (39.96±5.34µg/ml) was significantly higher than that of the placebo group (24.20±3.35µg/ml) on day 21 (P<0.05) **(Table 18 and Figure 16)**.

The results found that there was fluctuation of urinary GAG levels in placebo group **(Figure 17 and 18)**.

From a statistical point of view, the mean of urinary GAG level for placebo group (4.89±1.08µg/ml) was significantly (P<0.05) lower than that of the treatment group

(9.15±1.39µg/ml) on day 14 **(Table 19)**. In addition, there was a significant (P<0.05) difference of urine creatinine between placebo and treatment group on day 28 **(Table 19)**.

The overall of trend of GAG/creatinine ($x10^{-3}$) ratio in both placebo and treatment group is closed to the trend of urinary GAG level as shown above although there were no statistically significant differences in levels between placebo and treatment group **(Table 19, Figure 19 and Figure 20)**.

Table 18 Mean±SEM of plasma GAG levels (µg/ml) on day 0, 7, 14, 21, 28 and 56 in placebo and treatment group

Group	Plasma GAG (µg/ml)					
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 56**
Placebo	27.01±5.49	27.46±4.95	21.94±3.78	24.20±3.35	23.44±3.80	25.66±6.20
Treatment	27.46±3.90	31.89±6.02	36.22±6.75	39.96±5.34$^{\#}$*	39.91±6.74$^{\$}$	32.81±3.94

* P<0.05 when compared between placebo and treatment group
$^{\#}P<0.05$ when compared between day 0 and day 21 in treatment group
$^{\$}P<0.05$ when compared between day 0 and day 28 in treatment group

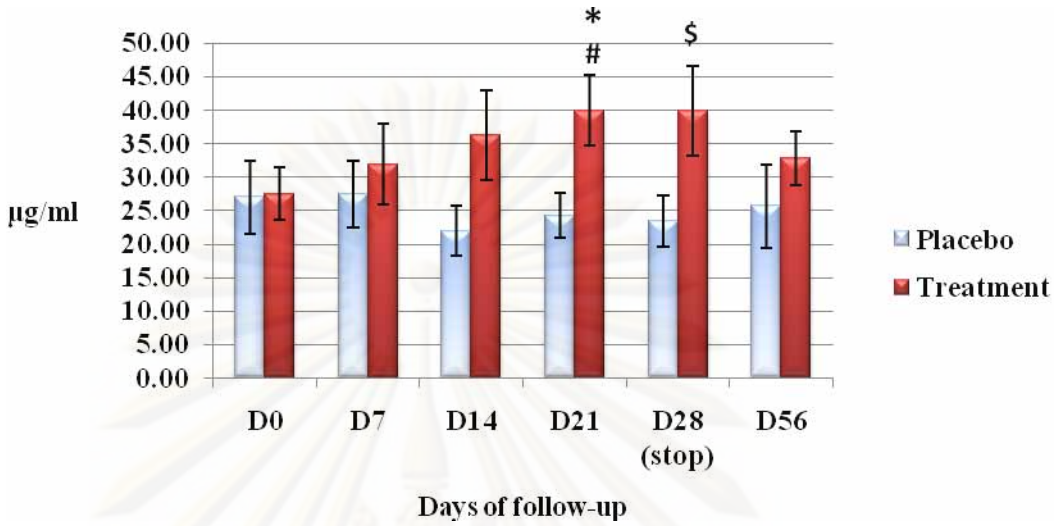


Figure 16 Mean±SEM of plasma GAG levels (µg/ml) when compared between placebo and treatment group on day 0, 7, 14, 21, 28 and 56

*$P<0.05$ when compared between placebo and treatment group
$^{\#}P<0.05$ when compared between day 0 and day 21 in treatment group
$^{\$}P<0.05$ when compared between day 0 and day 28 in treatment group
Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine
Stop = stop given placebo and N-acetyl-D-glucosamine

Table 19 Mean±SEM of urinary GAG, urine creatinine and GAG/creatinine ratio ($x10^{-3}$) levels on day 0, 7, 14, 21, 28 and 56 in placebo and treatment group

Day	Placebo Group			Treatment Group		
	Urinary GAG (µg/ml)	Urine Creatinine (mg%)	GAG/Creatinine ($x10^{-3}$)	Urinary GAG (µg/ml)	Urine Creatinine (mg%)	GAG/Creatinine ($x10^{-3}$)
D0	7.71±0.92	4.08±0.60	2.17±0.38	8.13±1.92	2.90±0.47	3.71±0.97
D7	7.76±1.20	3.48±0.38	2.40±0.47	10.80±2.30	3.31±0.49	3.30±0.57
D14	4.89±1.08	3.34±0.60	1.56±0.42	9.15±1.39*	3.16±0.52	4.30±1.44
D21	6.60±1.78	4.05±0.65	1.60±0.50	9.48±1.87	3.28±0.51	3.07±0.52
D28	8.94±4.50	4.40±0.34*	2.08±1.12	12.67±2.55	3.01±0.34	5.09±1.25
D56	8.13±2.77	4.32±0.49	1.93±0.63	13.65±2.69	3.57±0.62	4.55±1.16

*$P<0.05$ when compared between placebo and treatment group

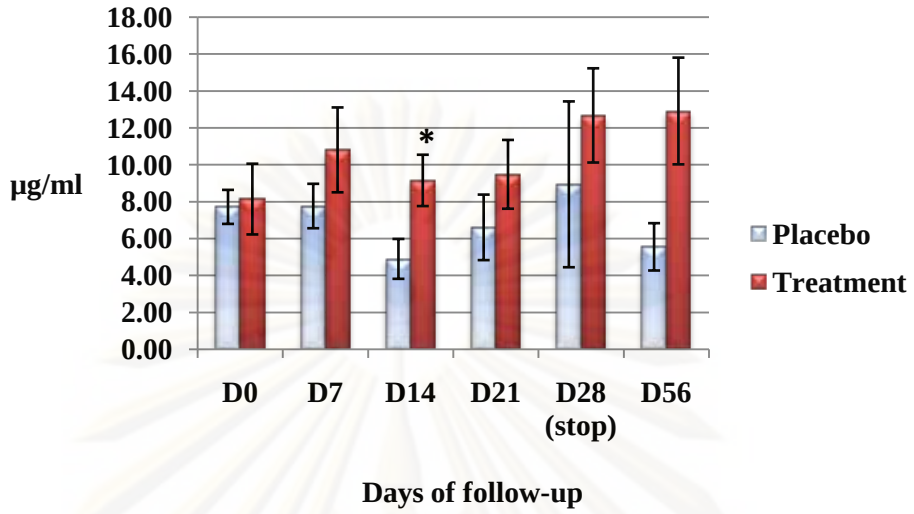


Figure 17 Mean±SEM of urinary GAG levels (µg/ml) when compared between placebo and treatment group on day 0, 7, 14, 21, 28 and 56

**P<0.05* when compared between placebo and treatment group
Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine
Stop = stop given placebo and N-acetyl-D-glucosamine

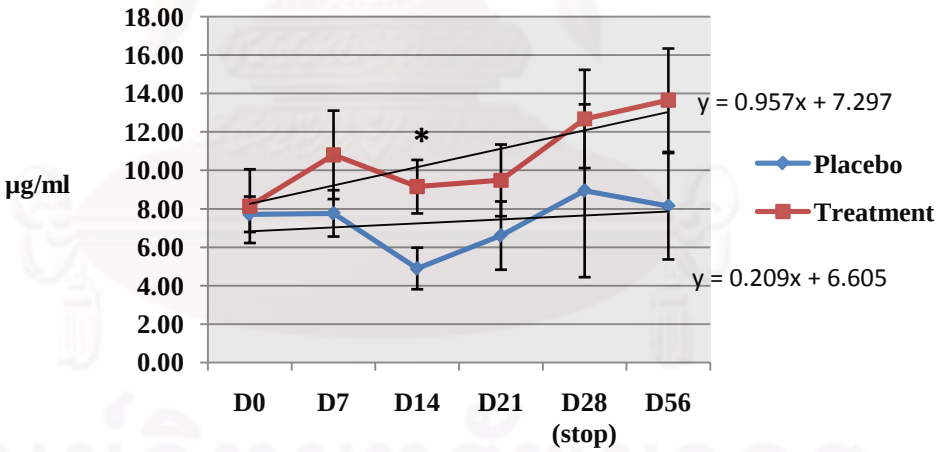


Figure 18 Mean±SEM of urinary GAG levels (µg/ml) when compared between placebo and treatment group on day 0, 7, 14, 21, 28 and 56

**P<0.05* when compared between placebo and treatment group
Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine
Stop = stop given placebo and N-acetyl-D-glucosamine

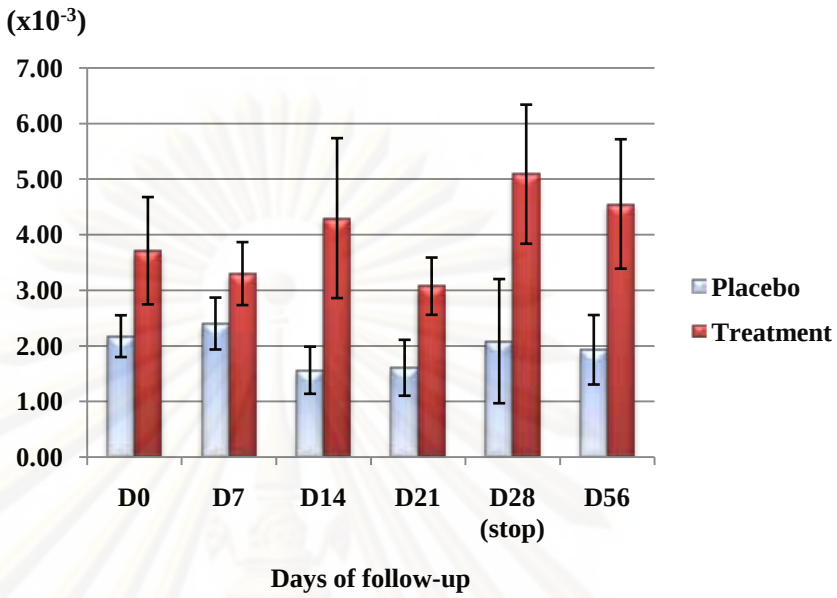


Figure 19 Mean±SEM of GAG/creatinine ($x10^{-3}$) when compared between placebo and treatment group on day 0, 7, 14, 21, 28 and 56

Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine
Stop = stop given placebo and N-acetyl-D-glucosamine

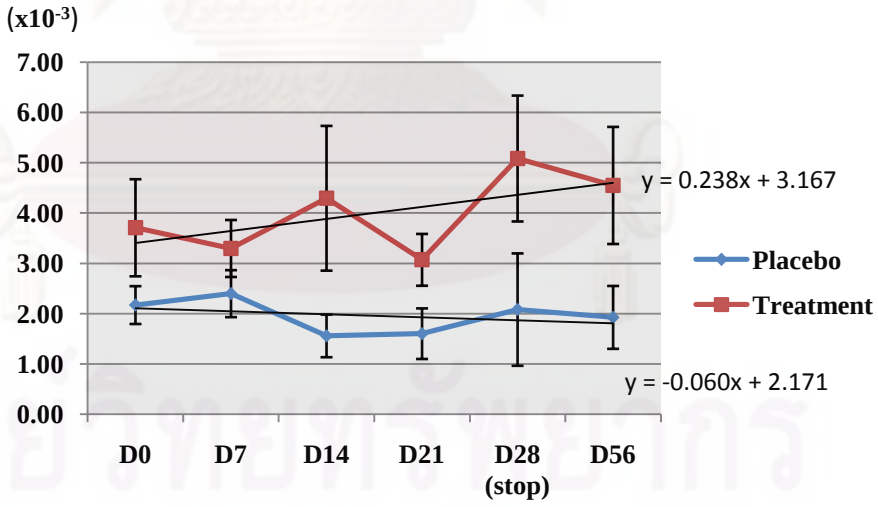


Figure 20 Mean±SEM of urine GAG/creatinine ($x10^{-3}$) when compare between placebo and treatment group on day 0, 7, 14, 21, 28 and 56

Placebo = cats received placebo
Treatment = cats received N-acetyl-D-glucosamine
Stop = stop given placebo and N-acetyl-D-glucosamine

Chapter V
Discussion

5.1 Signalments

The average age of cats with FIC in this study is 4.41±3.92 years old (age ranged from 1 to 18 yrs.) the same with previous studies (Kruger et al., 1996; Buffington et al., 1997; Lekcharoensuk et al., 2001; Hostutler et al., 2005). Cats between 2 to 7 years had increased risk for FLUTD (Kruger et al., 1996; Lekcharoensuk et al., 2001). Specific breed predispositions have not been identified in cats with FIC (Kruger et al., 1996). However, most FIC cats in this study are domestic short haired (DSH) which is the most popular breed of cat in Thailand. There were few pure breed cats with FIC such as Siamese and Persian. Castrated male cat has the highest number of FIC when compared with other gender. This result was consistent with others (Lekcharoensuk et al., 2001; Hostutler et al., 2005). In contrast, there was no intact female with FIC from the results. This finding may be because females had lower risk for FLUTD (Lekcharoensuk et al., 2001) and this may also occurred in FIC. Although, there were many previous researches about neutered cats and FIC, unfortunately, no reasons were explained about etiology between gender and FIC. The mean weight in the cats with FIC in this study was 4.62±0.97 kg indicated that FIC always occurs in overweight cats. Overweighed cats were at greater risk for developing FLUTD (Lekcharoensuk et al., 2001).

Our results also indicated that indoor and outdoor cats have equal chance of having FIC. Previous study have reported that FLUTD occurs more in the indoor cats (Buffington et al., 2006a). Cats with FIC have been postulated to have increased activity of their stress response system and decreased adrenocortical function (Westropp et al., 2003; Buffington, 2004). The indoor environment of some indoor cats were suggested to be very stressful so that indoor cats may be more stress than outdoor cats (Buffington et al., 2006a). However, results from this study showed that there are the same numbers of cats living outdoor to the number of cats living indoor. Conflict between cats with FIC and other cats within the same house has been shown to be one of the causes of stress (Westropp and Buffington, 2004). Most FIC cats in this study lived with more than one cat in the same household and could have been more stress than normal cats.

Data also showed that most FIC cats in this study consumed dry cat food more than mixed food and homemade diet. Dry cat food has been shown to be one of the risk factors for the formation of uroliths (Bartges and Kirk, 2006; Dru Forrester and Roudebush, 2007) and FIC (Markwell et al., 1999; Gunn-Moore and Shenoy, 2004).

Previous studies also found that cats with FIC tend to be fed with dry food than cats without FIC (Markwell et al., 1999; Gunn-Moore and Shenoy, 2004). Decreased water intake is also one of the risks for cat to develop FIC because of the possible supersaturation of urine in cats fed dry food and/or less water intake. This could cause more concentrated substances in the urine sample leading to irritation of the urinary bladder. Even though, all cats in this study received water ad libitum. Most owners found that FIC cats often drink less water and most of them feed dry food before became FIC. Therefore, nutritional management has been recommended to dilute urine of FIC cats for the purpose of decreasing concentration of the substances in urine, which irritate urinary bladder (Dru Forrester and Roudebush, 2007).

5.2 Complete blood count and serum chemistry test

Complete blood counts and serum chemistry profiles were within normal range for all cats in this study. There was only a statistically significant difference of ALT levels between placebo and treatment group on day 0 before given NAG and placebo. FIC cats receiving NAG did not have any abnormal complete blood count and serum chemistry findings. This suggested that given NAG to cats cause no side effects. The same trend was also observed by one study of FIC cats (Kruger et al., 1996) and consistent with the study in rats administrated N-acetylglucosamine for thirteen weeks (Lee et al., 2004). The amount of monocytes was also found to be significantly difference between placebo and treatment group on day 28 after cats received NAG. This result may be related to the effect of NAG on phagocyte activation of macrophage. The numbers of WBC observed in treatment group were higher than placebo group that may be due to the biological effect of NAG to the number of polymorphonuclear cells and their chemotactic activity (Suzuki et al., 1986).

5.3 Urinalysis

Results from the urinalysis in this study demonstrated the presence of hematuria and proteinuria in the urine sample of FIC cats on the first day of diagnosis (day 0). Hematuria and proteinuria without pyuria or bacteriauria can be found in urinalysis of cats with FIC (Kruger et al., 1996; Hostutler et al., 2005). Hematuria also occurs in FIC more than other causes such as urolithesis and UTI (Osborne et al., 1996a). At the end of the treatment (D28), it was found that 33% of FIC cats received NAG had decreased

number of red blood cells in the urine sediment. The amount of RBC in urine sediment increased after we stop given NAG to FIC cats for twenty-eight days (D56). This may be due to the action of NAG on the urinary bladder wall and its effect on the inflammation process because given NAG help to maintain GAGs level in the urinary bladder wall, thus prevent inflammation caused by FIC (Usami et al., 1998).

FIC cats in this study had concentrated and variable urine pH. Cats with FIC have been reported to have concentrated and acidic urine (Kruger et al., 1996). The presence of crystals is variable in the urine sediment and may have no clinical importance and pathologic significance in cats because crystals do not damage healthy urothelium (Hostutler et al., 2005). Urine that has been refrigerated or stored for hours also contains crystals in the urinary sediment and this phenomenon is exaggerated in urine that is highly concentrated (Sturgess et al., 2001; Albasan et al., 2003). From one previous study, it was found that 50% of FIC cats found struvite crystalluria (Kruger et al., 1996).

Few white blood cell and bacteria was also found in some urine samples. However, the urine culture of all cats in this study was all negative before FIC cats could enter into the study. Urine dipstick that detect white blood cell (WBC) often are positive in the absence of pyuria in cats (Hostutler et al., 2005). This false positive is common in cat and is caused by particulate material such as small crystals, cellular debris and liquid droplet that exhibit Brownian motion and may be misidentified as bacteria (Hostutler et al., 2005). On the other hand, contamination of the urine sample may occur depend on methods of urine collection, storage equipments and time of storage (false positive).

5.4 Visual Analogue Evaluation

Total VAS score in all questions between pre-treatment (day 0) and post-treatment (day 28) in placebo and treatment group were not significantly differences. However, there were the decreased trends of total VAS score on day 28 and day 56 in both groups. These data implied that FIC cats of both groups had decreased pain of urination as reported by the owners. The different of total VAS score between pre- and post treatment in placebo group was higher than that of the treatment group. Various studies indicated that cats with FIC can be self-cure with/without treatment (Kruger et al., 1996; Gunn-Moore and Shenoy, 2004; Bartges and Kirk, 2006; Dru Forrester and Roudebush, 2007; Kruger et al., 2009). The reason for this may be because the owners of both groups were educated about multimodal management in cats with FIC such as food, water, environment and stress before participate in this study. Studies have shown that

cats can be cured by changing of environment by owners (Gunn-Moore and Shenoy, 2004; Westropp and Buffington, 2004; Bartges and Kirk, 2006; Buffington et al., 2006a; Dru Forrester and Roudebush, 2007). In addition, another reason for this is that it may be because of the placebo effect that the owners get to feel better about their cats as they received medication for one month.

From three questions with the highest mean VAS score, the results demonstrated that main clinical signs of cats with FIC are increased frequency of urination (question 1), increase grooming around the perineum (question 5) and straining while urinating (question 7) as recorded subjectively by the owners. It is implied that these clinical signs persisted throughout the cause of the disease and takes time to resolve. Almost all owners assessed the results of treatment on day 28 (day of the stop of medication) as complete cured in both placebo and treatment group. This was subjectively judge of the outcome as related to the decreased in pain of urination that observed by the owners of both group. Some researchers also think that VAS questionnaire is subjective measurement and may not be highly precise and accurate to assess the true outcome of the treatment (Gould et al., 2001).

5.5 GAGs levels in plasma and urine

Urinary GAG levels of normal cats (44.26$\pm$6.16 µg/ml) and GAG/Cr ratio (14.23$\pm$3.47) are similar to the results from previous investigation on urinary GAG (34$\pm$23 µg/ml) and GAG/Cr ratio (9.4$\pm$5.2) (Buffington et al., 1996a). In that study, urine sample was measured by using 1,9–dimethyl-methylene blue (DMB) chloride that is the same method with this study. When compare GAG levels in normal and FIC cats, the mean urinary GAG level for FIC group was significantly lower than that of the normal group. This result is consistent with previous studies (Buffington et al., 1996a; Pereira et al., 2004). The reason for this is because there was an increased in permeability of the bladders of cats with FIC when compared with normal cats (Gao et al., 1994) and similar changes in urinary GAG was also observed in women with interstitial cystitis (Parsons and Hurst, 1990; Hurst et al., 1993).

From day 0 to day 28, there were steadily increased in plasma GAG level in the FIC cats received NAG and after stop NAG for one month plasma GAG level decreased. In contrast, there was a fluctuation of plasma GAG levels in placebo group and plasma GAG level were lowered than treatment group. It implied that received NAG orally can be absorbed through GI tract and pass into the circulatory system resulting in an

increased in plasma levels of GAG from day 0 to day 28 in NAG treatment group. This finding is consistent with Talent and Gracy (1996) that study in patients received NAG for osteoarthritis which had increased level of plasma GAG. The results showed that plasma GAG increased significantly in the treatment group on day 21. This phenomenon demonstrated that given NAG orally at 250 mg/kg daily, may take time up to 21 days to increase the plasma GAG levels significantly in FIC cats.

When consider urinary GAG, no previous studies on the effect of NAG administration in cats with FIC on GAG levels in plasma and urine has been reported. In this study, urinary GAG in cats received NAG orally is significant higher than cats received placebo on day 14 of the treatment. This finding was consistent with the decreased in number of RBC in urine sample on the same day (day 14). It may be because given NAG orally once a day can decrease the degree of hematuria in cats with FIC. NAG is the substrate for GAGs biosynthesis, but its half-life is relatively short (Talent and Gracy, 1996). There was one previous study about the metabolism of glucosamine and NAG which found that NAG was less completely oxidized resulting in more excretion in the urine (Kohn et al., 1962). NAG can also converted to glucosamine in vivo (Conchie and Hay, 1963; Talent and Gracy, 1996). Previous study about using endogenous glycosaminoglycans in treatment of interstitial cystitis in human patients, found that biotinylated pentosan polysulfate and heparin showed no or weak binding to rabbit and human bladder (Bhavanandan et al., 2001b). NAG can bind to lectins in the bladder epithelium (Bhavanandan et al., 2001a). Therefore, NAG is more suitable form to bind with epithelium than other GAGs when presented as endogenous.

Our results found no side effects of NAG when given to FIC cats at the dose of 250 mg/day for 28 days. Previous study in rats also found no toxicity from orally administering NAG at concentration of 5% in the rat's diet for 13 weeks (Lee et al., 2004). Even though, our result of given NAG oral supplementation did not give significant different on VAS score of cats with FIC evaluated by cats' owner as previous study (Gunn-Moore and Shenoy, 2004). Our results of increased urinary GAG and decreased in degree of hematuria of FIC cats showed improved of clinical sign and may have clinical benefit for FIC cats.

GAGs levels in plasma and urine were increased in treatment group. When we stop NAG treatment from one month, there was a recurrent of hematuria on day 56 in the treatment group.

5.7 Further suggestion

Cystoscope is a useful equipment to find the relationship of urinary GAGs levels and histopathology changing of urothelium bladder and to monitor the improvement of clinical sign of the FIC cats. Cystoscopy for every suspected FIC cats should be done. Alteration of structural or functional urothelial GAGs layer in cats with/without FIC are important to determine whether these abnormalities are specific cause of idiopathic disease or nonspecific effect occurring secondary to other etiologic mechanisms. Further study using cystoscope may be needed to assess the change of the urinary bladder epithelium in FIC cats receiving NAG.

REFERENCES

Akcay, T. and Konukoglu, D. 1999. Glycosaminoglycans excretion in interstitial cystitis. Int. Urol. Nephrol. 31(4): 431-435.

Albasan, H., Lulich, J.P., Osborne, C.A., Lekcharoensuk, C., Ulrich, L.K. and Carpenter, K.A. 2003. Effects of storage time and temperature on pH, specific gravity, and crystal formation in urine samples from dogs and cats. J. Am. Vet. Med. Assoc. 222(2): 176-179.

Anderson, J.W., Nicolosi, R.J. and Borzelleca, J.F. 2005. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food. Chem. Toxicol. 43(2): 187-201.

Apodaca, G. 2004. The uroepithelium: not just a passive barrier. Traffic. 5(3): 117-128.

Ashwell, G. and Harford, J. 1982. Carbohydrate-specific receptors of the liver. Annu Rev. Biochem. 51: 531-554.

Bade, J.J., Laseur, M., Nieuwenburg, A., van der Weele, L.T. and Mensink, H.J. 1997. A placebo-controlled study of intravesical pentosanpolysulphate for the treatment of interstitial cystitis. Br. J. Urol. 79(2): 168-171.

Barry, K.J. and Crowell-Davis S.L. 1999. Gender differences in the social behavior of the neutered indoor-only domestic cat. Appl. Anim. Behav. Sci. 64(3): 193-211.

Bartges, J.W. and Kirk, C.A. 2006. Nutrition and lower urinary tract disease in cats. Vet. Clin. North. Am. Small. Anim. Pract. 36(6): 1361-1376.

Bessey, F.K., Lowry, O.H. and Brock, M.J. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. J. Biol. Chem. 164:321–329.

Bhavanandan, V.P., Erickson, D.R., Herb, N., Sheykhnazari, M. and Ordille, S. 2001a. Use of glycosaminoglycans in the treatment of interstitial cystitis: a strategy to improve efficacy. Int. Congr. Ser. 1223: 227-237.

Bhavanandan, V.P., Puch, S., Guo, X. and Jiang, W. 2001b. Galectins and other endogenous carbohydrate-binding proteins of animal bladder. Adv. Exp. Med. Biol. 491: 95-108.

Birder, L.A. 2005. More than just a barrier: urothelium as a drug target for urinary bladder pain. Am. J. Physiol. Renal. Physiol. 289(3): F489-495.

Bovee, K.C., Reif, J.S., Maguire, T.G., Gaskell, C.J. and Batt, R.M. 1979. Recurrence of feline urethral obstruction. J. Am. Vet. Med. Assoc. 174(1): 93-96.

Buffington, C.A. 2002. External and internal influences on disease risk in cats. J. Am. Vet. Med. Assoc. 220(7): 994-1002.

Buffington, C.A. 2004. Comorbidity of interstitial cystitis with other unexplained clinical conditions. J. Urol. 172: 1242-1248.

Buffington, C.A., Blaisdell, J.L., Binns, S.P., Jr. and Woodworth, B.E. 1996a. Decreased urine glycosaminoglycan excretion in cats with interstitial cystitis. J. Urol. 155(5): 1801-1804.

Buffington, C.A., Chew, D.J. and DiBartola, S.P. 1996b. Interstitial cystitis in cats. Vet. Clin. North. Am. Small. Anim. Pract. 26(2): 317-326.

Buffington, C.A., Chew, D.J., Kendall, M.S., Scrivani, P.V., Thompson, S.B., Blaisdell, J.L. and Woodworth, B.E. 1997. Clinical evaluation of cats with

nonobstructive urinary tract diseases. J. Am. Vet. Med. Assoc. 210(1): 46-50.
Buffington, C.A. and Pacak, K. 2001. Increased plasma norepinephrine concentration in cats with interstitial cystitis. J. Urol. 165: 2051-2054.
Buffington, C.A., Westropp, J.L., Chew, D.J. and Bolus, R.R. 2006a. Clinical evaluation of multimodal environmental modification (MEMO) in the management of cats with idiopathic cystitis. J. Feline. Med. Surg. 8(4): 261-268.
Buffington, C.A., Westropp, J.L., Chew, D.J. and Bolus, R.R. 2006b. Risk factors associated with clinical signs of lower urinary tract disease in indoor-housed cats. J. Am. Vet. Med. Assoc. 228(5): 722-725.
Buffington, C.A. and Wolfe, S.A., Jr. 1998. High affinity binding sites for [3H] substance P in urinary bladders of cats with interstitial cystitis. J. Urol. 160(2): 605-611.
Cameron, M.E., Casey, R.A., Bradshaw, J.W., Waran, N.K. and Gunn-Moore, D.A. 2004. A study of environmental and behavioural factors that may be associated with feline idiopathic cystitis. J. Small. Anim. Pract. 45(3): 144-147.
Cassaro, C.M. and Dietrich, C.P. 1977. Distribution of sulfated mucopolysaccharides in invertebrates. J. Biol. Chem. 252(7): 2254-2261.
Chew, D.J., Buffington, C.A., Kendall, M.S., DiBartola, S.P. and Woodworth, B.E. 1998. Amitriptyline treatment for severe recurrent idiopathic cystitis in cats. J. Am. Vet. Med. Assoc. 213(9): 1282-1286.
Clasper, M. 1990. A case of interstitial cystitis and Hunner's ulcer in a domestic shorthaired cat. N. Z. Vet. J. 38(4): 158-160.
Conchie, J. and Hay, A.J. 1963. Mammalian glycosidases. 4. The intracellular localization of beta-galactosidase, alpha-mannosidase, beta-N-acetylglucosaminidase and alpha-L-fucosidase in mammalian tissues. Biochem. J. 87: 354-361.
Davis, E.L., El Khoudary, S.R., Talbott, E.O., Davis, J. and Regan, L.J. 2008. Safety and efficacy of the use of intravesical and oral pentosan polysulfate sodium for interstitial cystitis: a randomized double-blind clinical trial. J. Urol. 179(1): 177-185.
de Jong, J.G., Wevers, R.A., Laarakkers, C. and Poorthuis, B.J. 1989. Dimethylmethylene blue-based spectrophotometry of glycosaminoglycans in untreated urine: a rapid screening procedure for mucopolysaccharidoses. Clin. Chem. 35(7): 1472-1477.
de Lima, C.R., Aguiar, J.A. and Michelacci, Y.M. 2005. Reduced urinary excretion of sulfated polysaccharides in diabetic rats. Biochim. Biophys. Acta. 1741(1-2): 30-41.
Dietrich, C.P., Sampaio, L.O. and Toledo, O.M. 1976. Characteristic distribution of sulfated mucopolysaccharides in different tissues and in their respective mitochondria. Biochem. Biophys. Res. Commun. 71(1): 1-10.
Dru Forrester, S. 2007. Management of Feline Lower Urinary Tract Disease. Proceedings of the 2007 Hill's LUTD. Florida, USA, April. 45-52.
Dru Forrester, S. and Roudebush, P. 2007. Evidence-based management of feline lower urinary tract disease. Vet. Clin. North. Am. Small. Anim. Pract. 37(3): 533-558.
Elbadawi, A. 1997. Interstitial cystitis: a critique of current concepts with a new proposal for pathologic diagnosis and pathogenesis. Urology. 49. Suppl. 5A: 14-40.

Erturk, E., Kiernan, M. and Schoen, S.R. 2002. Clinical association with urinary glycosaminoglycans and urolithiasis. Urology. 59(4): 495-499.

Farndale, R.W., Buttle, D.J. and Barrett, A.J. 1986. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim. Biophys. Acta. 883(2): 173-177.

Gao, X., Buffington, C.A. and Au, J.L. 1994. Effect of interstitial cystitis on drug absorption from urinary bladder. J. Pharmacol. Exp. Ther. 271(2): 818-823.

Gould, D., Kelly, D., Goldstone, L. and Gammon, J. 2001. Examining the validity of pressure ulcer risk assessment scales: developing and using illustrated patient simulations to collect the data. J. Clin. Nurs. 10(5): 697-706.

Griffith, C.A., Steigerwald, E.S. and Buffington, C.A. 2000. Effects of a synthetic facial pheromone on behavior of cats. J. Am. Vet. Med. Assoc. 217(8): 1154-1156.

Gunn-Moore, D.A. 2003. Feline lower urinary tract disease. J. Feline. Med. Surg. 5(2): 133-138.

Gunn-Moore, D.A. and Cameron, M.E. 2004. A pilot study using synthetic feline facial pheromone for the management of feline idiopathic cystitis. J. Feline. Med. Surg. 6(3): 133-138.

Gunn-Moore, D.A. and Shenoy, C.M. 2004. Oral glucosamine and the management of feline idiopathic cystitis. J. Feline. Med. Surg. 6(4): 219-225.

Hauser, P.J., Dozmorov, M.G., Bane, B.L., Slobodov, G., Culkin, D.J. and Hurst, R.E. 2008. Abnormal expression of differentiation related proteins and proteoglycan core proteins in the urothelium of patients with interstitial cystitis. J. Urol. 179(2): 764-769.

Holm-Bentzen, M., Ammitzboll, T. and Hald, T. 1986. Glycosaminoglycans on the surface of the human urothelium. Neurourol. Urodynam. 5: 519.

Horwitz, D.F. 1997. Behavioral and environmental factors associated with elimination behavior problems in cats: a retrospective study. Appl. Anim. Behav. Sci. 52(1-2): 129-137.

Hostutler, R.A., Chew, D.J. and DiBartola, S.P. 2005. Recent concepts in feline lower urinary tract disease. Vet. Clin. North. Am. Small. Anim. Pract. 35(1): 147-170.

Hurst, R.E., Moldwin, R.M. and Mulholland, S.G. 2007. Bladder defense molecules, urothelial differentiation, urinary biomarkers, and interstitial cystitis. Urology. 69. Suppl. 4: 17-23.

Hurst, R.E., Parsons, C.L., Roy, J.B. and Young, J.L. 1993. Urinary glycosaminoglycan excretion as a laboratory marker in the diagnosis of interstitial cystitis. J. Urol. 149(1): 31-35.

Hurst, R.E., Rhodes, S.W., Adamson, P.B., Parsons, C.L. and Roy, J.B. 1987. Functional and structural characteristics of the glycosaminoglycans of the bladder luminal surface. J. Urol. 138(2): 433-437.

Hurst, R.E., Roy, J.B., Min, K.W., Veltri, R.W., Marley, G., Patton, K., Shackelford, D.L., Stein, P. and Parsons, C.L. 1996. A deficit of chondroitin sulfate proteoglycans on the bladder uroepithelium in interstitial cystitis. Urology. 48(5): 817-821.

Jezernik, K., Romih, R., Mannherz, H.G. and Koprivec, D. 2003. Immunohistochemical detection of apoptosis, proliferation and inducible nitric oxide synthase in rat urothelium damaged by cyclophosphamide treatment. Cell. Biol. Int. 27(10): 863-869.

Johansson, S.L. and Fall, M. 1990. Clinical features and spectrum of light microscopic changes in interstitial cystitis. J. Urol. 143(6): 1118-1124.

Jones, A.J., Papac, D.I., Chin, E.H., Keck, R., Baughman, S.A., Lin, Y.S., Kneer, J. and Battersby, J.E. 2007. Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal N-acetylglucosamine is similar in humans and cynomolgus monkeys. Glycobiology. 17(5): 529-540.

Jones, B.R., Sanson, R.L. and Morris, R.S. 1997. Elucidating the risk factors of feline lower urinary tract disease. N. Z. Vet. J. 45(3): 100-108.

Kaufman, J.E., Anderson, K. and Parsons, C.L. 1987. Inactivation of antiadherence effect of bladder surface glycosaminoglycans as possible mechanism for carcinogenesis. Urology. 30(3): 255-258.

Keay, S., Seillier-Moiseiwitsch, F., Zhang, C.O., Chai, T.C. and Zhang, J. 2003a. Changes in human bladder epithelial cell gene expression associated with interstitial cystitis or antiproliferative factor treatment. Physiol. Genomics. 14(2): 107-115.

Keay, S. and Warren, J.W. 1998. A hypothesis for the etiology of interstitial cystitis based upon inhibition of bladder epithelial repair. Med. Hypotheses. 51(1): 79-83.

Keay, S., Zhang, C.O., Shoenfelt, J.L. and Chai, T.C. 2003b. Decreased in vitro proliferation of bladder epithelial cells from patients with interstitial cystitis. Urology. 61(6): 1278-1284.

Kelly, G.S. 1998. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. Altern. Med. Rev. 3(1): 27-39.

Kjellen, L. and Lindahl, U. 1991. Proteoglycans: structures and interactions. Annu. Rev. Biochem. 60: 443-475.

Kohn, P., Winzler, J. and Hoffman, R.C. 1962. Metabolism of D-glucosamine and N-acetyl-D-glucosamine in the intact rat. J. Biol. Chem. 237: 304-308.

Kraijer, M., Fink-Gremmels, J. and Nickel, R.F. 2003. The short-term clinical efficacy of amitriptyline in the management of idiopathic feline lower urinary tract disease: a controlled clinical study. J. Feline. Med. Surg. 5(3): 191-196.

Kruger, J.M., Conway, T.S., Kaneene, J.B., Perry, R.L., Hagenlocker, E., Golombek, A. and Stuhler, J. 2003. Randomized controlled trial of the efficacy of short-term amitriptyline administration for treatment of acute, nonobstructive, idiopathic lower urinary tract disease in cats. J. Am. Vet. Med. Assoc. 222(6): 749-758.

Kruger, J.M. and Osborne, C.A. 1995. Recurrent, nonobstructive, idiopathic feline lower urinary tract disease: an illustrative case report. J. Am. Anim. Hosp. Assoc. 31(4): 312-316.

Kruger, J.M., Osborne, C.A., Goyal, S.M., Wickstrom, S.L., Johnston, G.R., Fletcher, T.F. and Brown, P.A. 1991. Clinical evaluation of cats with lower urinary tract disease. J. Am. Vet. Med. Assoc. 199(2): 211-216.

Kruger, J.M., Osborne, C.A. and Lulich, J.P. 1996. Management of nonobstructive idiopathic feline lower urinary tract disease. Vet. Clin. North. Am. Small. Anim. Pract. 26(3): 571-588.

Kruger, J.M., Osborne, C.A. and Lulich, J.P. 2009. Changing paradigms of feline idiopathic cystitis. Vet. Clin. North. Am. Small. Anim. Pract. 39(1): 15-40.

Kurth, K.H. and Parsons C.L. 2003. The interstitial cystitis syndrome: Intravesical and oral treatment. Eur. Urol. 2. Suppl. 4: 2-9.

Laguna, P., Smedts, F., Nordling, J., Horn, T., Bouchelouche, K., Hopman, A. and de la Rosette, J. 2006. Keratin expression profiling of transitional epithelium in the painful bladder syndrome/interstitial cystitis. Am. J. Clin. Pathol. 125(1): 105-110.

Laule, G.E. 2003. Positive reinforcement training and environmental enrichment: enhancing animal well-being. J. Am. Vet. Med. Assoc. 223(7): 969-973.

Lavelle, J., Meyers, S., Ramage, R., Bastacky, S., Doty, D., Apodaca, G. and Zeidel, M.L. 2002. Bladder permeability barrier: recovery from selective injury of surface epithelial cells. Am. J. Physiol. Renal. Physiol. 283(2): F242-253.

Lavelle, J.P., Meyers, S.A., Ruiz, W.G., Buffington, C.A., Zeidel, M.L. and Apodaca, G. 2000. Urothelial pathophysiological changes in feline interstitial cystitis: a human model. Am. J. Physiol. Renal. Physiol. 278(4): F540-553.

Lee, K.Y., Shibutani, M., Takagi, H., Arimura, T., Takigami, S., Uneyama, C., Kato, N. and Hirose, M. 2004. Subchronic toxicity study of dietary N-acetylglucosamine in F344 rats. Food. Chem. Toxicol. 42(4): 687-695.

Lekcharoensuk, C., Osborne, C.A. and Lulich, J.P. 2001. Epidemiologic study of risk factors for lower urinary tract diseases in cats. J. Am. Vet. Med. Assoc. 218(9): 1429-1435.

Lewis, S.A. 2000. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am. J. Physiol. Renal. Physiol. 278(6): F867-874.

Lilly, J.D. and Parsons, C.L. 1990. Bladder surface glycosaminoglycans is a human epithelial permeability barrier. Surg. Gynecol. Obstet. 171(6): 493-496.

Lloyd, A.G. 1961. The metabolism of exogenous N-acetyl-D-glucosamine 6-*O*[^{35}S]-sulphate in the normal rat. Biochem. J. 80(3): 572-578.

Lulich, J.P. 2007. Feline Lower Urinary Tract Disoders: Making the Correct Diagnosis. Proceedings of the 2007 Hill's LUTD. Florida, USA, April. 36-41

Lund, E.M., Armstrong, P.J., Kirk, C.A., Kolar, L.M. and Klausner, J.S. 1999. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. J. Am. Vet. Med. Assoc. 214(9): 1336-1341.

Markwell, P.J., Buffington, C.A., Chew, D.J., Kendall, M.S., Harte, J.G. and DiBartola, S.P. 1999. Clinical evaluation of commercially available urinary acidification diets in the management of idiopathic cystitis in cats. J. Am. Vet. Med. Assoc. 214(3): 361-365.

Michelacci, Y.M., Cadaval, R.A., Rovigatti, R.M. and Kohlman, O. 2001. Renal and urinary glycosaminoglycans in an experimental model of chronic renal failure in rats. Exp. Nephrol. 9(1): 40-48.

Mills, D.S. and White, J.C. 2000. Long-term follow up of the effect of a pheromone therapy on feline spraying behaviour. Vet. Rec. 147(26): 746-747.

Morell, A.G., Gregoriadis, G., Scheinberg, I.H., Hickman, J. and Ashwell, G. 1971. The role of sialic acid in determining the survival of glycoproteins in the circulation. J. Biol. Chem. 246(5): 1461-1467.

Moskowitz, M.O., Byrne, D.S., Callahan, H.J., Parsons, C.L., Valderrama, E. and Moldwin, R.M. 1994. Decreased expression of a glycoprotein component of bladder surface mucin (GP1) in interstitial cystitis. J. Urol. 151(2): 343-345.

Nikkila, M.T. 1989. Urinary glycosaminoglycan excretion in normal and stone-forming subjects: significant disturbance in recurrent stone formers. Urol. Int. 44(3): 157-159.

Ombra, M.N., Casula, S., Biino, G., Maestrale, G., Cardia, F., Melis, P. and Pirastu,

M. 2003. Urinary glycosaminoglycans as risk factors for uric acid nephrolithiasis: case control study in a Sardinian genetic isolate. Urology. 62(3): 416-420.

Osborne, C.A., Kruger, J.M. and Lulich, J.P. 1996a. Feline lower urinary tract disorders. Definition of terms and concepts. Vet. Clin. North. Am. Small Anim. Pract. 26(2): 169-179.

Osborne, C.A., Kruger, J.M., Lulich, J.P., Johnston, G.R., Polzin, D.J., Ulrich, L.K. and Sanna, J. 1996b. Prednisolone therapy of idiopathic feline lower urinary tract disease: a double-blind clinical study. Vet. Clin. North. Am. Small. Anim Pract. 26(3): 563-569.

Panin, G., Naia, S., Dall'Amico, R., Chiandetti, L., Zachello, F., Catassi, C., Felici, L. and Coppa, G.V. 1986. Simple spectrophotometric quantification of urinary excretion of glycosaminoglycan sulfates. Clin. Chem. 32(11): 2073-2076.

Parsons, C.L. 1994. A model for the function of glycosaminoglycans in the urinary tract. World. J. Urol. 12(1): 38-42.

Parsons, C.L. 2007. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. Urology. 69. Suppl. 4: 9-16.

Parsons, C.L., Boychuk, D., Jones, S., Hurst, R. and Callahan, H. 1990. Bladder surface glycosaminoglycans: an epithelial permeability barrier. J. Urol. 143(1): 139-142.

Parsons, C.L. and Hurst, R.E. 1990. Decreased urinary uronic acid levels in individuals with interstitial cystitis. J. Urol. 143(4): 690-693.

Parsons, C.L., Lilly, J.D. and Stein, P. 1991. Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J. Urol. 145(4): 732-735.

Parsons, C.L., Stauffer, C.W. and Schmidt, J.D. 1980. Bladder-surface glycosaminoglycans: an efficient mechanism of environmental adaptation. Science. 208(4444): 605-607.

Parsons, C.L., Stauffer, C.W. and Schmidt, J.D. 1988. Reversible inactivation of bladder surface glycosaminoglycan antibacterial activity by protamine sulfate. Infect. Immun. 56(5): 1341-1343.

Patton, C.J. and Crouch, S.R. 1977. Enzymatic determination of urea. Anal. Chem. 49:464-469.

Pereira, D.A., Aguiar, J.A., Hagiwara, M.K. and Michelacci, Y.M. 2004. Changes in cat urinary glycosaminoglycans with age and in feline urologic syndrome. Biochim. Biophys. Acta. 1672(1): 1-11.

Reche Junior, A. and Buffington, C.A. 1998. Increased tyrosine hydroxylase immunoreactivity in the locus coeruleus of cats with interstitial cystitis. J. Urol. 159(3): 1045-1048.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28(1): 56-63.

Rosamilia, A., Igawa, Y. and Higashi, S. 2003. Pathology of interstitial cystitis. Int. J. Urol. 10. Suppl: S11-15.

Sant, G.R., Kempuraj, D., Marchand, J.E. and Theoharides, T.C. 2007. The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis. Urology. 69. Suppl. 4: 34-40.

Scrivani, P.V., Chew, D.J., Buffington, C.A. and Kendall, M. 1998. Results of

double-contrast cystography in cats with idiopathic cystitis: 45 cases (1993-1995). J. Am. Vet. Med. Assoc. 212(12): 1907-1909.

Scrivani, P.V., Chew, D.J., Buffington, C.A., Kendall, M. and Leveille, R. 1997. Results of retrograde urethrography in cats with idiopathic, nonobstructive lower urinary tract disease and their association with pathogenesis: 53 cases (1993-1995). J. Am. Vet. Med. Assoc. 211(6): 741-748.

Setnikar, I. and Rovati, L.C. 2001. Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. Arzneimittelforschung. 51(9): 699-725.

Slobodov, G., Feloney, M., Gran, C., Kyker, K.D., Hurst, R.E. and Culkin, D.J. 2004. Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. J. Urol. 171(4): 1554-1558.

Sodikoff C.H. 2001. Red Blood Cells and White Blood Cells. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. St.Louis : Mosby. 100-124.

Sodikoff C.H. 2001. Serum chemical tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. St.Louis : Mosby. 5-18.

Sodikoff C.H. 2001. Urine tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. St.Louis : Mosby. 72-85.

Stahl, P.D. 1992. The mannose receptor and other macrophage lectins. Curr. Opin. Immunol. 4(1): 49-52.

Stockert, R.J. 1995. The asialoglycoprotein receptor: relationships between structure, function, and expression. Physiol. Rev. 75(3): 591-609.

Sturgess, C.P., Hesford, A., Owen, H. and Privett, R. 2001. An investigation into the effects of storage on the diagnosis of crystalluria in cats. J. Feline. Med. Surg. 3(2): 81-85.

Suzuki, K., Tokoro, A., Okawa, Y., Suzuki, S. and Suzuki, M. 1986. Effect of N-acetylchito-oligosaccharides on activation of phagocytes. Microbiol. Immunol. 30(8): 777-787.

Swenson, C.L., Boisvert, A.M., Kruger, J.M. and Gibbons-Burgener, S.N. 2004. Evaluation of modified Wright-staining of urine sediment as a method for accurate detection of bacteriuria in dogs. J. Am. Vet. Med. Assoc. 224(8): 1282-1289.

Talent, J.M. and Gracy, R.W. 1996. Pilot study of oral polymeric N-acetyl-D-glucosamine as a potential treatment for patients with osteoarthritis. Clin. Ther. 18(6): 1184-1190.

Thakral, R., Debnath, U.K., and Dent, C. 2007. Role of glucosamine in osteoarthritis. Curr. Orthop. 21(5): 386-389.

Thorne, I.D. and Resnick, M.I. 1984. A methodology for the characterization of urinary glycosaminoglycans. J. Urol. 131(5): 995-999.

Uldry, M., Ibberson, M., Hosokawa, M. and Thorens, B. 2002. GLUT2 is a high affinity glucosamine transporter. FEBS. Lett. 524(1-3): 199-203.

Usami, Y., Okamoto. Y., Takayama, T., Shigemasa., Y. and Minami., S. 1998. Effect of N-acetyl-D-glucosamine and D-glucosamine oligomers on canine polymorphonuclear cells in vitro. Carb. Polym. 36(2-3): 137-141.

Veranic, P. and Jezernik, K. 2000. The response of junctional complexes to induced desquamation in mouse bladder urothelium. Biol. Cell. 92(2): 105-113.

Veranic, P. and Jezernik, K. 2001. Succession of events in desquamation of

superficial urothelial cells as a response to stress induced by prolonged constant illumination. Tissue. Cell. 33(3): 280-285.
Westropp, J.L., Kass, P.H. and Buffington, C.A. 2006. Evaluation of the effects of stress in cats with idiopathic cystitis. Am. J. Vet. Res. 67(4): 731-736.
Westropp, J.L., Kass, P.H. and Buffington, C.A. 2007. In vivo evaluation of alpha(2)-adrenoceptors in cats with idiopathic cystitis. Am. J. Vet. Res. 68(2): 203-207.
Westropp, J.L. and Tony Buffington, C.A. 2004. Feline idiopathic cystitis: current understanding of pathophysiology and management. Vet. Clin. North. Am. Small. Anim. Pract. 34(4): 1043-1055.
Westropp, J.L., Welk, K.A. and Buffington, C.A. 2003. Small adrenal glands in cats with feline interstitial cystitis. J. Urol. 170: 2494-2497.
Zhang, C.O., Wang, J.Y., Koch, K.R. and Keay, S. 2005. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis. J. Urol. 174(6): 2382-2387.

APPENDICES

Appendix 1 Signalment, life style, number of cats in the same household, receiving of water, type of food and family history

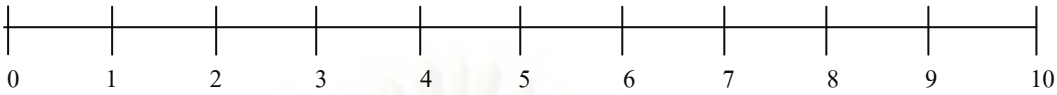
Number	Code	Group	Age	Breed	Sex	Weight	In/Outdoor	No.cat	Water	Type of Food	Family History
1	A1	Placebo	unk	DSH	M	4.8	Out	>1	Ad lib	homemade	no
2	A2	Placebo	7	DSH	Mc	4.36	Out	>1	Ad lib	mix	no
3	A3	Placebo	3	DSH	M	4.18	Out	>1	Ad lib	mix	yes
4	A4	Placebo	4	persia	M	3.9	Out	>1	Ad lib	mix	no
5	A5	Placebo	4	persia	M	3.8	In	1	Ad lib	dry	no
6	A6	Placebo	unk	DSH	Fs	4.3	Out	>1	Ad lib	dry	no
7	A7	Placebo	3.5	DSH	Mc	3.6	In	>1	Ad lib	mix	yes
8	B1	Treatment	18	DSH	Fs	5.6	In	>1	Ad lib	dry	no
9	B2	Treatment	5	DSH	M	5	In	>1	Ad lib	dry	yes
10	B3	Treatment	1	DSH	Mc	3.88	Out	>1	Ad lib	dry	no
11	B4	Treatment	2	DSH	M	4.3	In	>1	Ad lib	mix	yes
12	B5	Treatment	6	DSH	Mc	7.8	In	>1	Ad lib	homemade	no
13	B6	Treatment	1	Siamese	Mc	4.7	In	1	Ad lib	dry	no
14	B7	Treatment	3	DSH	Mc	3.38	Out	>1	Ad lib	mix	no
15	B8	Treatment	2	DSH	M	4.9	In	>1	Ad lib	dry	no
16	B9	Treatment	4	Siamese	Mc	4.6	Out	>1	Ad lib	dry	no
17	B10	Treatment	6	DSH	Mc	5.44	Out	>1	Ad lib	dry	no
18	B11	Treatment	unk	DSH	Mc	5	In	>1	Ad lib	dry	no
19	B12	Treatment	5	DSH	Mc	4.2	Out	>1	Ad lib	mix	no

Note: Unk= unknown

Appendix 2 **การประเมินสุขภาพแมวแบบ Visual Analogue Scale**

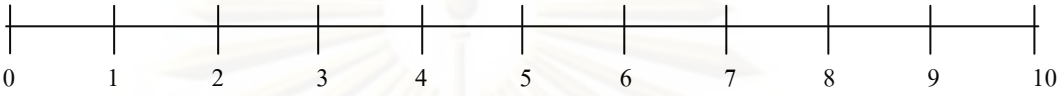
ทำเครื่องหมายกากบาท (X) ลงบนเส้นตรงตามหมายเลขที่ท่านพิจารณาจากอาการของแมวในแต่ละวัน

1.ปัสสาวะบ่อยขึ้นจากเดิม (ความถี่)

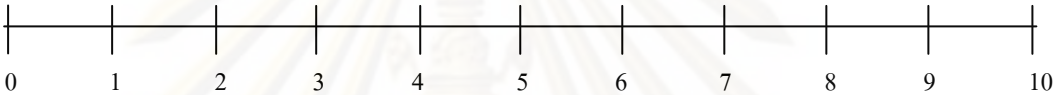


โปรดระบุปริมาณปัสสาวะโดยรวมต่อครั้ง ☐ มากขึ้น ☐ เท่าเดิม ☐ น้อยลง

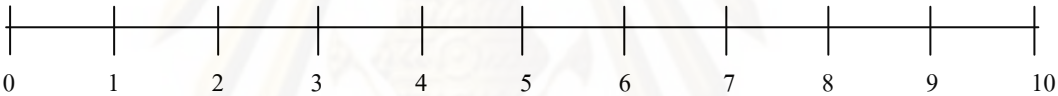
2.ส่งเสียงร้องขณะปัสสาวะ



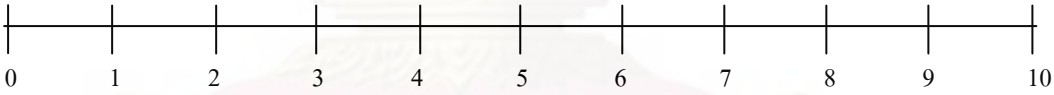
3.ปัสสาวะปนเลือดแบบที่สามารถสังเกตเห็นได้



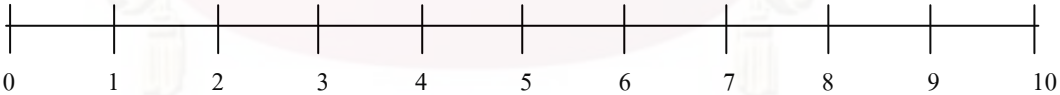
4.ปัสสาวะไม่เป็นที่



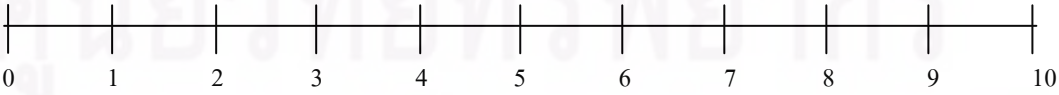
5.เลียบริเวณใกล้อวัยวะเพศหรือบริเวณใกล้ส่วนเชิงกราน (perineum) บ่อยขึ้น



6. พฤติกรรมผิดปกติ เช่น ก้าวร้าวมากขึ้น ชอบหลบซ้อน หวาดกลัว



7. ทำท่าจะปัสสาวะ แต่ปัสสาวะไม่ออก หรือออกน้อย



หมายเหตุ : 0 = ไม่มีอาการ หรือ ไม่สามารถสังเกตเห็นได้

> 0 ถึง <5 = มีอาการ แต่น้อยกว่าปกติตามลำดับ

5 = มีอาการเท่าเดิม หรือ มีอาการดังกล่าวเป็นปกติอยู่แล้วก่อนป่วย

> 5 ถึง <10 = มีอาการมากขึ้นตามลำดับ

10 = มีอาการมากที่สุด

Appendix 3 Mean±SEM for complete blood count in placebo group on day 0, 7, 14, 21, 28 and 56

Blood Results	Normal Value #	Placebo cats					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 56
Red Blood Cell ($x10^6$/µl)	5.22-8.46	6.22±0.77	5.46±0.39	7.28±1.53	6.59±0.62	6.29±0.88	6.33±0.55
Hematocrit (%)	25-45	35.14±2.39	37.14±2.29	36.86±2.79	38.57±1.90	38.71±3.15	35.50±3.28
White Blood Cell (/µl)	6,000-18,000	8,257±1,322	8,029±964	7,909±1,018	8,675±1,395	9,121±1,277	7,380±894
Neutrophils (/µl)	2,500-12,000	5,403±1,435	4,817±603	5,635±1,233	5,528±1,014	5,636±1,199	4,470±673
Bands (/µl)	0-300	16±10	61±29	14±14	23±23	28±20	0± 0
Eosinophils (/µl)	100-1,000	648±257	667±120	494±172	524±109	656±138	462±46
Lymphocytes (/µl)	1,500-7,000	2,063±3.57	2,292±394	2,234±361	2,569±443	2,684±354	2,135±390
Monocytes (/µl)	< 1,000	126±55	134±71	25±16	46±30	10±10	67±43

#Normal Reference Value from Sodikoff C.H. 2001. Red Blood Cells and White Blood Cells. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 100-124.

Appendix 4 Mean±SEM for complete blood count in treatment group on day 0, 7, 14, 21, 28 and 56

Blood Results	Normal Value #	Treatment cats					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 56
Red Blood Cell ($x10^6$/µl)	5.22-8.46	6.28±0.62	6.76±0.67	7.40±0.44	7.60±0.51	6.26±0.35	8.00±0.93
Hematocrit (%)	25-45	40.90±3.37	40.09±2.64	39.36±2.26	40.70±2.24	39.91±2.30	41.20±2.00
White Blood Cell (/µl)	6,000-18,000	9,504±1,194	9,468±942	12,964±2,246	7,625±729	9,479±1,075	9,130±1,236
Neutrophils (/µl)	2,500-12,000	6,737±945	6,995±863	8,560±1,548	4,403±415	5,842±662	5,556±932
Bands (/µl)	0-300	0±0	14±9	19±13	22±14	12±12	0± 0
Eosinophils (/µl)	100-1,000	505±144	411±118	796±127	454±164	529±177	558±114
Lymphocytes (/µl)	1,500-7,000	2,128±435	1,905±222	3,550±716	2,563±445	2,950±530	2,828±476
Monocytes (/µl)	< 1,000	105±33	174±67	21±17	120±42	74±23*	184±72

#Normal Reference Value from Sodikoff C.H. 2001. Red Blood Cells and White Blood Cells. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 100-124.

*$P<0.05$ when compared between placebo and treatment group

Appendix 5 Mean±SEM for blood chemistry values in placebo cats on day 0, 7, 14, 21, 28 and 56

Blood Chemistry	Normal Value#	Placebo cats					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 56
BUN (mg%)	10-30	24.73+2.57	26.36+3.76	24.13+2.85	23.97+2.14	22.77+2.94	25.52+0.88
Creatinine (mg%)	0.8-2	1.62+0.09	1.69+0.13	1.59+0.09	1.63+0.13	1.67+0.09	1.60+0.17
ALT (IU/L)	<80	27.74+6.50	50.06+8.76	35.94+9.44	33.99+8.43	39.84+10.84	37.76+5.80
ALP (IU/L)	<200	90.53+21.66	92.04+16.39	95.54+1.82	94.59+16.46	85.24+25.76	65.30+16.33

#Normal Reference Value from Sodikoff C.H. 2001. Serum Chemistry Tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 5-18.

Appendix 6 Mean±SEM for blood chemistry values in treatment cats on day 0, 7, 14, 21, 28 and 56

Blood Chemistry	Normal Value#	Treatment cats					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 56
BUN (mg%)	10-30	30.68±2.94	24.84±1.57	27.88±1.41	26.33±1.17	25.79±1.86	26.74±1.29
Creatinine (mg%)	0.8-2	1.84±0.1	1.87±0.12	1.87±0.09	1.80±0.11	1.80±0.12	1.85±0.14
ALT (IU/L)	<80	49.73±6.06	52.44±8.13	40.94±8.46	51.94±8.54	51.54±7.97	44.97±4.86
ALP (IU/L)	<200	90.54±20.24	77.41±12.53	74.79±13.06	75.71±15.61	79.38±15.61	83.89±18.00

#Normal Reference Value from Sodikoff C.H. 2001. Serum Chemistry Tests. Laboratory profiles of small animal diseases.: A guide to laboratory diagnosis. 3rd ed. Mosby-Year Book. St.Louis.p. 5-18.

Appendix 7 Alanine aminotransferase (ALT) (IU/L) between placebo and treatment group on day 0

Group	n	Mean	SD	SEM	t	Sig.
Placebo	7	27.7429	17.20706	6.50366	-2.341	0.032
Treatment	12	49.7333	21.00897	6.06477		

Appendix 8 Plasma GAG level (µg/ml) between placebo and treatment group on day21

Group	n	Mean	SD	SEM	t	Sig.
Placebo	7	24.2043	8.86106	3.34917	-2.500	0.025
Treatment	10	39.9620	16.88918	5.34083		

Appendix 9 Urinary GAG level (µg/ml) between placebo and treatment group on day14

Group	n	Mean	SD	SEM	t	Sig.
Placebo	7	4.8914	2.86117	1.08142	-2.174	0.045
Treatment	11	9.1473	4.61606	1.39179		

Appendix 10 Urine creatinine level (x100 mg%) between placebo and treatment group on day 28

Group	n	Mean	SD	SEM	t	Sig.
Placebo	6	4.3983	0.83127	0.33937	2.575	0.020
Treatment	12	3.0133	1.16987	0.33771		

Appendix 11 Plasma GAGs levels (µg/ml) that compare day 0 with day 21 in treatment group

Day	Mean	SD	SEM	D	SD_D	SEM_D	t	Sig.
Day 0	26.2844	12.2902	4.0967	-1.26578E1	16.1738	5.3913	-2.348	.047
Day 21	38.9422	17.5841	5.8614					

Appendix 12 Plasma GAGs levels (µg/ml) that compare day 0 with day 28 in treatment group

Day	Mean	SD	SEM	D	SD_D	SEM_D	t	Sig.
Day 0	27.4645	12.9344	3.8999	-1.35555E1	16.5621	4.9937	-2.715	.022
Day 28	41.0200	24.1454	7.2801					

Appendix 13 Complete blood count and blood chemistry values of each cat on day 0

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	5.19	30	8,200	5,166	2,378	0	328	328	33	1.8	12	50
2	A2	Placebo	10.38	35	10,550	5,167	3,270	0	0	2,110	18.5	1.53	64.5	50.3
3	A3	Placebo	5.75	33	6,900	4,071	1,932	69	276	552	35.6	2.07	31.7	49.3
4	A4	Placebo	3.85	30	4,600	2,530	1,564	0	46	460	21.1	1.5	21.5	139
5	A5	Placebo	6	32	4,600	2,760	1,380	46	230	184	24.1	1.3	23.4	153
6	A6	Placebo	6.74	48	8,400	4,452	3,192	0	0	756	21.3	1.49	20	161
7	A7	Placebo	5.6	38	14,550	13,677	728	0	0	146	19.5	1.65	21.1	31.1
8	B1	Treatment	6.85	45	5,600	3,920	1,400	0	0	280	45.9	2.42	56.2	118
9	B2	Treatment	6.1	38	4,200	3,108	966	0	42	84	28	2.1	59	102
10	B3	Treatment	5.24	35	7,100	4,047	2,769	0	0	284	30	1.7	40	62
11	B4	Treatment	10	55	9,400	6,110	2,256	0	94	752	21	1.8	75	148
12	B5	Treatment	4.5	29	7,100	5,680	923	0	355	142	45	2.15	64.7	275
13	B6	Treatment	9	60	6,400	4,288	1,792	0	256	64	48.4	2.25	39.6	114
14	B7	Treatment	2.96	28	10,000	9,100	700	0	0	200	25.6	1.93	30.4	12.1
15	B8	Treatment	5.12	38	13,500	12,690	540	0	0	270	29.4	1.62	20.2	31.5
16	B9	Treatment	4.04	ND	8,550	4,703	2,565	0	85	1,197	27.2	1.39	36.2	56
17	B10	Treatment	5.82	ND	8,650	5,882	1,903	0	87	778	28.5	1.45	37.1	62
18	B11	Treatment	9.02	34	16,700	9,018	5,678	0	167	1,670	19.8	1.68	94.9	55.2
19	B12	Treatment	6.72	47	16,850	12,301	4,044	0	169	337	19.4	1.57	43.5	50.7

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 14 Complete blood count and blood chemistry values of each cat on day 7

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	7	40	10,300	6,695	2,575	0	515	515	42.6	2.28	67.2	128
2	A2	Placebo	3.61	36	8,050	5,394	1,932	161	0	563	19.2	1.55	56.7	140
3	A3	Placebo	6.13	39	12,100	6,655	4,477	121	0	726	36.8	1.91	67.6	48.3
4	A4	Placebo	5.36	31	7,750	4,263	2,170	0	0	1,318	26.8	1.59	23.3	122
5	A5	Placebo	5.4	28	4,300	2,150	1,505	0	215	430	22.5	1.19	26.4	113
6	A6	Placebo	5.16	46	7,350	4,116	1,985	147	147	735	21.9	1.58	79.7	58.5
7	A7	Placebo	5.59	40	6,350	4,445	1,397	0	64	381	14.7	1.75	29.5	34.5
8	B1	Treatment	7.56	40	5,100	3,927	816	0	102	255	32.9	2.42	59.1	42.1
9	B2	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	B3	Treatment	11.25	48	7,500	3,675	2,625	75	300	825	29.2	1.51	46.7	75.8
11	B4	Treatment	9.2	45	10,100	7,070	2,828	0	0	202	22.2	1.73	96	113
12	B5	Treatment	3.7	25	10,500	8,190	1,785	0	525	0	16.7	2.57	22.5	111
13	B6	Treatment	8	52	8,000	6,000	1,520	80	400	0	31.1	2.21	40.4	145
14	B7	Treatment	6.36	30	8,100	5,508	2,430	0	0	162	27.2	2.22	56.1	135
15	B8	Treatment	5.87	30	8,400	6,720	1,512	0	0	168	25.5	1.53	20.8	31.4
16	B9	Treatment	4.95	45	6,150	3,567	1,968	0	0	615	27.4	1.66	42.7	61.1
17	B10	Treatment	6.24	36	12,150	10,328	1,337	0	243	243	20.5	1.37	36	54.7
18	B11	Treatment	4.06	42	16,000	12,000	3,040	0	0	960	19.7	1.82	106.1	51.8
19	B12	Treatment	7.13	48	12,150	9,963	1,094	0	0	1,094	20.8	1.53	50.4	30.6

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 15 Complete blood count and blood chemistry values of each cat on day 14

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	14.45	28	7,363	5,080	2,062	0	74	147	29.9	1.82	20	88.7
2	A2	Placebo	4.08	37	8,350	4,843	2,088	0	0	1,419	23.3	1.6	90.5	162
3	A3	Placebo	10.8	41	9,800	9,408	3,528	98	98	588	35.1	1.85	33.3	111
4	A4	Placebo	5	31	5,300	3,127	1,219	0	0	636	21.5	1.47	23.6	151
5	A5	Placebo	3.49	30	6,650	2,727	3,591	0	0	333	28.5	1.22	19	38.2
6	A6	Placebo	7.89	46	5,150	3,296	1,494	0	0	206	16.7	1.39	27.9	69.5
7	A7	Placebo	5.25	45	12,750	10,965	1,658	0	0	128	13.9	1.76	37.3	48.4
8	B1	Treatment	8.56	35	4,200	2,100	1,596	84	0	420	36.2	2.41	44.1	106
9	B2	Treatment	9.54	50	9,400	4,888	3,384	0	188	752	31.3	1.8	57.4	118
10	B3	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	B4	Treatment	8.3	40	12,000	8,520	2,760	120	0	600	31.5	1.52	101	142
12	B5	Treatment	6.95	25	26,650	17,322	7,995	0	0	1,332	25.3	1.99	22.14	58.6
13	B6	Treatment	8	40	21,950	13,389	7,463	0	0	1,097	24.5	2.28	16.4	74.3
14	B7	Treatment	5.1	32	4,550	3,003	1,046	0	45	455	25.6	2.18	69.7	135
15	B8	Treatment	6.93	42	18,400	14,904	2,392	0	0	1,104	26.4	1.7	29.2	31.2
16	B9	Treatment	8.98	47	6,350	4,128	1,524	0	0	699	26.5	1.55	13.6	44.4
17	B10	Treatment	7.54	40	7,500	5,400	1,800	0	0	300	32.7	1.67	15.8	53.9
18	B11	Treatment	5.2	34	14,050	9,273	4,356	0	0	422	27.4	1.72	22	20.2
19	B12	Treatment	6.3	48	17,550	11,232	4,739	0	0	1,580	19.3	1.72	59	39.1

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 16 Complete blood count and blood chemistry values of each cat on day 21

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	9	30	9,975	5,187	4,289	0	0	499	30.5	1.81	34.4	93
2	A2	Placebo	8.12	36	6,350	3,493	2,222	0	0	835	18.3	1.57	77.7	106
3	A3	Placebo	5.06	40	5,450	3,924	1,253	0	0	273	29.3	1.99	34.3	145
4	A4	Placebo	6	36	5,500	3,520	1,430	0	165	385	26.3	1.32	14.2	55.2
5	A5	Placebo	4.93	40	7,800	4,680	2,184	0	156	780	18.8	1.23	17.7	130
6	A6	Placebo	7.58	45	9,750	6,923	2,633	0	0	98	17.2	1.38	43.6	20
7	A7	Placebo	5.44	43	15,900	10,971	3,975	159	0	795	27.4	2.09	16	112.9
8	B1	Treatment	9.87	35	5,400	3,348	1,674	108	0	270	35.1	2.4	55.9	50.9
9	B2	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	B3	Treatment	6.6	48	7,500	4,800	2,400	0	225	75	26	1.7	89	112
11	B4	Treatment	9.3	43	8,400	5,796	2,268	0	84	252	27.4	1.74	88.8	147
12	B5	Treatment	5	32	10,000	4,900	4,500	0	0	300	21	1.88	21.3	46.5
13	B6	Treatment	8	54	6,500	1,950	4,225	0	325	0	27.2	2.24	9.54	83.6
14	B7	Treatment	8	31	6,800	4,760	1,496	109	204	340	28	1.93	60.5	140
15	B8	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	B9	Treatment	9.42	38	5,800	3,828	754	0	0	1,218	24.1	1.54	41	107
17	B10	Treatment	7.29	42	5,500	3,685	1,210	0	55	550	25.2	1.52	39.6	6.9
18	B11	Treatment	6.63	42	7,550	4,304	2,492	0	302	0	23.8	1.76	38.3	20.4
19	B12	Treatment	5.87	42	12,800	6,656	4,608	0	0	1,536	25.5	1.28	75.5	42.8

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 17 Complete blood count and blood chemistry values of each cat on day 28

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	3.85	28	13,100	7,729	4,323	131	0	262	30.5	1.73	16.2	96.2
2	A2	Placebo	4.92	39	6,000	3,420	1,740	0	0	840	14.3	1.7	58.9	156
3	A3	Placebo	7.12	41	6,700	4,355	2,010	67	0	268	26.9	1.88	96.3	196
4	A4	Placebo	5.83	36	9,150	4,758	3,021	0	0	1,281	24.7	1.21	20.1	53.1
5	A5	Placebo	6.17	34	7,100	2,911	3,408	0	0	781	13.6	1.74	30.5	32.1
6	A6	Placebo	11.03	55	7,250	4,350	2,103	0	73	725	17	1.53	21.9	52.5
7	A7	Placebo	5.12	38	14,550	11,931	2,183	0	0	437	32.4	1.93	35	10.8
8	B1	Treatment	8.09	44	5,250	3,675	1,155	0	52	315	30.9	2.53	50.2	106.2
9	B2	Treatment	6.4	43	6,100	4,148	1,525	0	122	305	21.7	1.7	40.4	107
10	B3	Treatment	7	54	7,500	4,050	3,225	0	150	75	22	1.5	110	159
11	B4	Treatment	8.23	45	7,800	5,616	1,716	0	0	468	32.2	1.94	58.4	126
12	B5	Treatment	6.63	25	14,850	6,682	5,643	148	0	2,376	19.1	2.14	36.7	54.1
13	B6	Treatment	5.29	40	11,250	4,725	6,187	0	112	225	26.1	2.49	36.7	167
14	B7	Treatment	5	30	4,100	2,091	1,517	0	246	246	28.9	2.05	49.8	86.6
15	B8	Treatment	4.39	ND	13,200	9,900	2,772	0	132	396	21.7	1.36	85.5	23.8
16	B9	Treatment	7.1	40	7,950	5,963	1,749	0	80	159	39.7	1.34	13.4	45.8
17	B10	Treatment	5.5	38	8,300	6,723	913	0	0	664	28.4	1.62	16.8	9.65
18	B11	Treatment	6.03	38	12,750	7,268	4,590	0	0	383	21	1.4	46.5	50.1
19	B12	Treatment	5.4	42	14,700	9,261	4,410	0	0	735	17.8	1.52	74.1	17.3

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 18 Complete blood count and blood chemistry values of each cat on day 56

Number	Code	Group	RBC ($x10^6$/µl)	Hct (%)	WBC (/µl)	Neutrophils (/µl)	Lymphocytes (/µl)	Bands (/µl)	Monocytes (/µl)	Eosinophils (/µl)	BUN (mg%)	Cr (mg%)	ALT (IU/L)	ALP (IU/L)
1	A1	Placebo	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	A2	Placebo	6.33	29	5,400	2,700	2,160	0	0	540	22.6	1.44	58	64.2
3	A3	Placebo	5.73	32	6,100	4,270	1,464	0	0	366	26.3	1.98	43.7	127
4	A4	Placebo	6.45	ND	10,250	6,868	1,538	0	205	410	25.1	1.1	28.6	46.6
5	A5	Placebo	8.23	37	8,600	4,386	3,612	0	0	602	25.6	1.99	29.3	32.1
6	A6	Placebo	4.9	44	6,550	4,127	1,900	0	131	393	28	1.49	29.2	56.6
7	A7	Placebo	6.34	39	13,150	9,863	3,814	263	0	526	35.7	1.51	23.8	31.3
8	B1	Treatment	10.1	45	3,900	2,184	1,131	0	39	507	29.2	2.72	62.1	157
9	B2	Treatment	9	54	3,800	2,128	1,330	0	114	228	26.3	1.67	34.4	90.3
10	B3	Treatment	6.3	41	7,300	2,847	3,869	0	146	438	24.3	1.61	48.3	105
11	B4	Treatment	13.34	40	15,250	10,675	4,270	0	0	305	27.7	1.74	46.2	48.7
12	B5	Treatment	5.6	32	14,500	9,135	3,335	0	725	1,305	27.6	2.31	47.6	51.3
13	B6	Treatment	7	44	11,700	7,137	3,978	0	351	234	24.7	2.39	53.8	206
14	B7	Treatment	4.7	32	8,950	7,428	1,342	0	0	179	23.9	1.54	25.6	53.6
15	B8	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	B9	Treatment	11.89	40	7,750	5,115	1,473	0	310	853	36.2	1.44	18.3	39.6
17	B10	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	B11	Treatment	5.48	42	10,400	4,264	5,304	0	0	832	26.6	1.57	68.2	45.8
19	B12	Treatment	6.61	42	7,750	4,650	2,248	0	155	698	20.9	1.5	45.2	41.6

Note: ALP = alkaline phosphatase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, Hct = hematocrit, Cr = Creatinine, ND = Not determined

Appendix 19 Urinalysis of each cat on day 0

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	1.035	6	3	neg.	4	neg.	TNTC	TNTC	neg.	neg.	neg.
2	A2	Placebo	>1.050	7	3	neg.	neg.	neg.	0-1	1-2	neg.	struvite rare	neg.
3	A3	Placebo	>1.050	7	3	2	4	neg.	50-100	3-5	neg.	Struvite rare	TSC rare
4	A4	Placebo	1.040	6	3	3	4	neg.	100-200	3-5	neg.	neg.	neg.
5	A5	Placebo	>1.050	6	3	2	3	neg.	neg.	neg.	neg.	neg.	fat droplet
6	A6	Placebo	>1.050	9	3	3	4	neg.	TNTC	neg.	neg.	struvite3+, ca^{2+} oxalate rare	TSC rare
7	A7	Placebo	1.028	9	3	3	4	neg.	10-20	3-5	neg.	struvite 2+	debris, mucous
8	B1	Treatment	1.021	5	2	neg.	neg.	neg.	neg.	neg.	neg.	neg.	SQC rare, RBC clumps
9	B2	Treatment	>1.050	7	3	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10	B3	Treatment	>1.050	7	2	1	3	neg.	5-10	neg.	neg.	struvite rare	neg.
11	B4	Treatment	1.045	8	3	1	4	neg.	50-100	2-3	neg.	struvite rare	neg.
12	B5	Treatment	1.015	6	3	1	4	neg.	neg.	20-30	neg.	neg.	rod motile bacteria 3+, wbc clump
13	B6	Treatment	1.026	9	neg.	3	4	neg.	TNTC	neg.	neg.	struvite 2+, ca^{2+} oxalate 1+	neg.
14	B7	Treatment	1.04	6	3	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
15	B8	Treatment	1.032	8	3	2	4	2	10-20	TNTC	neg.	neg.	SQC rare
16	B9	Treatment	1.026	9	3	1	neg.	neg.	neg.	neg.	neg.	neg.	SQC rare
17	B10	Treatment	>1.050	7	3	1	4	neg.	20-30	5-10	neg.	neg.	neg.
18	B11	Treatment	1.049	8	3	1	neg.	neg.	neg.	neg.	neg.	struvite rare	neg.
19	B12	Treatment	1.049	6	3	3	4	neg.	TNTC	3-5	neg.	neg.	neg.

Appendix 20 Urinalysis of each cat on day 7

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	1.04	6	3	3	4	neg.	neg.	TNTC	neg.	neg.	rod bacteria
2	A2	Placebo	1.042	7	3	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
3	A3	Placebo	>1.050	8	3	1	2	neg.	neg	neg	neg	struvite 3+	RBC clump
4	A4	Placebo	>1.050	9	3	2	neg.	neg.	1-2	neg.	neg.	struvite 2+	SQC rare
5	A5	Placebo	>1.050	6	3	1	2	neg.	neg.	neg.	neg.	neg.	neg.
6	A6	Placebo	>1.050	8	3	1	4	neg.	TNTC	neg.	neg.	neg.	SQC rare, debris
7	A7	Placebo	1.029	6	3	2	4	neg.	10-20	1-2	neg.	struvite 3+	neg.
8	B1	Treatment	>1.050	6	2	1	neg.	neg.	neg.	neg.	neg.	struvite rare	neg.
9	B2	Treatment	>1.050	8	3	2	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet
10	B3	Treatment	>1.050	8	3	1	4	neg.	TNTC	5-10	neg.	amorphous 3+, struvite 3+	rod bacteria
11	B4	Treatment	>1.050	8	3	2	4	neg.	TNTC	2-3	neg.	neg.	neg.
12	B5	Treatment	1.015	7	3	1	3	neg.	30-50	5-10	neg.	neg.	rod bacteria
13	B6	Treatment	1.026	6	2	1	neg.	neg.	neg.	30-50	neg.	neg.	rod bacteria
14	B7	Treatment	1.042	5	3	1	1	neg.	neg.	neg.	neg.	neg.	neg.
15	B8	Treatment	1.046	6	3	1	neg.	neg.	2-3	TNTC	neg.	neg.	neg.
16	B9	Treatment	1.016	6	2	neg.	1	neg.	neg.	neg.	neg.	neg.	SQC 10/HPF
17	B10	Treatment	>1.050	9	3	1	neg.	neg.	neg.	neg.	neg.	amorphorous rare	neg.
18	B11	Treatment	1.049	8	3	1	neg.	neg.	5-10	1-2	neg.	struvite 1+	neg.
19	B12	Treatment	1.049	6	3	3	4	neg.	TNTC	TNTC	neg.	neg.	neg.

Appendix 21 Urinalysis of each cat on day 14

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	>1.050	7	3	2	4	neg.	neg.	neg.	neg.	neg.	neg.
2	A2	Placebo	1.042	7	3	1	neg.	neg.	neg.	2-3	neg.	struvite 1+	neg.
3	A3	Placebo	>1.050	9	2	2	2	neg.	neg.	neg.	neg.	struvite 1+	TSC rare
4	A4	Placebo	>1.050	8	3	1	4	neg.	neg.	neg.	neg.	struvite 1+	neg.
5	A5	Placebo	>1.050	6	3	1	1	neg.	neg.	0-1	neg.	neg.	fat droplet, SQC rare
6	A6	Placebo	>1.050	7	3	neg.	4	neg.	10-20	neg.	neg.	neg.	neg.
7	A7	Placebo	1.01	8	3	3	4	neg.	10-20	neg.	neg.	struvite 1+	neg.
8	B1	Treatment	>1.050	5	1	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
9	B2	Treatment	>1.050	7	4	2	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10	B3	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	B4	Treatment	1.041	8	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
12	B5	Treatment	1.019	6	2	1	4	neg.	neg.	neg.	neg.	neg.	neg.
13	B6	Treatment	1.018	7	1	1	4	neg.	1-2	2-3	neg.	neg.	rod motile bacteria, TSC clump
14	B7	Treatment	1.038	6	3	1	4	neg.	5-10	1-2	neg.	neg.	renal tubular cell
15	B8	Treatment	1.04	8	3	2	4	neg.	TNTC	TNTC	neg.	neg.	neg.
16	B9	Treatment	1.042	9	3	2	4	neg.	TNTC	1-2	neg.	struvite 3+	amorphorous rare
17	B10	Treatment	>1.050	6	3	1	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet
18	B11	Treatment	1.05	7	3	1	2	neg.	neg.	neg.	neg.	neg.	cocci rare, debris
19	B12	Treatment	>1.050	7	3	1	1	neg.	neg.	neg.	neg.	struvite 1+	neg.

Appendix 22 Urinalysis of each cat on day 21

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	>1.050	8	3	2	3	neg.	neg.	neg.	neg.	neg.	neg.
2	A2	Placebo	>1.050	9	2	1	neg.	neg.	neg.	neg.	neg.	struvite rare	neg.
3	A3	Placebo	1.038	7	3	1	neg.	neg.	neg.	neg.	neg.	struvite rare	neg.
4	A4	Placebo	1.05	6	3	3	4	neg.	TNTC	30-50	neg.	struvite rare, Ca^{2+} oxalate rare Hippuric acid 10-20/LPF	SQC rare
5	A5	Placebo	1.048	8	neg.	1	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet
6	A6	Placebo	>1.050	8	3	neg.	neg.	neg.	2-3	neg.	neg.	neg.	SQC rare
7	A7	Placebo	1.018	6	3	3	4	neg.	30-50	10-20	neg.	neg.	neg.
8	B1	Treatment	1.038	5	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
9	B2	Treatment	>1.050	7	3	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10	B3	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	B4	Treatment	>1.050	7	3	1	3	neg.	neg.	neg.	neg.	neg.	neg.
12	B5	Treatment	1.014	7	2	1	4	neg.	neg.	neg.	neg.	neg.	rod motile bacteria (numerous), TSC
13	B6	Treatment	1.041	7	1	1	4	neg.	neg.	neg.	neg.	neg.	RBC clump, TSC
14	B7	Treatment	1.042	7	3	1	1	neg.	neg.	neg.	neg.	neg.	neg.
15	B8	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	B9	Treatment	1.05	7	3	neg.	3	neg.	neg.	neg.	neg.	struvite 3+	debris
17	B10	Treatment	>1.050	6	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
18	B11	Treatment	>1.050	6	3	neg.	neg.	neg.	neg.	1-2	neg.	amorphorous	rod 2+
19	B12	Treatment	>1.050	6	3	1	4	neg.	30-50	neg.	neg.	neg.	neg.

Appendix 23 Urinalysis of each cat on day 28

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	>1.050	7	3	2	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet
2	A2	Placebo	>1.050	8	2	1	neg.	1	neg.	neg.	neg.	struvite rare	neg.
3	A3	Placebo	>1.050	9	3	1	2	neg.	neg.	neg.	neg.	Ca^{2+} Oxalate, struvite rare	SQC rare
4	A4	Placebo	>1.050	8	3	2	4	neg.	TNTC	3-5	neg	struvite rare	SQC rare
5	A5	Placebo	>1.050	5	3	2	neg.	neg.	neg.	neg.	neg.	neg.	SQC rare
6	A6	Placebo	>1.050	6	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
7	A7	Placebo	1.028	7	3	3	4	neg.	TNTC	30-50	neg.	neg.	neg.
8	B1	Treatment	>1.050	5	neg.	1	neg.	neg.	neg.	neg.	neg.	neg.	neg.
9	B2	Treatment	>1.050	8	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10	B3	Treatment	>1.050	7	3	neg.	neg.	neg.	neg.	neg.	neg.	struvite rare	neg.
11	B4	Treatment	>1.050	7	3	3	4	neg.	neg.	neg.	neg.	struvite 2+	sperm, RBC clumps
12	B5	Treatment	1.02	7		2	neg.	neg.	neg.	1-2	neg.	neg.	rod motile bacteria
13	B6	Treatment	1.024	6	2	1	neg.	neg.	neg.	30-50	neg.	neg.	rod motile bacteria
14	B7	Treatment	1.039	6	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	SQC 1-2cells/HPF
15	B8	Treatment	>1.050	7	3	2	neg.	neg.	neg.	neg.	neg.	struvite rare, amorphorous	neg.
16	B9	Treatment	1.049	7	3	1	2	neg.	3-5	1-2	neg.	neg.	neg.
17	B10	Treatment	>1.050	6	3	1	neg.	4	neg.	neg.	neg.	neg.	neg.
18	B11	Treatment	>1.050	6	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	cocci bacteria
19	B12	Treatment	>1.050	5	3	1	4	neg.	neg.	neg.	neg.	neg.	neg.

Appendix 24 Urinalysis of each cat on day 56

Number	Code	Group	Sp.Gr.	pH	WBC (strip)	Protein	Blood	Glucose	RBC (/HPF)	WBC (/HPF)	Cast	Crystals	Others
1	A1	Placebo	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	A2	Placebo	>1.050	7	3	1	neg.	neg.	neg.	neg.	neg.	neg.	SQC rare
3	A3	Placebo	>1.050	6	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet, TSC rare
4	A4	Placebo	>1.050	6	3	2	neg.	neg.	neg.	neg.	neg.	neg.	neg.
5	A5	Placebo	1.05	8	3	2	neg.	neg.	neg.	neg.	neg.	neg.	fat droplet
6	A6	Placebo	>1.050	7	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
7	A7	Placebo	1.028	7	3	1	4	neg.	100-200	20-30	neg.	neg.	neg.
8	B1	Treatment	1.028	5	3	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
9	B2	Treatment	>1.050	7	3	2	neg.	neg.	neg.	neg.	neg.	neg.	neg.
10	B3	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	B4	Treatment	1.015	8	3	3	4	neg.	2-3	TNTC		struvite 1+	TSC 1-5/HPF
12	B5	Treatment	1.024	7	3	1	4	neg.	3-5	5-10	neg.	neg.	rod motile bacteria (numerous), WBC clumping
13	B6	Treatment	1.034	7	3	2	neg.	neg.	neg.	10-20	neg.	neg.	rod motile bacteria (numerous)
14	B7	Treatment	1.04	7	2	1	4	neg.	10-20	neg.	neg.	neg.	neg.
15	B8	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	B9	Treatment	>1.050	9	3	1	4	neg.	100	1-2	neg.	struvite 3+	neg.
17	B10	Treatment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	B11	Treatment	1.042	6	3	neg.	4	neg.	10-20	1-2	neg.	neg.	cocci bacteria 3+
19	B12	Treatment	>1.050	6	3	1	4	neg.	100-200	neg.	neg.	struvite 1+	neg.

Appendix 25 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 0

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	54.01	6.57	1.94	3.39
2	A2	Placebo	34.08	11.53	5.34	2.16
3	A3	Placebo	19.60	4.35	5.82	0.75
4	A4	Placebo	9.74	8.94	2.97	3.01
5	A5	Placebo	15.92	8.34	5.86	1.42
6	A6	Placebo	25.57	8.85	2.95	3.00
7	A7	Placebo	30.15	5.40	3.65	1.48
8	B1	Treatment	20.73	7.01	2.15	3.26
9	B2	Treatment	46.84	5.15	6.36	0.81
10	B3	Treatment	ND	ND	ND	ND
11	B4	Treatment	30.47	3.69	4.33	0.85
12	B5	Treatment	41.52	7.88	1.28	6.16
13	B6	Treatment	10.57	23.52	2.38	9.88
14	B7	Treatment	32.73	4.56	2.45	1.86
15	B8	Treatment	18.71	4.81	1.83	2.63
16	B9	Treatment	9.53	7.28	2.02	3.60
17	B10	Treatment	44.55	5.88	2.42	2.43
18	B11	Treatment	21.44	17.00	1.94	8.76
19	B12	Treatment	25.02	2.70	4.7	0.57

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 26 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 7

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	30.03	2.48	3.64	0.68
2	A2	Placebo	43.69	3.80	3.17	1.20
3	A3	Placebo	6.41	9.52	5.42	1.76
4	A4	Placebo	14.47	9.46	2.56	3.70
5	A5	Placebo	26.59	10.10	4	2.53
6	A6	Placebo	38.28	9.35	3.09	3.03
7	A7	Placebo	32.73	9.60	2.46	3.90
8	B1	Treatment	ND	9.42	1.72	5.48
9	B2	Treatment	ND	ND	ND	ND
10	B3	Treatment	46.00	27.37	7.41	3.69
11	B4	Treatment	31.22	6.68	4.26	1.57
12	B5	Treatment	61.96	4.81	2.17	2.22
13	B6	Treatment	7.61	14.89	2.3	6.47
14	B7	Treatment	29.66	16.70	3.23	5.17
15	B8	Treatment	0.81	4.05	1.95	2.08
16	B9	Treatment	22.27	2.78	2.39	1.16
17	B10	Treatment	44.92	12.58	4.36	2.88
18	B11	Treatment	25.30	16.35	3.63	4.50
19	B12	Treatment	49.12	3.20	3.03	1.06

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 27 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 14

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	26.77	8.23	2.56	3.21
2	A2	Placebo	24.71	4.44	5.13	0.87
3	A3	Placebo	15.57	4.03	3.91	1.03
4	A4	Placebo	8.65	8.08	2.69	3.00
5	A5	Placebo	13.02	6.21	3.69	1.68
6	A6	Placebo	27.35	3.00	4.9	0.61
7	A7	Placebo	37.48	0.25	0.48	0.52
8	B1	Treatment	21.44	12.59	0.7	17.99
9	B2	Treatment	77.67	5.53	5.27	1.05
10	B3	Treatment	ND	ND	ND	ND
11	B4	Treatment	66.84	5.21	6.51	0.80
12	B5	Treatment	55.14	6.31	2.48	2.54
13	B6	Treatment	9.99	11.35	2.18	5.21
14	B7	Treatment	25.81	14.19	2.9	4.89
15	B8	Treatment	18.94	6.16	2.51	2.45
16	B9	Treatment	10.60	3.27	2.37	1.38
17	B10	Treatment	37.55	7.36	2.01	3.66
18	B11	Treatment	33.94	10.20	2.78	3.67
19	B12	Treatment	40.47	18.45	5.09	3.62

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 28 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 21

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	26.78	4.00	4.41	0.91
2	A2	Placebo	29.22	4.21	4.67	0.90
3	A3	Placebo	21.21	9.25	5.31	1.74
4	A4	Placebo	15.17	10.86	2.57	4.23
5	A5	Placebo	13.35	4.41	3.16	1.40
6	A6	Placebo	39.40	13.45	6.64	2.03
7	A7	Placebo	24.30	0.03	1.58	0.02
8	B1	Treatment	58.14	8.52	2.06	4.14
9	B2	Treatment	ND	5.53	4.66	1.19
10	B3	Treatment	49.14	ND	ND	ND
11	B4	Treatment	53.89	ND	ND	ND
12	B5	Treatment	68.19	5.11	1.5	3.41
13	B6	Treatment	27.93	16.63	3	5.54
14	B7	Treatment	22.30	8.55	3.29	2.60
15	B8	Treatment	ND	ND	ND	ND
16	B9	Treatment	18.09	3.28	3.07	1.07
17	B10	Treatment	34.48	5.02	2.41	2.08
18	B11	Treatment	26.02	14.00	2.92	4.79
19	B12	Treatment	41.44	18.65	6.60	2.83

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 29 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 28

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	21.71	30.18	4	7.55
2	A2	Placebo	33.04	1.02	4.66	0.22
3	A3	Placebo	28.70	4.12	4.05	1.02
4	A4	Placebo	10.01	ND	ND	ND
5	A5	Placebo	9.63	11.56	5.95	1.94
6	A6	Placebo	32.99	3.78	3.61	1.05
7	A7	Placebo	27.99	2.95	4.12	0.72
8	B1	Treatment	47.11	16.29	1.64	9.93
9	B2	Treatment	58.05	4.93	3.16	1.56
10	B3	Treatment	27.66	36.27	3.29	11.02
11	B4	Treatment	64.76	5.74	5.29	1.09
12	B5	Treatment	54.93	6.86	2.12	3.24
13	B6	Treatment	10.40	17.21	1.17	14.71
14	B7	Treatment	38.80	9.05	3.19	2.84
15	B8	Treatment	8.69	11.57	3.48	3.32
16	B9	Treatment	35.60	5.07	2.26	2.24
17	B10	Treatment	86.62	8.81	2.34	3.76
18	B11	Treatment	20.98	11.25	4.02	2.80
19	B12	Treatment	25.28	19.01	4.2	4.53

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 30 Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) of each cat on day 56

Number	Code	Group	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Cr (mg%)	GAG/Cr ($x10^{-3}$)
1	A1	Placebo	ND	ND	ND	ND
2	A2	Placebo	39.81	2.66	5.14	0.52
3	A3	Placebo	31.39	7.56	5.46	1.38
4	A4	Placebo	12.56	4.11	2.64	1.55
5	A5	Placebo	9.19	7.85	4.04	1.94
6	A6	Placebo	35.33	18.46	4.34	4.25
7	A7	Placebo	ND	ND	ND	ND
8	B1	Treatment	30.76	12.81	1.06	12.08
9	B2	Treatment	45.37	3.12	6.25	0.50
10	B3	Treatment	28.01	28.47	ND	ND
11	B4	Treatment	54.76	7.11	5.97	1.19
12	B5	Treatment	22.44	6.44	1.84	3.50
13	B6	Treatment	19.80	26.06	3.76	6.93
14	B7	Treatment	25.26	9.55	3.16	3.02
15	B8	Treatment	ND	ND	ND	ND
16	B9	Treatment	20.45	11.54	3.3	3.50
17	B10	Treatment	ND	ND	ND	ND
18	B11	Treatment	32.71	11.10	1.79	6.20
19	B12	Treatment	48.49	20.26	5.01	4.04

Note: GAG = glycosaminoglycan, Cr = creatinine, ND = Not determined

Appendix 31 Signalment, Plasma GAG (µg/ml), urinary GAG (µg/ml), urine creatinine (mg%) and GAG/creatinine ratio ($x10^{-3}$) in normal cats

Number	Code	Age	Breed	Sex	Weight	Plasma GAG (µg/ml)	Urinary GAG (µg/ml)	Urine Creatinine (mg%)	GAG/Creatinine ($x10^{-3}$)
1	C1	unk	DSH	M	unk	28.02±3.85	36.44±2.7	4.97	7.33
2	C2	unk	DSH	F	unk	37.04±2.30	10.42±0.54	2.13	4.89
3	C3	unk	DSH	M	2.8	19.92±2.44	ND	ND	ND
4	C4	unk	DSH	M	3.5	29.55+1.63	ND	ND	ND
5	C5	unk	DSH	M	5	16.60±3.83	ND	ND	ND
6	C6	unk	DSH	F	unk	17.46±2.92	14.04±0.16	5.68	2.47
7	C7	unk	DSH	F	4.62	33.08±3.31	ND	ND	ND
8	C8	unk	DSH	M	unk	11.57±4.82	48.69±0.02	ND	ND
9	C9	unk	DSH	unk	unk	30.49±3.55	50.82±1.45	1.57	32.37
10	C10	unk	DSH	unk	unk	16.75±2.84	ND	ND	ND
11	C11	unk	DSH	unk	unk	ND	69.43±1.93	3.61	19.23
12	C12	unk	DSH	Fs	unk	ND	50.18±3.75	3.77	13.31
13	C13	unk	DSH	Fs	unk	ND	60.36±5.16	2.24	26.95
14	C14	unk	DSH	Fs	unk	ND	40.94±5.44	7.17	5.71
15	C15	unk	DSH	unk	unk	ND	61.26±1.91	3.88	15.79

Note: Unk= unknown, ND = Not determined

BIOGRAPHY

Miss Jinnapat Panchaphanpong was born on 5th November 1982 in Bangkok, Thailand. She graduated with Doctor of Veterinary Medicine (D.V.M) from Faculty of Veterinary Science, Mahidol University, Thailand, in 2007 and Bachelor of Economics (Business Economics), Sukhothai Thammatirat Open University, Thailand, in 2005. Then, she studied in Master of Science in Veterinary Medicine, Companion Animal Medicine Division, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Thailand in 2007. She also is a practitioner in private small animal hospital.