

# Semen Characteristics and Cardiac Enzymes in Healthy Male Cats Fed with Commercial Cat Food Containing *Yucca schidigera* <sup>[1][2]</sup>

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## Abstract

This study has been designed to examine the effect on cardiovascular enzymes, testosterone and semen characteristics of the *Yucca schidigera* extract (YSE), used as a feed additive in commercial cat food for the purpose of decreasing faecal odour in male cats. In eighteen healthy male cats, while the biomarker cardiac troponin I (cTnI), known as the "gold standard" for cardiovascular assessment in cats was used, creatin kinase-MB (CK-MB), total cholesterol and triglyceride levels were also investigated as aiding parameters. The level of cTnI was 0.16±0.03 ng/ml before feeding without YSE. It was found 0.20±0.04 ng/ml after feeding with YSE. Similarly, results of the study demonstrated that there was no difference statistically in cardiovascular or lipidic parameters after feeding on this type of cat food for 12 weeks. The level of testosterone was 0.29±0.01 ng/ml before feeding without YSE. It was found 0.21±0.02 ng/ml after feeding with YSE. (P>0.05). Therefore, semen analysis revealed a statistically significant increase in sperm motility alongside a significant decrease in sperm abnormalities. The mean volume, concentration, motility and total sperm defect rates were 118.61±8.55 µl, 197.78±12.83×10<sup>6</sup>/ml, 83.33±1.06% and 27.22±1.33% before feeding without YSE, respectively. These values were 213.06±21.29 µL, 300.56±16.59×10<sup>6</sup>/ml, 90.00±1.07% and 18.67±0.59% after feeding with YSE, respectively. The differences between these values were statistically significant (P<0.001). It was concluded at the end of the study that the commercial cat food containing *Yucca schidigera* extract could be beneficial effects of the sperm volume, concentration, motility and total morphological defect rates in healthy male cats.

**Keywords:** Male cat, *Yucca schidigera*, Semen, Cardiac enzymes

## *Yucca schidigera* İçeren Ticari Kedi Mamaları ile Beslenen Sağlıklı Erkek Kedilerde Sperma Özellikleri ve Kardiyak Enzim Analizleri

### Özet

Bu çalışma, erkek kedilerde fekal kokuyu azaltmak amacıyla ticari kedi mamalarına bir yem katkı maddesi olarak kullanılan *Yucca schidigera* ekstraktının (YSE) kardiyovasküler enzimler, testosteron ve spermatolojik özellikler üzerindeki etkisini incelemek için dizayn edilmiştir. Onsekiz adet sağlıklı erkek kedide, kedilerde kardiyovasküler değerlendirme için "altın standart" olarak bilinen biyobelirtec kardiyak troponin I (cTnI), kreatin kinaz-MB (CK-MB), total kolesterol ve trigliserid düzeyleri incelendi. cTnI düzeyi YSE içermeyen beslenmeden önce 0.16±0.03 ng/ml bulundu. Bu değer YSE ilavesi ile beslendikten sonra 0.20±0.04 ng/ml olarak tespit edildi. Benzer şekilde, 12 hafta boyunca beslenme sonrası kardiyovasküler veya lipidik parametrelerde istatistiksel fark olmadığı gözlemlendi. Testosteron düzeyi YSE içermeyen beslenmeden önce 0.29±0.01 ng/ml olarak saptandı. YSE ile beslendikten sonra ise 0.21±0.02 ng/ml olarak bulundu. (P>0.05). Buna karşın, YSE ile beslenme sonrası spermatolojik incelemelerde spermatozoon anomalilerinde önemli oranda bir azalma ile birlikte sperm motilitesinde istatistiksel olarak anlamlı bir artış saptandı. Ortalama sperma hacmi, konsantrasyon, motilite ve toplam spermatozoon anomalileri YSE içermeyen beslenmeden önce sırasıyla; 118.61±8.55 µL, 197.78±12.83×10<sup>6</sup>/ml, 83.33±1.06% ve 27.22±1.33% olarak saptandı. Bu değerler YSE ile beslendikten sonra sırasıyla; 213.06±21.29 ul, 300.56±16.59×10<sup>6</sup>/ml, %90.00±1.07 ve %18.67±0.59 olarak bulundu. Bu değerler arasındaki fark istatistiksel olarak anlamlı bulundu (P<0.001). Bu çalışmanın sonunda, *Yucca schidigera* ekstraktı içeren ticari kedi maması ile beslenen sağlıklı erkek kedilerde sperm hacmi, konsantrasyon, motilite ve toplam morfolojik bozukluk oranları üzerine yararlı etkileri olabileceği kanısına varıldı.

**Anahtar sözcükler:** Erkek kedi, *Yucca schidigera*, Sperma, Kardiyak enzimler



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## INTRODUCTION

Use of natural herbal extracts known as “Phytomedicines” is growing on the basis of both their aid in the treatment of various diseases as well as their health benefits. Addition of herbal extracts to animal food products is preferred due to their low cytotoxicity and residual properties. Across the globe, and particularly in the US, the herbal market is on the rise in the fields of human and animal health. Negativities caused by development of resistance and residue in relation to feed additive antibiotics has led the animal nutrition industry to focus on herbal extracts and oils. There are over 80.000 plants known for their bioactive characteristics <sup>[1]</sup>. Worldwide use of these plants in the veterinary field has been documented. In Italy <sup>[2]</sup>, reported 280 ethno veterinary plants, while <sup>[3]</sup> listed ~ 200 plants with ethno veterinary use in Southern Africa. For instance, it is known that aromatic herbs such as; fennel, thyme, sage, eucalyptus and cinnamon are used in egg poultry and pet food <sup>[4]</sup>. Echinacea, flaxseed, garlic, ginger, ginseng and yucca are also among herbal supplements given to horses <sup>[5]</sup>. In recent years, to prevent significant economic losses due to mould spoilage in pet food products and human salmonellosis contamination related to these foods, natural plant oils and extracts such as rosemary, oregano and lemongrass have been used <sup>[6]</sup>. *Yucca schidigera* extract has been used as a feed additive for a long time in human, equine, livestock and pet diets. The Federal Drug Administration of the USA has described the extract of this plant as safe for human consumption <sup>[7,8]</sup>. According to the European Commission <sup>[9]</sup> (EC 1831, 2003), regarding feed additives in animal nutrition, phytogetic compounds have been categorized as “sensory additives” and, in particular, as flavouring compounds, i.e. substances the inclusion of which in feeding stuffs increases feed smell and palatability. In present day pet industry, *Yucca schidigera* is also used as an alternative diet formulation, particularly to reduce spreading of faecal odour <sup>[10]</sup>.

Scientists have claimed that the *Yucca* plant is a phytogetic feed additive (PFA) containing steroids, saponins and glycocomponents. Essential oils and spices have distinct biological functions, such as; antiprotozoal, antimicrobial, or antioxidant properties <sup>[8]</sup>. Numerous previous studies have demonstrated the antibacterial effects of *Yucca schidigera* extract on the inhibition of gram-positive bacteria <sup>[11]</sup>. *Yucca* extract supplements have proved to have beneficial effects on the quality of carcass and meat in storage, as well as improving resistance of the immune system against diseases and reducing mortality. *Yucca* products have also been reported to stimulate the laying rate, egg weight and decrease serum glucose, cholesterol and triglyceride levels in laying quails <sup>[12]</sup>. *Yucca* contains resveratrol, polyphenolics and other stilbenes (yuccaols A, B, C, D and E). Therefore, *yucca* is used as an antioxidant and free-radical scavenger <sup>[13]</sup>. The positive effects of antioxidants on stress and production of free radicals

have been among the most important research topics in recent years regarding human and animal reproduction. Furthermore, since it was established that it decreased faecal odour in pet animals, *Yucca schidigera* has become very popular as an alternative diet formulation. In other studies aimed at the use of *yucca* extracts as an additive, weight gain, growth and reproduction in animals were investigated. However, despite its widespread use in pet nutrition, studies on male animal reproduction, in particular, are scarce. Research directed at the cardiovascular system is also insufficient. The most recent long-term large-scale epidemiological study has revealed a strong relationship between low testosterone levels and deaths related to cardiovascular diseases <sup>[14]</sup>. The purpose of this study is to contribute to recent studies aiming at drawing attention to the relationship between the reproductive and cardiovascular systems. In this study, effects on testosterone, cardiac enzymes and semen characteristics in healthy male cats feeding with commercial cat food containing *Yucca schidigera* extract has been assessed.

## MATERIAL and METHODS

### Animals

Eighteen healthy shorthair male cats from, approximately 24-36 months old and 3.5-4.5 kg bodyweight, were used. The cats were initially fed a commercial cat food for 12 weeks, which was produced by the Eagle Pack Natural Pet Food Company (Indoor adult dry cat food, Tewksbury, MA, USA) and did not contain the *Yucca schidigera* plant. Following this, a feeding programme was undertaken with the same cat food containing 150 ppm *Yucca schidigera* extract (DK Powder-35, produced by Desert King Int. USA) for 12 weeks and exposed to a 12-h light/dark cycle, at room temperature. Cats consumed average 60-65 g commercial dry cat food daily and were given drinking water *ad libitum*. The study was approved by the Animal Experimentation Ethics Committee of Istanbul University (Protocol 76/2009). All cat owners were given detailed information and signed a research consent form. Food ingredients was shown in *Table 1*.

**Table 1.** Chemical and basic composition of commercial cat food\*

**Tablo 1.** Ticari kedi mamasının temel ve kimyasal içeriği\*

Nutritional Level	Analysis Values
Moisture	10.0%
Crude protein	30.0%
Crude fat	14.0%
Crude fiber	7.3%
Crude ash	4.9%
Mineral matter	2.0%
Metabolizable energy	967 kcal/kg

\* taurine, magnesium, omega 3 and omega 6 fatty acids, vitamin A, D<sub>3</sub> and E

### Semen Analysis

All cats produced ejaculates with 80% progressively motile sperm; sperm counts were within normal limits. Semen samples were collected from the animals before eating (control) and after a period of 12 weeks feeding with commercial cat food containing YSE. Semen collection was performed from January and April using an electroejaculator (P-T Electronics, Boring, OR, USA) under general anesthesia. The volume of the semen was measured using an adjustable automatic micropipette (10-1.000  $\mu$ L) and the value was recorded in microliters. Sperm motility was assessed subjectively (3  $\mu$ L sample) using phase-contrast microscope at x200 magnification by viewing at least 3 fields. An aliquot (5  $\mu$ L) of semen was diluted with 10% formol saline and evaluated for total number of sperm using a hemocytometer chamber (Neubauer, Boeco, Hamburg, Germany). A spermac<sup>®</sup> stain kit (Stain enterprises, Onderstepoort, Republic of South Africa) was employed in sperm morphologic observations. The preparation was examined by light microscope at x1.000 magnification by counting 200 cells (acrosome, head, mid-piece, tail and total abnormal spermatozoa rate) as described by Baran et al.<sup>[15]</sup>.

### Blood Serum Analysis

Blood samples were collected from the animals before eating (control) and after a period of 12 weeks feeding with commercial cat food containing YSE. In serum samples; CK-MB, Total cholesterol and Triglyceride levels were measured with the enzymatic method using the Abbott C8000 autoanalyser and identical commercial kits.

Cardiac troponin-I (cTnI) and total testosterone levels were determined using the IMMULITE<sup>®</sup> commercial kit (solid-phase, competitive chemiluminescent enzyme immunoassay) with an immulite one immunoassay analyzer (DPC, USA). Due to the commercial kit properties, readings below 0.20 ng/ml could not be measured quantitatively

and were considered to be negative. For cTn-I, values of 0.20 mg/ml and above were considered to be positive and measured quantitatively.

### Statistical Analysis

Statistical analysis was performed using the SPSS for Window version 21.0 programme (SPSS Inc., Chicago, IL, USA). Analysis was performed using Student's t-test. P<0.05 was considered statistically significant.

## RESULTS

No difference was observed among the male cats with regard to testosterone levels and mid-piece sperm morphology after with YSE feeding (P>0.05). The motility rate, volume and concentration after with YSE feeding were observed higher than before with YSE feeding (P<0.001).

Results revealed that there was a statistically significant positive effect on motility, volume and morphological properties following feeding on pet food containing *Yucca schidigera* extract (Table 2).

No statistically significant difference was found between the enzymes CK-MB, cTn-I, total cholesterol and triglyceride examined to assess the cardiovascular system, before and after feeding (Table 3).

## DISCUSSION

It is necessary to investigate the health effects of adding natural herbal extracts to animal diets. Haematologic parameters examined during research can provide information on clinical and nutritional status. These parameters also aid in diagnosing the metabolic condition of tissues and organ function disorders. In the present study, the haematologic parameters used were; total cholesterol, triglyceride, troponin and ck-mb levels. The cholesterol-lowering action mechanism of herbal extracts containing

**Table 2.** Means of spermatological traits and testosterone levels of male cats in before (control) and after with *Yucca schidigera* extract feeding

**Tablo 2.** *Yucca schidigera* ekstraktlı beslenme öncesi (kontrol) ve sonrasında erkek kedilerin testostere ve spermatolojik özelliklerinin ortalamaları

Parameters	Before	After	Significance
Testosterone (ng/ml)	0.29±0.01	0.21±0.02	NS
Volume (microliter)	118.61±8.55 <sup>a</sup>	213.06±21.29 <sup>b</sup>	P<0.001
Concentration (x10 <sup>6</sup> /ml)	197.78±12.83 <sup>a</sup>	300.56±16.59 <sup>b</sup>	P<0.001
Motility (%)	83.33±1.06 <sup>a</sup>	90.00±1.07 <sup>b</sup>	P<0.001
Acrosome (%)	15.11±0.90 <sup>b</sup>	10.83±0.83 <sup>a</sup>	P<0.01
Head (%)	6.22±0.68 <sup>b</sup>	3.83±0.33 <sup>a</sup>	P<0.01
Mid-piece (%)	2.11±0.47	1.33±0.34	NS
Tail (%)	3.78±0.41 <sup>b</sup>	2.67±0.38 <sup>a</sup>	P<0.05
Total (%)	27.22±1.33 <sup>b</sup>	18.67±0.59 <sup>a</sup>	P<0.001

<sup>a,b</sup> Different superscripts within the same line demonstrate significant differences (n=18); Means±SE, NS: Not significant

**Table 3.** Means of creatine kinase-MB, troponin, total cholesterol and triglyceride levels in before (control) and after with *Yucca schidigera* extract feeding  
**Tablo 3.** *Yucca schidigera* ekstraktlı beslenme öncesi (kontrol) ve sonrasında kreatin kinaz-MB, troponin, toplam kolesterol ve trigliserit düzeylerinin ortalamaları

Stage	Creatine Kinase-MB (ng/ml)	Troponin (ng/ml)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
Before control feeding	197.89±7.56	0.16±0.03	109.44±5.81	28.33±3.25
After YSE feeding	194.94±7.58	0.20±0.04	116.56±6.73	24.56±2.76
Significance	NS	NS	NS	NS

Means±SE, P>0.05 NS: Not significant, (n=18)

sterol is based on decreasing and preventing cholesterol absorption. Some present views state that, plant sterols cause cholesterol to drop in broilers by limiting blood cholesterol increase and allowing its secretion via bile [4]. Similarly, Aazami et al.<sup>[16]</sup> pointed out the cholesterol-lowering effect of saponins present in yucca, either directly by way of inhibiting small intestinal absorption of cholesterol or indirectly by mucosal cell desquamation or preventing reabsorption of bile acids. There are studies demonstrating the presence of a cholesterol-lowering effect in goats receiving *Yucca schidigera* powder compared to the control group [17]. Mardalena et al.<sup>[18]</sup> reported that *Yucca schidigera* powder possesses an anti-oxidant function and this lowered cholesterol by preventing lipid oxidation through its ability to capture free radicals and radical peroxy salts. In this study, while the commercial food containing yucca extract was not seen to have a triglyceride- and cholesterol-lowering effect in cats, other studies suggest that the use of the herb may originate from differences in dosage.

Plant compounds cause diverse effects in the cardiovascular system. There are those that cause cardiac arrhythmias, haemorrhagic syndrome and cardiac necrosis and this effect is often associated with plant secondary compounds such as glycosides [19,20]. In this study, in order to assess cardiac function, the authors utilized cardiac markers used as biomarkers. In veterinary medicine, cardiac troponins are used in the diagnosis and prognosis of myocardial injury originating from the damage in myocardial cells. Circulating cardiac troponin I, in particular, is known to be the most important biomarker - the "gold standard" - in the diagnosis of myocardial injury in cats. It has been reported that, it is significantly raised compared to the control group in hypertrophic cardiomyopathy, the most important cardiac disease in cats. Also, while a relationship could not be found between histopathological investigation and diagnosis, it was established that the significantly raised cTnI level was useful and this was confirmed by other veterinary practices participating in the study [21,22].

While cTnI levels are low to non-detectable in healthy mammals, raised cTnI levels have been reported to be useful myocardial injury biomarkers in calves, cattle, horses, dogs and lambs [23-28]. In the present study, the cTnI level was seen to be normal in healthy cats and no change was observed in parameters when the cats were fed food

containing *Yucca schidigera* extract.

Creatin Kinase myocardial band (CK-MB) is another cardiac biomarker and it has been reported that it is raised in chronic coronary artery occlusion in animals, as well as in cases of post-surgical tissue damage and renal failure. However, due to its higher sensitivity between cardiac parameters, cTnI is favoured over CK-MB and troponin C and T [22]. Based on this information, while the authors preferred cTnI since it is the most sensitive parameter in response to cardiac disorders in cats, in this study, in view of the fact that it may aid in cardiac function assessment, CK-MB values were also examined and found to be within normal limits.

Consistent with other studies [14] in which testosterone level and cardiac function are related, hormone and cardiac parameters were seen to be within normal levels in the present study. Today, the *Yucca schidigera* plant is used as an additive in pet food products. However, there are few studies directed at its reproductive activity. In this study, semen characteristics examined in the andrological assessment of male cats were; volume (ml), concentration (sperm count/ml), motility (%) and abnormal spermatozoa count (%). In healthy male cats fed food containing yucca extract, while sperm volume, concentration and motility showed a statistically significant increase; acrosome, head, tail and total morphological abnormalities were found to be statistically lower than pre-administration values. In a study [13] determining reproductive activity in rabbits, the powder extract of the whole plant was used at doses of 5 g/100 kg and 20 g/100 kg. Both doses were found to be high compared to semen and progressive motility in the control group. The results of the study are similar to the sperm characteristics recorded in the present study. On this subject, there is data regarding the anti-oxidants and free radical scavengers present in yucca improving spermatological responses by aiding in the suppression of reactive oxygen species. Also, in a study carried out by Baláži et al.<sup>[29]</sup> it has been demonstrated that yucca intake in female rabbits aids in achieving the best birth rate by increasing progressive motility of spermatozoa in genitalia.

Detectability of natural herbal extracts after addition to food is a priority when determining their effects on health. In a study [30] carried out in Japan, it was reported that only 5-6% of yucca extract used as a food additive contained saponin and over 60% was constituted of

unknown substances. In this study it was also reported that, the extract of this plant is recorded in the food additive list in Japan and is included in food as a shelf life extender. However, no specification has been stated of this widely used plant as a food additive. In Turkey, it is used without any standardization or toxicological assessments. The present study, on the other hand, confirmed that examined parameters showed there to be no negative effects on the cardiovascular system, that the normal levels were preserved in analyses related to testosterone and heart, and concluded that it has a beneficial effect on reproduction.

Addition of crude plant extracts or plant mixtures to food is based mostly on traditional knowledge. It is necessary for this information to be supported by scientific research. There is a need for scientific research directed towards the health effects of plant active components. In research, dosage, toxicological risk assessment and standardization must be considered and inclusion of synthetic drugs and feed additives into feeding must be in this direction.

In keeping with present day animal production processes, the main scope in pet food industry is to promote better quality of life through an eco-friendly clean and natural mission. The current study shows that the used herbal extract has no long-term negative effects. Moreover, it has positive effects on semen characteristics in male cats. Nevertheless, there are deficiencies regarding suitable dosage, determining phytochemical composition and mechanism of action in the use of feed additives.

It was concluded at the end of the study that the commercial cat food containing *Yucca schidigera* extract (150 ppm) could be beneficial effects of the sperm volume, concentration, motility and total morphological defect rates in healthy male cats.

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