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Evaluation of the efficacy of IQV04 for weight loss in overweight and obese dogs.

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Abstract

Introduction: Overweight and obesity are worldwide problems. Because of the higher risk of associated diseases, it is better to prevent and treat overweight and obesity. Beside weight loss programs, there are different medications and supplements available. After promising results from human health studies on the efficacy of Litramine, a similar product, named IQV04, is developed for dogs to support weight loss. The objective of this study was to evaluate the efficacy of IQV04 for weight loss in overweight and obese dogs.

Materials and methods: Nineteen colony dogs were randomly divided in three groups, in this placebo controlled and double-blinded study. All dogs received once a day a standardised maintenance meal for their resting energy requirement (RER) with one of the three pills (IQV04 concentration A, IQV04 concentration B and placebo). Body weight and pelvic circumference were measured weekly. By start and end of the study, physical examination, body condition score, complete blood count, serum biochemical analysis and fecal examination was done.

Results: Results suggested that there was a difference in body weight reduction between the three groups. The rate of weight loss in the placebo, IQV04 concentration A and IQV04 concentration B group were 2.45 ± 0.51, 2.85 ± 1.84 and 3.03 ± 1.26% body weight reduction per week, respectively. However, this difference did not reach significance. Also there was no significant difference found in pelvic circumference reduction per week.

Conclusion: Further studies with larger patient pool are required to achieve more statistic power.

Keywords: Obesity, overweight, dogs, weight loss, IQV04

Introduction:

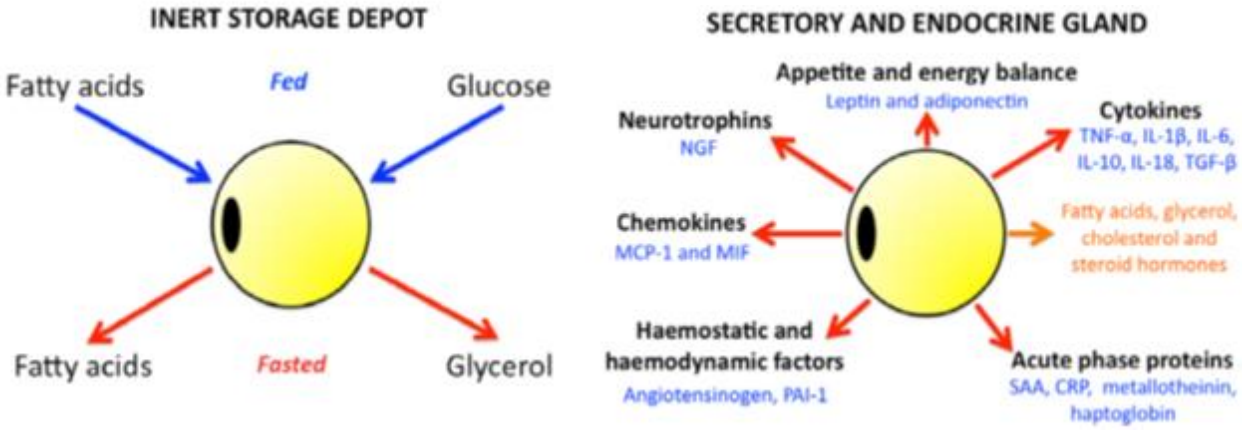
Overweight and obesity are worldwide problems among humans and the prevalence of obese people has more than doubled since 1980 (World Health Organization, 2015). This problem is not only seen in humans, even animals suffer from it. In recent studies,
the growing incidence of overweight and obese dogs is thought to be in the range of 41-61% of the general population (Courcier et al., 2010; McGreevy et al., 2005; Ricci et al., 2007), this is an increase proven by the fact that earlier studies found a prevalence ranging from 23% to 38% (Colliard et al., 2006; Donoghue et al., 1991; Edney & Smith, 1986; Lund et al., 2006). In a study of Corbee (2012) it is notable that show dogs have a lower incidence of overweight and obesity (18.6%) when compared to the preceding recent outcomes of general populations. This difference can be explained by the fact that these dogs need to be in a good condition for the show, which for most breeds implies being in an ideal body condition. Another explanation of the lower incidence of overweight show dogs is that these dogs are intact (Corbee, 2012).

The terms ‘obesity’ and ‘overweight’ are often used interchangeably, even though this is not correct. Overweight is defined as an abnormal fat accumulation and obesity is defined as an

excessive fat accumulation. In human medicine the difference is made by measuring the body mass index (BMI), where overweight is a BMI ≥ 25 kg/m^2 and obesity is a BMI ≥ 30 kg/m^2 (World Health Organization, 2015). The BMI is not used to define overweight and obesity in veterinary medicine. In dogs, overweight is defined as an increase of 10 to 20% of the ideal weight and obesity is defined as an increase of more than 20% (Toll et al., 2010). In this report, the terms 'obesity' and 'overweight' are used as defined by Toll et al. (2010).

Obesity is a state of positive energy balance, generally the result of either excessive dietary intake or inadequate energy use. The positive energy balance causes an increase in white adipose tissue mass by adipocytes. Adipose tissue functions as an active endocrine organ by secreting important hormones and adipokines (figure 1), for example leptin and adiponectin are two hormones produced by adipose tissue. Therefore, when adiposity increases, leptin secretion increases too. Leptin can stimulate increasing energy expenditure by diet-induced thermogenesis and activates the hypothalamus to reduce food intake by decreasing appetite (Laflamme, 2012). Lack of a normal feedback mechanism to downregulate food intake or increase metabolic energy expenditure, is a known pathophysiological mechanism in obese humans. This phenomenon is known as leptin resistance. To date there is no evidence of leptin resistance as a contributing factor to obesity in dogs (Wynne et al., 2005). While leptin levels rise when adiposity increases, adiponectin levels decrease. Adiponectin stimulates basal energy expenditure and improves insulin sensitivity (Ishioka et al., 2006; Laflamme, 2012).

Adipokines, like tumour necrosis factor, interleukin and others, produced by adipose tissue, can influence a diversity of biological processes, like inflammation, glycaemic control and



cardiovascular function (German et al., 2010).

Figure 1 – The biological function of adipose tissue
IL, interleukin; TNF- α, tumour necrosis factor- α; TGF-β, transforming growth factor-β; CRP, C-reactive protein; SAA, serum amyloid A; PAI-1, plasminogen activator inhibitor-1; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor (German et al., 2010).

Dysregulation of adipokines seems to contribute to the development of several diseases associated with obesity. Numerous studies support an association of obesity with orthopaedic diseases most notably osteoarthritis (German, 2006; Kealy et al., 1997; Kealy et al., 2000; Laflamme, 2005; Marshall et al., 2009), reduction of weight seems to have a positive result on lameness in dogs with osteoarthritis (Impellizeri et al., 2000; Marshall et al., 2010). Besides osteoarthritis, increased incidence of fractures, cranial cruciate ligament rupture, hip dysplasia and intervertebral disc disease are reported in overweight dogs (Brown et al., 1996; Edney & Smith, 1986; Smith et al., 2001; van Hagen et al., 2005). Cardiovascular and pulmonary disease are also associated with obesity. In small dogs obesity is an important risk factor for tracheal collapse development (White & Williams, 2008). Obesity can aggravate

laryngeal paralysis, brachycephalic airway obstruction syndrome and heatstroke (German, 2006) and it seems to be associated with portal vein thrombosis as well (van Winkle & Bruce, 1993). When considering endocrine and metabolic diseases, adipokines seem to contribute to insulin resistance leading to diabetes mellitus (German et al., 2009b; Lund et al., 2006). It has been proven that weight loss in obese dogs leads to a decrease in insulin resistance and reduction in plasma adipokines (German et al., 2009b). Other reported diseases associated with obesity are hypothyroidism, hyperadrenocorticism and insulinoma (Burkholder & Toll, 2000; Lund et al., 2006). Disorders in the urinary tract which associated with obesity are urethral sphincter mechanism incompetence and developing of calcium oxalate urolithiasis (Henegar et al., 2001; Lekcharoensuk et al., 2000). Accumulations of adipose tissue around the birth canal increase the risk of dystocia (Edney & Smith, 1986). An increased risk to develop neoplasia like transitional cell carcinoma of the bladder (Glickman et al., 1989) or mammary carcinoma (Sonnenschein et al., 1991) are also reported in overweight or obese dogs. The relationship between overweight or obesity with a reduction in animal's life span is reported in a long term research study of Kealy et al. (2002). In this study ad libitum-fed dogs tended to be overweight had a decreased longevity (Kealy et al., 2002). The study of German et al. (2012) examined the quality of life in obese dogs and showed that obese dogs have a reduced quality of life but after successful weight loss the quality of life improves (German et al., 2012). In addition to the numerous diseases obese and overweight dogs are at risk to develop, obesity is also a restriction in the clinical evaluation. For example, adipose tissue impedes auscultation of thorax, palpation of abdomen and ultrasonography (German, 2006), besides that there is the increased anesthetic risk, especially due to estimation of anesthetic dose and prolonged operating time, reported in obese animals (Clutton, 1988; van Goethem et al., 2003).

The major cause of overweight and obesity is the positive inequality between energy intake and energy expenditure. However, there are some diseases and factors which play a role in the development of obesity, for example an underlying disease is hypothyroidism, with a decrease metabolism and activity levels. Other examples are hyperadrenocorticism or pharmaceuticals, for instance glucocorticoids, which cause cortisol-induced polyphagia (German, 2006). Another risk factor is breed, there are studies suggesting that some breeds have a higher predisposition to become overweight or obese (Corbee, 2012; Edney & Smith, 1986; Lund et al., 2006; McGreevy et al., 2005). Recently, researchers found a gene deletion in Labrador Retrievers, which is associated with increased body weight, adiposity and greater food motivation (Raffan et al., 2016). Many studies suggested neutering and old age as an important risk factor for obesity, due to decrease in metabolic rate and decrease in activity, respectively (Courcier et al., 2010; Laflamme, 2005; Lund et al., 2006; McGreevy et al., 2005). There are risk factors associated with the owners of the dogs which may increase obesity, like type of diet (Kienzle et al., 1998; Lund et al., 2006), bodyweight of the owner (Nijland et al., 2010) and socio-economic status of the owner (Courcier et al., 2010; Kienzle et al., 1998). It is shown that obese dogs receive a higher number of meals during the day and more snacks than normal dogs (Kienzle et al., 1998) and when owners are obese it is more likely that their dog will be overweight or obese too (Colliard et al., 2006; Nijland et al., 2010). There also seems to be a link with the owners socio-economic status, when an owner is poor, the dogs tend to weight too much (Courcier et al., 2010; Kienzle et al., 1998).

Because of the increased risk of the previously mentioned diseases, it is better to prevent and treat overweight and obesity. Veterinarians play an important role in the prevention of obesity, they should, for example, recommend owners to reduce their food supply after neutering (McGreevy et al., 2005). The main objective of treatment for overweight and obese animals is weight loss, this increases insulin sensitivity (German et al., 2009b) and improves lameness in obese dogs with osteoarthritis (Marshall et al., 2010). A common way to achieve

weight loss is by using a dietary therapy. Different purpose-formulated weight reduction diets already exist on the market. The advantage of purpose-formulated weight reduction diets is that the energy content is low, while the diet is complete and balanced (German, 2016b). In addition to restriction of food energy intake, increasing the Maintenance Energy Requirement through additional exercise and other behavioural management has a positive effect on weight loss (German, 2006; Gossellin et al., 2007; Laflamme & Kuhlman, 1995). By additional exercise like walking, swimming, playing and use of puzzle feeders, the loss of adipose tissue will be stimulated and the lean tissue mass remains (German, 2006; German, 2016b). Behavioural management includes avoiding additional food intake, like treats and snacks, and specifically measuring the amount of food given on a scale to avoid accidental overfeeding while using to generously filled measuring cups (German, 2016b). To minimize hunger, prevent loss of lean tissue mass, and help reduce rebound weight gain, it is important not to lose weight too quickly. A 1 to 2% of body weight loss weekly is recommended ((Centraal Bureau voor de Statistiek, 2012; Laflamme & Kuhlman, 1995; Landelijk Informatie Centrum Gezelschapsdieren, 2010). The duration to reach the target body weight depends, among other things, on the extent of overweight and on the motivation and discipline of the owner.

However, the treatment of overweight and obese animals is not always as straightforward as it might appear. Some pet owners may be reluctant to admit that their animal is suffering from overweight or obesity while, others may be unable to resist their animal begging for food. Many pet owners need help with the weight reduction of their animal, with simple guidance in the amount of food given and exercise with concurrent check-ups of the weight and BCS of the animal. Though, weight-reduction programs can be often unsuccessful, mainly because of non-compliance with feeding and exercise recommendations of owners (Gossellin et al., 2007). The study of German et al. (2015) shows a drop out in weight loss programs of 40% for two reasons, because the dogs were euthanized (8%) or because the owner choose to stop with the weight loss program (32%) (German et al., 2015). The median rate of weight loss in colony dogs is 1 to 2.8% of body weight per week (Borne et al., 1996; Diez et al., 2002; Laflamme & Kuhlman, 1995) which is compared to the median rate of weight loss in client-owned dogs of 0.5 to 1% of body weight per week (German et al., 2007; German et al., 2015), more successful. The explanation for this difference in weight loss rate is likely due to owner compliance (German, 2016b).

In human medicine many drugs, like dinitrophenol, clenbuterol and phenethylamines, have been promoted for weight-reduction over the years. However, there are a lot of risks and side-effects like hyperthermia, cataracts, increased of blood pressure, tachycardia and death (Gossellin et al., 2007). The veterinary pharmaceutical industry have introduced dirlotapide (Slentrol®) and mitratapide (Yarvitan®) as weight loss promoting medication for dogs. Both drugs are microsomal triglyceride transfer protein (MPT) inhibitors. As several studies have shown, MTP inhibitors decreases intestinal fat absorption and reduces food intake, which lead to significant weight loss (Dobenecker et al., 2009; Wren et al., 2007). However, when administrating dirlotapide the decrease of intestinal fat absorption contributed to only approximately 10% of the weight loss. The other 90% of the weight loss could be attributed to appetite suppression. By discontinued use of dirlotapide, food intake will increase dramatically, so there is a big risk of weight rebound (Wren et al., 2007). Mitratapide is no longer authorised in Europe.

In addition to medications, there are multiple supplements for body weight reduction available in veterinary medicine. One specific supplement is important in the context of this study, namely Litramine. Data from human health studies on the efficacy of Litramine are promising. Litramine is a natural fiber complex derived from the plant *Opuntia ficus indica.* In

a study to investigate the efficacy and safety of Litramine in humans, a significant difference in mean body weight between the treatment and placebo group was demonstrated (Chong et al., 2014; Grube et al., 2013). After these promising results a similar product, named IQV04, is developed for dogs to support weight loss. This product contains the active ingredients such as *Opuntia ficus indica*, *Phaseolus vulgaris* extract (white kidney bean extract) and L-carnitine tartrate. *Opuntia ficus indica* is a fiber complex extracted from the leaves and produced with a proprietary manufacturing process. It is proven to reduce the absorption of dietary fat. White kidney bean (*Phaseolus vulgaris*) extract and L-carnitine tartrate are intended to reduce the absorption of dietary carbohydrate and increase energy metabolism.
The weight loss, as showed in previous human studies, would be caused by binding of the dietary fat. The fiber from *Opuntia ficus indica* prevents absorption of dietary fat in the intestinal digestion tract, by making fat-fiber complexes. The weight loss effect could probably also be ascribed to swelling of the fiber which due to dilatation of the stomach, results in release of satiety signals (Chong et al., 2014).
The aim of this study was to determine the efficacy of IQV04 on overweight and obese dogs by measuring body weight and pelvic circumference. Our hypothesis was that IQV04 could provide a significant difference in the rate of weight loss on overweight and obese dogs in comparison with placebo.

Materials and methods

Study design

The study was a placebo controlled, double-blinded study, and had a randomized block design. The duration of the study was twelve weeks, starting on day 0. The participating dogs were randomly divided into three groups, one placebo control, one treatment group who received IQV04 in the normal concentration (A) and one treatment group who received IQV04 in the double concentration (B). The randomization was generated with the aid of an online tool[1].
This study was conducted according to Dutch legislation on animal welfare.

Animals

Twenty colony dogs of the University clinic of Companion Animals Utrecht participated in this study. The placebo group consisted of 6 dogs, the treatment group with concentration A also consisted of 6 dogs, and the treatment group with concentration B consisted of 7 dogs. All dogs were healthy adults (over 1.5 years old), intact male or intact female (not pregnant), beagle or crossbreed beagle, up to date on vaccinations and de-worming and had a BCS of ≥ 6 of 9 (see table 1). For all parameters there was no statistically significant difference between the three groups.

The dogs were included in the study if they were not treated with concurrent medication or supplements, if there were no abnormalities in their health based on the physical examination, complete blood count, serum biochemical analysis and fecal examination. All dogs were housed in kennels with an inside and outside area, alone or with one other dog. During feeding, all the dogs were separated from other dogs. The dogs were allowed free access of drinking water throughout the entire study period. The dogs were allowed outside on grass daily for about two hours.

[1] www.randomization.com, consulted on 11-1-2016.

Criterion	Placebo	IQV04 concentration A	IQV04 concentration B	*p*-value[1]
Number	6	6	7	-
Age (year)	6.5 ± 3.6	5.8 ± 2.9	6.3 ± 3.5	0.941

Breed	Beagle (4) Crossbreed beagle (2)	Beagle (5) Crossbreed beagle (1)	Beagle (6) Crossbreed beagle (1)	0.707
Gender	Male (1) Female (5)	Male (1) Female (5)	Male (2) Female (5)	0.852
Start BW (kg)	14.9 ± 2.1	14.2 ± 1.8	16.8 ±3.0	0.149
Start PC (cm)	54.8 ± 6.0	53.9 ± 6.7	56.6 ± 7.0	0.749
Start BCS (of 9-point scale)	7.2 ± 0.7	7.2 ± 1.2	6.0 ± 1.2	0.835

Table 1 – Start characteristics of the dogs, all data are expressed as Mean ± Standard Deviation
[1] *P – value* quoted are for ANOVA between the groups
BW, body weight; PC, pelvic circumference; BCS, body condition score; kg, kilogram; cm, centimeter.

Treatment

The investigated product, IQV04, is a complementary dietary feeding supplement in tablet form. The composition is *Opuntia ficus indica* extract, *Phaseolus vulgaris* extract, L-carnitine, wheat endosperm extract, biotin, zinc, copper, magnesium stearate, calcium phosphate, bentonite, yeast and pork liver flavour. The control product is a similar tablet, containing magnesium stearate, calcium phosphate, bentonite, yeast and a pork liver flavour. The concentration A of IQV04 is 50% lower than concentration B of IQV04. At this moment, the exact amount of substances of IQV04 cannot be disclosed due to IP-restrictions.

The bottles with pills had the same label. Three different numbers were used to distinction the three groups. Personnel and investigators did not know the identity of the three numbers.

All three products were given once a day before the recommended meal and given directly to the dog for oral administration. Dogs with a body weight of ≤ 20kg received 1 tablet a day. Dogs with a body weight of 20-40kg received 2 tablets a day and dogs with a body weight of >40kg received 4 tablets. Doses were calculated based on current body weight and if necessary adjusted. If the dog vomited after administration, they were not re-dosed.

Parameters

Body weight

The primary parameter was change in body weight (% body weight reduction per week), so body weight was measured every week at the same moment and using the same electronic scale (Avery Weigh-Tronix, model E1005) with an accuracy of 0.1kg. The percentage body weight (BW) reduction per week was calculated by the following formula:

$$BW\ reduction\ (\%\ per\ week) = \left(\frac{\frac{start\ BW\ (kg) - end\ BW\ (kg)}{participated\ weeks}}{start\ BW\ (kg)} \right) x\ 100\%$$

Pelvic circumference

The pelvic circumference was measured with a measuring tape, with an accuracy of 0.5cm, at a point cranial to the ilium, at the level of the sixth lumbar vertebra every week at the same moment. To look to the difference between the three groups, a similar formula as for body weight was used. The percentage pelvic circumference (PC) reduction per week was calculated by the following formula:

$$PC\ reduction\ (\%\ per\ week) = \left(\frac{\frac{start\ PC\ (cm) - end\ PC\ (cm)}{participated\ weeks}}{start\ PC\ (cm)} \right) x\ 100\%$$

Body condition score

The body condition score was determined on a 9-point scale according to Laflamme 1997 (table 2) by inspection and palpation on day 0 and 85 or when reached target weight (Laflamme, 1997). All the dogs were scored by a veterinary student and a board certified nutritionist. Dogs with a BCS of 6, 7, 8 or 9 of 9-point scale were allowed to participate in the study. Overweight was defined as a BCS >5 and obesity was defined as a BCS >7.

	BCS	Description
'Too thin'	1 of 9	"Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass. "
'Too thin'	2 of 9	"Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass. "
'Too Thin'	3 of 9	"Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck."
'Ideal'	4 of 9	"Ribs are easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident."
'Ideal'	5 of 9	"Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side."
'Too heavy'	6 of 9	"Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent."
'Too heavy'	7 of 9	"Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present."
'Too heavy'	8 of 9	"Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distention may be present."
'Too heavy'	9 of 9	"Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention."

Table 2 – Canine Body Condition Scoring System (Laflamme, 1997; The World Small Animal Veterinary Association, 2011). BCS, body condition score

Estimation of target body weight

The target body weight was calculated from starting body condition score and body weight (BW). In dogs each subsequent point (≥6) on the 9-point scale represents an increment of 10% of body weight (= % overweight). For example, a dog with a BCS of 7 of 9 is approximately 20% heavier (= % overweight) than its ideal weight (Brooks et al., 2014; German et al., 2009a). This leads to the following formula:

$$Target\ BW = current\ weight\ x \left(\frac{100 - \%\ overweight}{100}\right)$$

Diet

During the twelve week study period the dogs received a standardised maintenance food of Hill's Science Plan TM once daily (table 3). All bags of food were from the same batch.

This diet has been chosen because it is a balanced diet with a normal amount of fat (15.03% fat as fed). In the previously described human study (Chong et al., 2014), weight loss was caused by the binding with fat, therefore to evaluate the efficacy of IQV04 there should be available fat in the diet.

Hills Science Plan ™ Canine Adult Advanced Fitness ™ Medium Chicken	
ME content	373 kcal/100

	Kcal/100g
Moisture	2.28 g
Crude protein	5.84 g
Crude fat	447.72 mg
Crude fibre	0.4 g
Crude carbs	12.95 g
Ingredients	Chicken (Chicken 26%, Total poultry 39%): Maize, poultry meat meal, maize gluten meal, animal fat, digest, vegetable oil, minerals, beet pulp, flaxseed, rice, vitamins and trace elements. With natural preservative and natural antioxidants.

Table 3 – Composition of the diet (Hill's, 2016b)
ME, metabolisable energy; g, gram; kg, kilogram; Kcal, kilocalorie; mg, milligram

During the first week of the study the dogs were gradually switched to the new diet by mixing their old food with the new food to avoid any gastrointestinal disruptions (German, 2016a). The previous diet was the same diet with a similar composition, but had another flavour, namely lamb & rice.
The entire study duration the diet will be given as per targeted body weight (BW). The amount of the food was calculated by Resting Energy Requirement (RER) according to the American Animal Hospital Association guideline:

$$RER\ (kcal) = 70\ x\ target\ BW^{0.75}$$ (Brooks et al., 2014)

$$Daily\ amount\ of\ food\ (g) = \frac{RER\ (kcal)}{3.73\ (kcal/g)}$$

Every week, the recommended daily meal was measured by an electronic kitchen scale for each dog and bowls for 7 days for each dog were prepared for the animal caretaker.

Procedure
All dogs were examined clinically on day 0 and day 85 or when reached target weight. This examination included physical examination including body weight, BCS and pelvic circumference; complete blood count and blood biochemistry and fecal examination. During the twelve weeks the animal caretakers kept a diary with abnormalities in food intake, in product intake, in general health and in general behaviour of the dog. Every week the dogs were weighted and the pelvic circumference was measured by the same person on Thursday at 5:00 PM.

Statistical analyses
As there is no useful pre-clinical data to estimate an appropriate sample size for obtaining statistical power, an initial sample size of 30 dogs should be recruited (each group n=10). In these study were only 20 dogs included. Further investigation will be necessary to recruit the last 10 dogs. After these 30 dogs the sample size will be reassessed to reach the primary effectiveness endpoint.
Computer software (IBM SPSS Statistics version 24) was used for all tests. First, normality was determined by the Shapiro-Wilk and Kolmogorov-Smirnov method as well as by visual evaluation of the histograms. The Levene's test was performed to test for homogeneity. All data were normally distributed and homogeneous, therefore, a one-way between groups analysis of variance (ANOVA) was performed. The level of significance was set at $P < 0.05$ for two-sided analyses.

Results

Animals
In total nineteen dogs were included in the analyses, one dog (group IQV04 concentration A) was excluded due adjustment of the amount of feed. All nineteen dogs did not show

abnormalities in physical examination, complete blood count, serum biochemical analysis and fecal examination on day 85 (or when reached target weight) of the study.
During the study, one dog (in group IQV04 concentration A) had surgery because of an anterior cruciate ligament lesion. The morning before the anaesthesia the dog was fasted and received his recommended diet and pill of that day in the evening instead of the morning. After the operation the dogs stayed for eleven days on the surgical ward. While staying on this ward the dog received his recommended diet and pills every day. Another dog (in the placebo group) had dental cleaning but stayed on the surgical ward for only one day. Also this dog received his recommended diet and pills in the evening instead of the morning because of the anaesthesia.
Four weeks after the start of this study the animal caretakers reported that all the dogs showed increased food-seeking behaviour, that the dogs had eaten grass during the daily outside moments and that the dogs showed signs of coprophagia. Around week eight of the study these reported behaviours stabilized, but persisted during the rest of the study.

Treatment
All the nineteen dogs received the tablets according to the prescribed dosage (100% compliance), however one dog did not take the pills voluntarily. Therefore, the pill was placed as far back as possible in the dog´s mouth. During the participation of the study eighteen dogs received 1 tablet a day (all dogs were ≤ 20kg). One dog received the first two weeks 2 tablets (weighted 20,9kg and 20,3kg respectively) and the remaining time 1 tablet (weighted ≤ 20kg).

Parameters

Body weight - % BW reduction per week
To evaluate the effectiveness of two concentrations on percentage of body weight reduction per week, a one-way between groups analysis of variance (ANOVA) was conducted (see table 4 and figure 2).
The ANOVA assumptions of normality (Shapiro-Wilk test) and homogeneity (Levene's test) of variance were not violated, and the F test was not significant, $F(2, 16) = 0.329$, $p = 0.724$. It should be noted, however, that $\eta^2=0.04$, which can be characterized as medium.

% BW reduction per week				**95% CI**	
Group	N	Mean	SD	Lower bound	Upper bound
Placebo	6	2.45	0.51	1.91	2.98
Concentration A	6	2.85	1.84	0.92	4.79
Concentration B	7	3.03	1.26	1.87	4.20

Table 4 – BW reduction (%/week)
N, number; CI, Confidence Interval; BW, body weight; SD, Standard Deviation

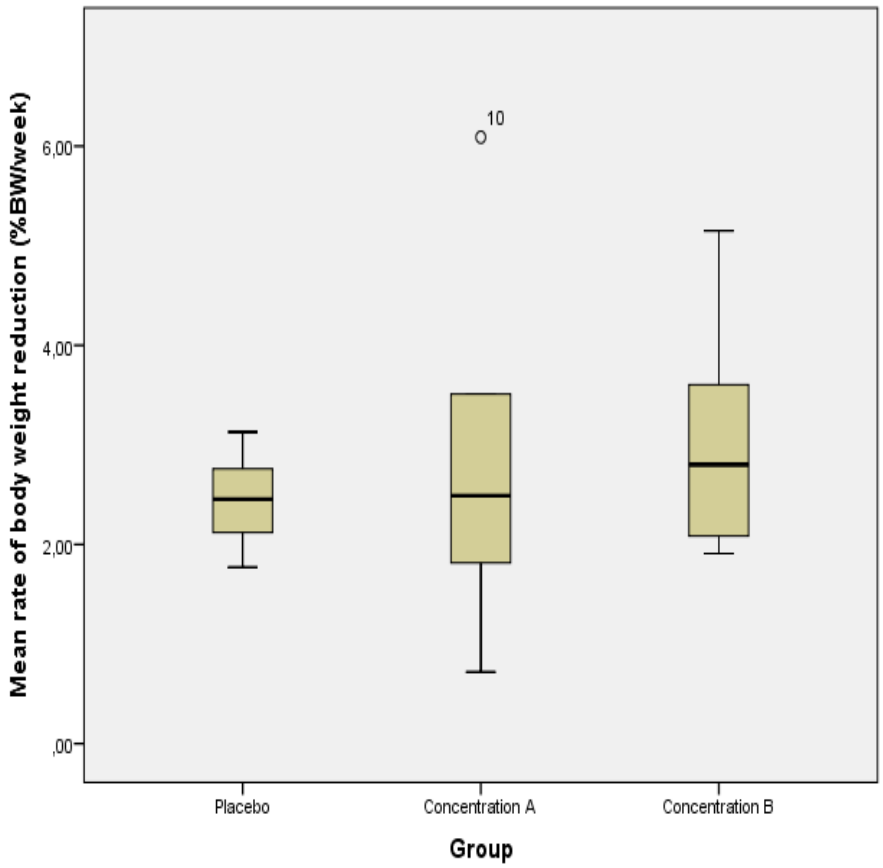


Figure 2 – Boxplot of the mean rate of BW reduction (%/week)
The horizontal line in the box is the median, the top and bottom of the box is the inter-quartile range, the vertical line shows the highest and lowest scores in the distribution (not the outliers). The separated point is the outlier.

Pelvic circumference - % PC reduction per week

To evaluate the effectiveness of two concentrations on percentage of pelvic circumference reduction per week, a one-way between groups analysis of variance (ANOVA) was conducted (see table 5 and figure 3).

The ANOVA assumptions of normality (Shapiro-Wilk test) and homogeneity (Levene's test) of variance were not violated, and the F test was not significant, $F(2, 16) = 0.108$, $p = 0.898$. It should be noted, however, that $\eta^2=0.01$, which can be characterized as medium.

% PC reduction per week				**95% CI**	
Group	N	Mean	SD	Lower bound	Upper bound
Placebo	6	2.46	0.38	2.07	2.87
Concentration A	6	2.65	0.93	1.67	3.63
Concentration B	7	2.56	0.63	1.97	3.14

Table 5 – PC reduction (%/week)
N, number; CI, Confidence Interval; PC, pelvic circumference; SD, Standard Deviation

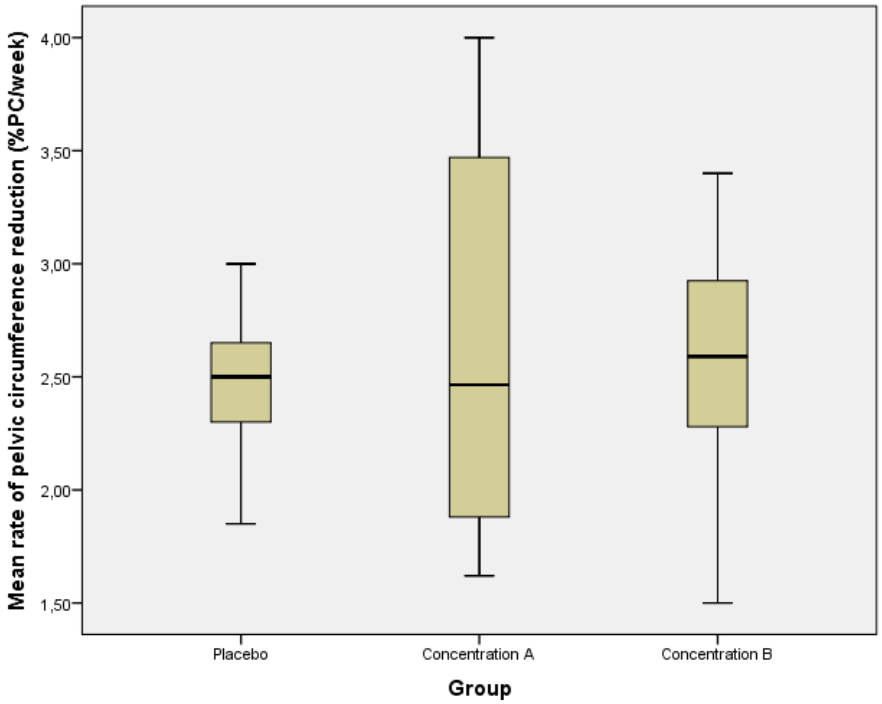


Figure 3 – Boxplot of PC reduction (%/week)
The horizontal line in the box is the median, the top and bottom of the box is the inter-quartile range, the vertical line shows the highest and lowest scores in the distribution.

Body condition score
The mean end body condition score was 4.5 ± 0.5, 4.5 ± 1.4 and 4.7 ± 1.3 for placebo, IQV04 concentration A and IQV04 concentration B, respectively.

Duration to reach target body weight
Of the 19 dogs, 7 dogs (placebo (2), IQV04 concentration A (3), IQV04 concentration B (2)) did not reach the target weight. The other 12 dogs reached target weight before the end of this study. The mean duration of participation of the study for placebo group was 8.7 ± 3.6 weeks, IQV04 concentration A participated 8 ± 4.7 weeks and IQV04 concentration B 6.4 ± 4.1. No significant difference between groups was found in duration to reach target body weight ($p = 0.615$).

Discussion
The current study evaluated the efficacy of IQV04 for weight loss in overweight and obese dogs. Both percentage body weight reduction per week and pelvic circumference reduction per week were not statistically significant different between the three groups.

Body weight
The rate of weight loss in the placebo, IQV04 concentration A and IQV04 concentration B group were 2.45 ± 0.51, 2.85 ± 1.84 and 3.03 ± 1.26% body weight reduction per week, respectively.
Compared to previous weight loss studies in dogs (Borne et al., 1996; Diez et al., 2002; German et al., 2007; German et al., 2015; Laflamme & Kuhlman, 1995), these outcomes showed higher reduction of weight loss. This overall effect can probably be attributed the use of colony dogs and standardized conditions. In this study we did not depend on pet owners and the subsequent potential non-compliance with feeding (Gossellin et al., 2007). As previously mentioned, a drop out rate of 32% in weight loss programs by the choice of the owner have

been reported, for example due to failure to comply with the weight loss program or refusing help shortly after enrolment (German et al., 2015). Opposite, the results of this current study are possibly not representative of the client-owned dog population and a clinical trial is necessary to investigate the effect of IQV04 in client-owned dogs.

An important limitation of this study is the number of test subjects. As described in material and methods, there was no pre-clinical data to estimate an appropriate sample size for obtaining statistical power. The initial sample size of 30 dogs was not achieved, instead of that, in this study a total of 19 dogs were included. This is a low number of subjects, in comparison to the previous human Litramine studies with 49 and 123 test subjects (Grube et al., 2013; Grube et al., 2015). The number of test subjects in each group may not be adequate to pick up a statistically significant difference in weight loss between the three groups and this study may be underpowered (Sexton et al., 2008).

Even though there was no significant difference in the amount of weight loss between the three groups, the dogs in group B have the highest mean percentage body weight reduction per week. Whether or not the effect of IQV04 is dose dependent in dogs remains to be investigated. In previous studies with humans (Grube et al., 2013; Grube et al., 2015), there were no information about the exact amount of the active product. Unfortunately, a comparison of effective dosage between dogs and humans is not possible due to the before mentioned IP restrictions concerning the concentration used.

In this study the body weight of the dogs were measured on the same scales every week on the same time of day. The scale had a capable of accuracy to 0.1 kg. These conditions are required to rule out potential sources of error (Gossellin et al., 2007).

Ideally, weight loss caused by adipose tissue loss while lean tissue mass remains. Unknown remains whether the weight loss in this study was caused by loss of adipose tissue or also lean tissue mass loss. A precise way to measure body composition is dual energy X-ray absorptiometry (DEXA). It has been used for determining body fat and lean body mass (Laflamme, 1997). In this study DEXA was not used, which was another limitation of this study.

In human medicine, weight loss of more than 2% per week is unhealthy because a greater loss of lean tissue mass (Weinsier et al., 1984; Weinsier et al., 1995). Results from animal studies are variable, Floerchinger et al. (2015) showed a retaining of the lean mass tissue during weight loss of 1.4% a week, measured by dual energy X-ray absorptiometry (Floerchinger et al., 2015). By contrast German et al. (2007) presented both adipose tissue loss and lean tissue mass during weight loss in dogs (German et al., 2007). The discrepancy between the two studies can be explained by the significant associating with the amount of lean tissue loss with the overall percentage weight loss in dogs who lost more than 20% of their start body weight (German et al., 2007; German, 2016b).

Pelvic circumference

Besides measuring body weight, every week the pelvic circumference was measured to evaluate the efficacy of IQV04. The rate of pelvic circumference reduction in the placebo, IQV04 concentration A and IQV04 concentration B group were 2.46, 2.65 and 2.56 % pelvic circumference reduction per week, respectively.

In previous studies of weight loss, measuring of pelvic circumference rarely used. However, studies which used pelvic circumference by evaluation of weight loss showed a statistically significant decrease in pelvic circumference with decrease in body weight (Dobenecker et al., 2009; Marshall et al., 2010).

The limitation of low size of test subjects as described by body weight, also applied to the pelvic circumference. Further research with more dogs is necessary to make conclusions of differences in pelvic circumference reduction between the three groups.

Body condition score

The body condition score (BCS) was used to be able to score, uniformly a large number of dogs of a variety of breeds. It provides a semi quantitative assessment of body composition and is a non-invasive, low cost, reliable, practical means of assessing body composition (Laflamme, 1997). The BCS evaluates body fat by using both visual and tactile cues (Roudebush et al., 2008). There are different scales to evaluate the BCS. The 9-point scale, as used by the Word Small Animal Veterinary Association, is highly recommended (The World Small Animal Veterinary Association, 2011). The nine point scale (table 2) is preferable because the discriminating ability of a system depends, in part, on the number of units used. To measure the body composition, the 9-point BCS system had been shown to be reliable and consistent using DEXA (German et al., 2010; Laflamme, 1997).

In this study the scoring was done by a Dutch and European specialist in Veterinary and Comparative Nutrition. Though, experience is not even necessary to reliably score the BCS. German et al. (2006) looked to the agreement among experienced and unexperienced operators and reported an agreement (German et al., 2006). In this study the target weight was based on the BCS. Each subsequent point (≥6) on the 9-point scale represents a change of 10% of body weight. This is the best estimated method to calculate the target body weight. However, there is variability with this method (German et al., 2009a). Certainly, if the body condition sore is 9 of 9, relating to have 40% more body weight than ideal weight, because grossly obese dogs have often an overestimate of the ideal weight (Brooks et al., 2014). So, during the weight loss period, dogs should be scored regular and if necessary adjustments in target weight should be made.

Duration to reach target body weight

There was no significant difference between the groups in duration to reach target body weight. However, this is not very surprising because each dog had an individual amount of weight to lose depending on percentage overweight. The seven dogs which did not reach the target weight in the study period and their BCS were 7 of 9, 8 of 9 and 9 of 9 for 1 dog, 4 dogs and 2 dogs, respectively. The estimated duration can be calculated. For example, a dog of 15kg with a BCS of 7 of 9 is 20% heavier. The target body weight of this dog is 12kg. With a weight loss of 0.5 to 2 % a week, this dog will reach his target body weight in 10 (3kg / (15kg x 2%)) to 40 (3kg / (15 kg x0.5%)) weeks (Toll et al., 2010). Dogs which reached their target body weight, stopped with the study. The disadvantage of this requirement, is that dogs with a BCS of 6 of 9 participated a shorter period in the study than dogs with a BCS of 8 of 9 or 9 of 9. There were even dogs who only participated two weeks. To prevent this in further research, only dogs with a BCS ≥ 7 of 9 should be included.

Diet

In this study a standardized maintenance food of Hill's Science Plan was chosen. As mentioned earlier, the human studies to evaluate the efficacy of Litramine described that the weight loss was caused by the binding with fat (Grube et al., 2013; Grube et al., 2015), therefore the diet should contain a normal percentage of fat (15.03% fat as fed). Most of specific weight loss diets contains less fat, like Hills's Prescription Diet ™ Canine r/d ™ with Chicken, with a crude fat of 10.8% as fed (Hill's, 2016a). Toll et al. (2010) recommended a maximum of 9% and 14% fat for dogs will lose weight and dogs preventing rebound weight gain, respectively (Toll et al., 2010). The 15.03% of the diet used in this study is slightly above the limit of prevention of rebound weight gain.

A limitation of this study is that during the first week of the study the dogs were gradually switched to the new diet instead of the week before the study started. To rule out influences on the results, it is better to launch an acclimation period where the dogs switched to their

new diet, before starting the study. However, the previous diet was the same diet only with another flavor. The composition of the previous diet is showed in table 6 (appendix 1).
All the dogs were fed for appropriate weight loss by using RER according to the American Animal Hospital Association guidelines (Brooks et al., 2014). Hence, all the dogs lost weight independent of the treatment, as shown by the placebo group with an average weight loss of 2.45% per week. The average weight loss of the highest concentration of IQV04 is 3.03% per week, unknown what part caused by diet and what part caused by the treatment of IQV04. Perhaps it would have been better to feed all the dogs by their daily energy requirement (DER). The subjective DER is determined by activity or production. For example the DERs of a neutered bitch and lactating bitch of the same weight are very different (Thatcher et al., 2010). Using the DER instead of RER would aid in determining which part of the weight loss is can be attributed to the use of IQV04, but also because this dogs loss more weight than the recommended 1 to 2 % per week (Laflamme & Kuhlman, 1995; Toll et al., 2010). If a higher percentage weight loss in dogs also causes a greater loss of lean tissue mass like in humans (Weinsier et al., 1984; Weinsier et al., 1995), then this higher percentage weight loss does not have any advantages and it was better to feed the dogs by DER. As mentioned previously, use of DEXA can help to make distinction in body composition and demonstrate potential loss in lean tissue mass.

Treatment
The efficacy of Litramine was already investigated with promising results in human clinical trials with obese subjects. IQV04 contains the same active ingredient, *Opuntia ficus indica*, as Litramine. The weight loss by the subjects of the human studies was ascribed to the fat binding complex and satiety mechanism (Chong et al., 2014).
Remarkable was the food-seeking behaviour of all the dogs in the current study, as reported by the animal caretakers. During the daily outside moments the dogs were eating grass and showed even coprophagia. This behaviour started around week four and stabilized around week eight and persisted during the rest of the study. This behaviour can be explained by hunger. This is in sharp contrast to the results of the human studies with Litramine, in which the subjects 'experienced an increase satiety feeling' (Grube et al., 2015). Partially, this may be attribute to placebo effects in human studies or to the decreased amount of food in the study presented here. In weight loss programs, food-seeking behaviour has been previously reported. In such weight loss programs, this adverse behaviour is tried to avoid by improving the satiety. As to slow food intake for more time to release hormones of gastrointestinal tract that affect the hunger center in the brain (German, 2016a). This can be arranged by puzzle feeders (German et al., 2015; German, 2016a). Another method to improve satiety is a diet with high protein and fiber concentrations, these diets showed a decrease voluntary food intake in dogs (Weber et al., 2007) with additional effect that such diets promote greater fat loss during weight loss period (German et al., 2010).
A further limitation of this current study was the duration of the evaluation of IQV04. IQV04 was evaluated for only a maximum of twelve weeks, therefore it lacks a long-term evaluation and additional studies are required to make conclusions of this effect.

Animals
As earlier discussed, this study is done with colony dogs instead of client-owned dogs. To demonstrate the efficacy of a product, standardized conditions are beneficial. However the results will possible not be fully representative of client-owned dogs. The use of client-owned dogs should be considered when further research will be done.
Due to a miscommunication in the beginning of the study the amount of food was increased with 10% when dogs reached their target body weight, or more if the dog was still losing weight after increasing with 10% food. Meanwhile, the pills were given until the end of the

study. However, after eight weeks eleven dogs had already reached their target body weight or were even lower than the target weight. The diets of the dogs were adjusted according to the before mentioned protocol and dogs which reached their target body weight, stopped with the study. The disadvantage of this alteration is that the final measurements of the eleven dogs were performed after week eight, instead of the week when first reached target weight. However body weight, pelvic circumference and body condition score were performed after reached target weight and were include in the final analyses.

Another limitation of this study was that during the weight loss period the exercise was not assessed. All the dogs went outside on grass for about two hours. However, dogs have variability in activity levels, which may affect weight loss. The overall weight loss program includes, beside the weight loss period, also weight control over extended period of time and avoiding rebound (German et al., 2015). Those two parts were not evaluate in this current study. This has also to do with the lacking long-term evaluations.

In summary, this study evaluated the efficacy of IQV04 on weight loss in dogs. Most of the obese dogs reached their target weight and there was a difference in body weight reduction between the three groups. However this difference did not reach significance. Therefore, further research is needed, with larger patient group to achieve more statistical power.

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Appendix 1

Hill's Science Plan ™ Canine Adult Advanced Fitness ™ Medium Lamb and rice	
ME content	375 kcal/100
	kcal/100g
Moisture	2.13 g
Crude protein	5.87 g
Crude fat	4 g
Crude fibre	505.67 mg
Crude carbs	13.12 g
Ingredients	Lamb & Rice (minimum Lamb 26 %; minimum Rice 4 %): Ground maize, lamb meal, soybean meal, animal fat, maize gluten meal, ground rice, digest, vegetable oil, flaxseed, L-lysine hydrochloride, salt, potassium chloride, DL-methionine, taurine, L-tryptophan, vitamins and trace elements. Naturally preserved with mixed tocopherols, citric acid and rosemary extract.

Table 6 – Composition of the diet (Hill's, 2016c)
ME, metabolizable energy; g, gram; kg, kilogram; kcal, kilocalorie; mg, milligram