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Research article

Effectiveness of standardized red orange extract (*Citrus sinensis*) for weight reduction in canine obesity

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Abstract

Obesity is the most common nutritional disease in both humans and dogs, currently characterized as a low-intensity inflammatory state. The anthocyanins found in red oranges can assist in combating weight gain and reducing body fat accumulation by reducing lipogenesis and modulating inflammation. This study aimed to compare the effectiveness of standardized dried red orange extract in reducing overweight in obese dogs compared to chromium picolinate and a placebo. In total, 23 animals were used in the current study and divided randomly into three experimental groups. All dogs received the same hypocaloric diet and supplementation: standardized *Citrus sinensis* red-orange extract (G1, n=9), chromium picolinate (G2, n=7), and a placebo-control group (G3, n=7). The dogs were managed by their owners for 90 days. In the distribution of the body condition score (BCS) determined at the beginning of the study, 47.8% of the animals had BCS of 6; 26.1% had BCS of 7; 8.7% had BCS of 8, and 17.4% had BCS of 9. During the program, most of the owners reported being unable to involve other residents of their households in the dogs' treatment. At the end of the experiment, the G1 group had a significant reduction in weight and BCS ($p<0.01$), as well as in HDL cholesterol levels ($p<0.05$) when compared to the other groups. It was concluded that incorporating dried red-orange extract into the diet of overweight and obese dogs can lead to weight loss and improved BCS.

Keywords: Companion animals, *Citrus sinensis*, Dogs, Nutraceuticals, Obesity, Red orange extract

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Introduction

Obesity is currently the most common nutritional disease in companion animals, and in dogs, it occurs when the body weight is at least 15% above the ideal weight (Burkholder and Toll, 2000). In both humans and companion animals, the medical significance of obesity lies in its effect on the mortality and morbidity of associated diseases (Suarez et al., 2022). Overfeeding is the main reason for developing the disease in humans and dogs, which can lead to a positive energy balance, resulting in excess body fat storage (Carciofi et al., 2005).

In its current concept, obesity has been seen as a low-intensity inflammatory state directly or indirectly associated with cardiovascular, respiratory, and orthopedic problems and metabolic disorders such as reduced glucose tolerance, diabetes mellitus, and dyslipidemia (German, 2010).

Natural agents, like red-orange *Citrus sinensis* (L.) Osbeck has been researched as an alternative for reducing excess adipose tissue. It is rich in phenolic compounds, vitamin C, and anthocyanins, which act as powerful antioxidants, modulating inflammation generated by excess adipose tissue and inhibiting lipid peroxidation (Rodrigues et al., 2020; Briskey et al., 2022). Recent studies have shown that the standardized red-orange extract can downregulate adipogenic genes and enzymes, along with modulating adiponectin secretion and leptin release. Consumption of red oranges (especially Moro juice) has also been found to curb body weight gain, improve insulin sensitivity, and reduce serum levels of triglycerides and total cholesterol in mice (Titta et al., 2010; Salamone et al., 2012).

While severe overweight conditions in companion animals are readily noticeable, detecting subtle changes presents a challenge for many methods. This makes monitoring weight loss programs and early interventions complex. The assessment of body condition aims to determine whether animals are underfed or overfed.

Table 1: Body condition scores in dogs (Laflamme, 2006).

Body condition	Scores	Description
Underfed	❶	Ribs, lumbar vertebrae, pelvic bones, and all bony prominences are visible from a distance. There is no discernible body fat. Evident loss of muscle mass.
	❷	Ribs, lumbar vertebrae, and pelvic bones are easily visible. No palpable fat. Some other bony prominences may be visible. Minimal loss of muscle mass.
	❸	Ribs are easily palpable and may be visible without palpable fat. Top of lumbar vertebrae visible. Pelvic bones start to become visible. Waist and abdominal tuck are evident.
Ideal	❹	Ribs are easily palpable with minimal fat covering. From a top view, the waist is easily observed. Abdominal tuck evident.
	❺	Palpable ribs without excess fat covering. Abdomen tucked when viewed from the side.
Overfed	❻	Palpable ribs with slight excess fat covering. The waistline is visible when viewed from the top but not pronounced. Abdominal tuck is apparent.
	❼	Palpable ribs with difficulty; intense fat covering. Evident fat deposits over the lumbar area and base of the tail. Absence of waist or only slightly visible. Abdominal tuck may be present.
	❽	Inability to palpate the ribs located under a very dense fat cover or ribs palpable only with firm pressure. Heavy fat deposits over the lumbar area and base of the tail. No waistline. No abdominal tuck. Significant abdominal distension may be present.
	❾	Massive fat deposits over the chest, spine, and base of the tail. Fat deposits on the neck and limbs. Abdominal distension is evident.

Numerous techniques are available for evaluating body composition, including advanced methods like dual-energy X-ray absorptiometry (DEXA), magnetic resonance, neutron activation analysis, and computed tomography. However, only a few of these options are appropriate for animal use (Diez and Nguyen, 2006; Andrade Junior et al., 2020).

Using body weight as an indicator of body composition is limited by significant variation among breeds, age ranges, and sex, and it is not recommended as the sole form of evaluation as it does not assess fat mass or muscle mass. Dogs with the same body weight can have different body compositions. Body weight can only quantify the percentage of weight excess by comparing it to the ideal weight, which is often a theoretical value (Diez and Nguyen, 2006). Body condition score (BCS) is used to subjectively and semi-quantitatively assess an animal's body fat and muscle mass. The BCS (Table 1) uses numerical scales based on patient inspection and palpation, employing scales from one to nine to reduce subjectivity (Laflamme, 2006).

The long-term weight control of obese pets can represent a frustrating clinical challenge destined to fail unless an organized approach with practical strategies is adopted (Center, 2003; Rodrigues et al., 2020).

There needs to be more research focusing on exploring novel weight loss solutions for dogs beyond the commonly recommended methods of a well-rounded diet and regular physical activity. This study aimed to evaluate the weight-reducing properties of red-orange extract, compared to chromium picolinate and a balanced diet alone, for animals.

Materials and methods

Ethical approval

This study was approved by the Ethics Committee on Human Research, COMEP/UNICENTRO (protocol 5.180.123), and by the Committee on Ethics in the Use of Animals (CEUA) with protocol number 01/2022.

A questionnaire was created using the Google Forms platform, consisting of 16 objective multiple-choice questions. It was designed to be easy to understand and was administered to overweight or obese dog owners at two veterinary clinics in the city of Guarapuava-PR.The owners were informed about the research and voluntarily responded to the questionnaire. The questionnaire was provided from January 21^{st} to March 15^{th}, 2022. Forty-five owners responded to the questionnaire.

Selection of the animals

The inclusion criteria for participation in the project were: dogs aged between 2 and 7 years, healthy, without comorbidities with a body condition score (BCS) between 6 and 9, of both sexes, spayed or not, and weighing between 5 and 40 kg. The owners were asked about the body condition of their dogs, and those who agreed to participate in the project (n=23) signed an informed consent form.

Exclusion criteria encompassed dogs with a history of significant medical issues, including cardiovascular, neurological, renal, gastrointestinal, immune, endocrine, or hematological abnormalities, and those with a BCS$\leq$5. Animals receiving systemic or topical glucocorticoids and anticonvulsants were also excluded.

Table 2: Data from experimental groups (mean±standard deviation)

Group	Number	Mean age (years)	Sex (%)		Body condition scores [a]
			Males	Females	
Morosil®	9	5.89±1.96	22.2	77.8	7.0±1.39
Chromium picolinate	7	4.43±2.07	14.3 *	71.4 14.3 *	7.0±0.9
Placebo	7	4.21±1.58	28.6*	42.9 28.6*	6.0±1.11
Total	23	4.93±1.97	8.7 13.0*	65.2 13.0*	6.96±1.15

[a]Body condition scores after Laflamme (2006). *Indicates the percentage of non-castrated animals

Table 3: Difficulties reported by owners during the program.

Question		Group							
		Morosil®		Chromium picolinate		Placebo		Total	
Are owners following the program correctly?	Response	Yes	No	Yes	No	Yes	No	Yes	No
	Frequency	6	3	1	6	4	3	11	12
	%	66.7	33.3	16.7	83.3	57.2	42.8	47.8	52.2
Did owners manage to involve other household members in treating their dogs?	Frequency	4	5	3	4	2	5	9	14
	%	44.5	55.5	42.8	57.2	28.5	71.5	39.1	60.9
Do owners consider that their dogs have been hungry?	Frequency	2	7	1	6	3	4	7	16
	%	22.2	77.8	14.2	85.8	42.8	57.2	30.4	69.6

On day 60 of the study, a second questionnaire consisting of three multiple-choice questions was administered to the owners. This secondary assessment aimed to gain insight into the challenges they encountered during their animals' weight loss program.

At the program's commencement and conclusion, the dogs were weighed, and anamnesis data and BCS were documented on an evaluation form. Blood samples were collected at the program's onset and conclusion through punctures to the cephalic or jugular vein, with 5 ml of blood drawn and placed in anticoagulant-free tubes. The samples were appropriately labeled and refrigerated before transport to the Animal Lab Laboratory in Guarapuava-PR.

Serum biochemical parameters assessed included total cholesterol, its fractions (HDL, LDL, and VLDL), and triglycerides. An automated biochemical analyzer (IDEXX Catalyst One ®, Maine, USA) was used for analysis.

Experimental groups

The animals were randomly divided into three groups. Group 1 (G1, n=9) received a specific and individualized diet for each dog, along with dry red orange extract (Morosil®) at a dose of 200 mg/animal/day orally (PO), as directed by the supplier (Galena, 2021). The standardized extract was provided by Bionap S. R. L. (Belpasso, Catania, Italy).

Group 2 (G2, n=7) received a specific and individualized diet for each dog, along with chromium picolinate (Sigma-Aldrich, Saint Louis, USA) at a dose of 0.01 mg/kg/day PO (NRC, 2006). Group 3 (G3, n=7) was assigned an individualized diet for each dog, supplemented with maltodextrin (Sigma-Aldrich, Saint Louis, USA) at a dosage of 200 mg/animal/day orally, intended as a placebo. At this dose, maltodextrin holds no nutritional value and does not induce changes in blood biochemical parameters. Thus, G3 functioned as the control group.

The Morosil®, chromium picolinate, and placebo were manipulated and distributed to the participants in similar sachets, identical in appearance and function, and without identification, with the instruction to be given to the dogs together with their first meal of the day. In all three cases, a salmon-flavored palatabilizer was used.

Energy requirements and animal monitoring

The feed used in this experiment contained 10% moisture, 35.5% crude protein, 8% ether extract, 7.5% ash, 15% crude fiber, 1.3% calcium, 0.6% phosphorus, 0.66% potassium, 1.5% omega-6, and 0.3% omega-3. The feed was provided to the owners free of charge, and each owner received a measuring cup adjusted for their dog to facilitate the proper daily feeding amount, divided into one, two, or three portions, according to their convenience. The amount of feed offered to the animals corresponded to 70% of their maintenance energy requirement (NRC, 2006), calculated for their target weight, standardized as their current weight minus 10%.

The experimental groups were managed by the owners themselves, who received instructions to follow the weight loss program for 90 days. It was also recommended that the dogs not be given treats and other foods during the experimental period. The follow-up was done through monthly weighting of the animals, interviews with the owners, and periodic contact via telephone to encourage them to continue with the program.

Table 4: The initial and final average weight (mean± standard deviation) evaluated by repeated measures ANOVA at a significance level of 5% post-Tukey test.

Group	Number	Average initial weight	Average final weight	F*	p-value
Morosil®	9	20.7±12.6	19.5±12.3	9.42	<0.01
Chromium picolinate	7	16.4±10.1	15.2±8.95	4.71	0.013
Placebo	7	11.1±5.48	10.9±5.64	0.308	0.820

*F; value obtained from the repeated measures analysis of variance..

Table 5: The initial and final body condition score (BCS) expressed as mean± standard deviation assessed through the Friedman test and multiple Durbin-Conover comparisons with 5% significance.

Group	Number	Initial BCS	Final BCS	p-value
Morosil®	9	7±1.39	6±1.27	<0.001
Chromium picolinate	7	7±0.9	6±0.976	0.078
Placebo	7	6±1.11	6±0.690	0.078

Statistical analysis

The interview data were tabulated in Microsoft Excel® spreadsheets, and the results were expressed by descriptive statistics, presenting the values in frequency. A repeated-measures ANOVA test was used to evaluate the variation in the animals' weight. Significance was considered at 5% post-Tukey's test. The Friedman test was used to evaluate the animals' body condition score. Significance was considered at 5% post multiple comparisons of Durbin-Conover. For the blood biochemistry tests, the Wilcoxon test was used with a significance level of 5%. The statistical analysis was performed using the Jamovi software (Anonymus, 2020).

Results

The data from the studied dogs are presented in Table 2. The average age of the study population (n=23) was 4.93±1.97 years old. In the group that received the dry extract of red orange (n=9), the average age was 5.89±1.96 years. In the group that received chromium picolinate (n=7), the average age was 4.43±2.07 years, and in the placebo group (n=7), the average age was 4.21±1.58 years.

The BCS of the animals was assessed at the initial consultation, and 47.8% of the animals were found to have a BCS of 6; 26.1% had a BCS of 7; 8.7% had a BCS of 8 and 17.4% had a BCS of 9. In the evaluation of the BCS of the animals in the Morosil® group, an average BCS of 7±1.39 was found. Analyzing the BCS frequencies revealed that 44% of the animals had a BCS of 6; 22.2% had a BCS of 7, and 33.3% had a BCS of 9.

In the group that received chromium picolinate, the average BCS was 7±0.9, with a distribution of 42.9% of animals with a BCS of 6; 28.6% with a BCS of 7, and 28.6% with a BCS of 8. In the control group, which received a placebo, the average BCS was 6.96±1.15, with 57.1% of animals having a BCS of 6; 28.6% with a BCS of 7, and 14.3% with a BCS of 9.

At the 60-day mark of the program, a second interview was conducted with the participating owners, in which they were asked about the main difficulties encountered up to that point (Table 3). Less than half of the participants (47.8%) stated that they followed the program correctly. One of the major difficulties reported was the inability to involve all household members in treating their animals (60.9%). Additionally, 30.4% of the interviewed guardians believed that their dogs were feeling hungry.

Comparing the initial and final weighings of the animals (Table 4), the group that received Morosil® (n=9) showed a statistically significant weight loss (p<0.01) compared to the group that received chromium picolinate (n=7; p=0.013) and the placebo-controlled group (n=7; p=0.82).

In the comparison of the BCS among the groups (Table 5), a statistically significant difference was observed in the reduction of BCS between the initial and final measurements in the group that received red-orange extract (n=9; p<0.001) when compared to the chromium picolinate group (n=7; p=0.078) and the placebo group (n=7; p=0.078).

In the comparison of biochemical parameters among the three treatments (Table 6), there was no statistically significant difference, except for the HDL cholesterol of the group that received Morosil® (n=9; p<0.05).

Discussion

Based on the results obtained, most of the dogs were adults, as evidenced in other epidemiological studies (Carciofi et al., 2005; Courcier et al., 2010; Aptekmann et al., 2014; Teixeira et al., 2020). As the animal ages, lean mass is replaced with fat mass, and it is advised to reduce energy intake by 10 to 15% from the age of seven, according to the animal's body condition, as suggested by Aptekmann et al. (2014). Castration has been cited as a risk factor for the development of obesity in previous studies, and the hypothesis for this increased risk is due to a decrease in basal metabolic rate, changes in eating behavior, and reduced physical activity (Courcier et al., 2010; Aptekmann et al., 2014).

A previous study suggested that dogs living in households with more members were more prone to developing obesity because there would always be a temptation, especially for children to offer treats or leftovers, leading to energy intake above what is desirable(Bland et al., 2009). Rohlf et al. (2010) emphasize

Table 6: Biochemical parameters (mean±standard deviation) by group.

Parameters[a]	Morosil®		Chromium picolinate		Placebo	
	Initial	Final	Initial	Final	Initial	Final
Cholesterol	168±94.7	131±72.0	159±28.6	132±38.7	146±37.1	128±31.9
HDL	126.6±58.0	101.3±43.3[b]	86.3±28.3	81.6±27.5	101.1±29.4	80.7±18.2
LDL	48.8±35.8	35.3±28.4	58.9±38.1	35.9±21.0	35.9±10.6	36.4±14.3
VLDL	19.5±21.4	16.4±10.25	14.3±10.8	14.4±8.34	8.86±3.21	11.14±7.19
Triglycerides	97.6±106.5	100.8±95.5	70.1±54.9	92.4±65.3	42.1±18.3	55.9±38.2

[a]Reference values (mg/dL): Cholesterol (108-270), HDL Cholesterol (40-78), LDL Cholesterol (31-71), VLDL Cholesterol (<25), Triglycerides (20-150) (Vaden, 2009). [b]p-value <0.05 for the Wilcoxon test.

the need for counseling of the tutors, encouraging the whole family to be involved in the dog's weight loss. Simply pointing out that the dog is overweight and that something needs to be done about it is unlikely to lead to satisfactory results. For many families, the act of feeding and providing treats to dogs is a way of showing affection. Many owners believe that food restriction causes suffering to their dogs, and they prefer to see their animals "happy", even though they know that excess weight reduces life expectancy. This would be a way to justify their animals' body condition and rationalize the lack of interest in solving the problem (Larsen and Villaverde, 2016).

In the study conducted by Carciofi et al. (2005), 50% of the interviewed owners believed that their dogs felt hungry during the weight loss program. The behavior of "begging for food" can be interpreted as hunger, increasing the chances of providing treats and leftovers, or even increasing the amount of food.

Mawby et al. (2004) demonstrated the reliability of BCS when comparing four different body condition assessment methods using DEXA, deuterium dilution, BCS, and biometry in 23 healthy dogs. There was a good correlation (r2=0.92) between the percentage of fat obtained by DEXA and BCS. DEXA is a technique used to measure body fat and lean tissue by using two different energy X-rays (70 and 140 kVp) that enable differentiation of the type and amount of tissue, as well as bone mineral density, fat mass, and lean mass of the body. The low coefficient of variation indicates that it is a very precise, fast, and safe technique, but due to the high equipment cost, it is only used experimentally in research with small animals (Shoveller et al., 2014).

No studies were found that evaluated the effect of red orange extract on weight control in obese dogs. Similar results were found in research with humans, such as in Cardile et al. (2015), in which 60 adults were supplemented with 400 mg of Morosil® or placebo for 12 weeks. At the end of the experiment, a significant reduction ($p<0.05$) in body weight, body mass index, and abdominal circumference of the adults supplemented with Morosil® was reported. Briskey et al. (2022) evaluated 98 men and women receiving Morosil® or a placebo for six months in a randomized, double-blind, placebo-controlled study. Following the final assessment with DEXA, the authors noted a substantial decrease in fat mass and abdominal fat compared to the placebo group. The authors attributed the phyto-complex within Moro orange, primarily composed of flavonoids like anthocyanins, naringenin, and hesperidin, with the ability to diminish adipocyte lipid accumulation and modulate cytokine release, thereby alleviating oxidative stress and inflammation.

In a study that evaluated the effect of Moro orange juice supplementation in rats fed a high-fat diet for 12 weeks, the results showed a decrease in weight gain, triglycerides, and total cholesterol, as well as an improvement in hepatic steatosis (Salamone et al., 2012). In another study, Jeusette et al. (2010) found a significant difference in plasma concentrations of cholesterol and triglycerides in cats supplemented with flavonoids (naringenin) compared to the non-supplemented group. According to the researchers, flavonoids help limit weight gain, decrease triglyceride synthesis and accumulation in the liver, lower total cholesterol, VLDL, and glucose concentrations, and improve insulin sensitivity. However, in the study by Briskey et al. (2022), no difference was found in the biochemical parameters of 98 human patients supplemented with Morosil® over six months.

Conclusion

In the present study, the use of red orange extract (Morosil®) resulted in a reduction of weight and BCS in obese dogs. The extract is thought to reduce inflammation and oxidative stress associated with adipocyte hypertrophy. However, additional research is necessary to determine if the supplementation can be recommended as a truly effective method for treating and preventing obesity in dogs.

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Conflict of Interest. Emerson Milla is a Master's degree student at the Postgraduate Studies Program in Veterinary Sciences, Department of Veterinary Medicine, Universidade Estadual do Centro Oeste, Guarapuava, Paraná, Brazil. The authors declare no conflict of interest.

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IK; visualization, EM and IK; supervision, EM and IK; project administration, EM; funding, EM.

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