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Effects of Isoflavones on Metabolism and Weight Management in Dogs

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Abstract

Overweight and obesity are associated with increased risk of many chronic diseases in dogs. Estrogen plays an important role in regulating energy and fat metabolism and maintaining normal body fat in both male and female animals. Spaying and neutering resulted in the loss of estrogens, decreased energy metabolism and increased incidence of overweight and obesity in dogs. This study tested the hypothesis that isoflavones may mimic estrogen in reducing weight gain and that a combination of soy isoflavones, conjugated linoleic acid and L-carnitine may have synergistic effects on weight gain prevention in dogs.

Glossary of Abbreviations
CLA: Conjugated Linoleic Acid
DEXA: Dual Energy X-Ray Absorptiometry
IVGTT: Intravenous Glucose Tolerance Test
MER: Maintenance Energy Requirement
^{1}H NMR: One-Dimensional Proton Nuclear Magnetic Resonance
O-PLS-DA: Orthogonal-Partial Least Squares-Discriminant Analysis
PCA: Principle Component Analysis
RIA: Radio Immuno Assay
TBI Probe: Triple Broadband Inverse Probe

Introduction

A dog is considered overweight when its body weight exceeds its ideal body weight, and an overweight dog is classified as obese when its body weight exceeds its ideal body weight by 15% or more.[1] About 25% to 34% of dogs in the U.S. are overweight or obese.[2,3] The prevalence of overweight and obese dogs in Europe is about 24% to 44%, depending on different surveys.[4,5] In Australia, a study showed that 41% of dogs were overweight or obese.[6]

The risk factors for dog overweight and obesity include excessive food intake, reduced activity/exercise, age, spaying/neutering, and breed predisposition.[3,5] Lund et al.[3] reported that in the U.S., intact male dogs had the lowest prevalence (23.6%) of overweight and obesity, followed by intact females (27.3%), while spayed female dogs (38.2%) and neutered male dogs (37.5%) had the highest prevalence of overweight and obesity. Spaying/neutering increased the prevalence of overweight and obesity in female and male dogs by 40% and 59%, respectively.[3]

The increased risk of overweight and obesity in old dogs and spayed/neutered dogs mainly may be due to a significant decrease in daily energy metabolism.[5,7] Interestingly, Lovejoy et al.[8] reported that compared with premenopausal women, menopause onset resulted in significant decrease in both energy expenditure and fat oxidation and significant increase in total body and visceral fat. These data indicated that spaying/neutering in dogs leads to changes in energy metabolism similar to those induced by natural menopause onset in women. In fact, studies in rodents showed that estrogen plays an important role in regulating energy and fat metabolism and maintaining normal body fat in both male and female animals.[9]

Chronic overweight and obesity can increase the risk of several chronic diseases including diabetes, hypertension, pulmonary and cardiovascular disease, cancer, and degenerative joint disease.[3,10] Therefore, diets that help prevent and treat excessive weight gain and obesity are needed. Reduced energy metabolism makes it easier to gain weight and more difficult to lose weight. Lund et al.[3] suggested that young and middle-aged spayed and neutered dogs should be the primary targets of weight management to reduce the prevalence of overweight and obesity in dogs. Reduced energy metabolism is a key determinant for overweight and obesity in spayed/neutered and old dogs due to the loss of estrogen. Therefore, nutritional modulation of energy metabolism appears to be a potential strategy to prevent excessive weight gain in spayed/neutered and old dogs.

Isoflavones are natural plant compounds that belong to the phytoestrogen class and include at least one of three chemical compounds: daidzein, genistein and glycitein. Soy isoflavones exist in four chemical forms: aglycone, glucoside, acetylglucoside and malonylglucoside.[11] Most of the soy isoflavones are in the glucoside form in nonfermented soybean products. Intestinal glucosidases hydrolyze isoflavone glucosides and release

aglycone isoflavones. Aglycone isoflaovones are absorbed or metabolized in the intestine. The metabolites of isoflavones (equol, dihydrodaidzein and O desmethylangiolensin from daidzein; p-ethyl phenol from genistein) are then absorbed.[12]

Isoflavones exist exclusively in legumes with the soybean containing the highest amount of isofoavones in glucoside forms. Soy products with isoflavones have been consumed in Asian countries for centuries. Many health benefits have been associated with regular consumption of soy products. For instance, soy consumption has been shown to reduce the risk of cardiovascular disease, breast and prostate cancer; relieve hot flashes associated with menopausal estrogen deficiency; retard osteoporosis in postmenopausal women; reduce total amount of cholesterol, LDL cholesterol and triglycerides in plasma; preserve cognitive functions in postmenopausal women; and improve symptoms of hypertension.[12-16]

Sites et al.[17] reported that daily supplement of soy protein enriched with isoflavones prevented the increase in subcutaneous and total abdominal fat compared with an isocaloric casein placebo in postmenopausal women. These data suggest that soy isoflavones may be effective in reducing body fat buildup and preventing overweight and obesity in animals.

Conjugated linoleic acid (CLA) has been shown to reduce body fat and to increase lean body mass in several animal species.[18-20] The possible mechanisms by which CLA may affect body composition include increased lipolysis in adipose tissue and enhanced fatty-acid oxidation in both adipose tissue and skeletal muscle.[21,22] In addition, L-carnitine has been shown to reduce body fat in growing pigs. It was suggested that L-carnitine facilitates the transport of long-chain fatty acid into mitochondria for beta oxidation.[23]

The objectives of the study were to test the hypothesis that isoflavones may mimic estrogen in regulating the energy metabolism and reducing weight gain in spayed/neutered dogs and that a combination of soy isoflavones, CLA and L-carnitine may have synergistic effects on weight gain prevention in dogs.

Table 1. Nutrient Composition of Diets

	Control	Isoflavone	Cocktail
Nutrient composition (% as fed)			
Moisture	9.0	8.6	8.8
Ash	6.9	7.2	7.1
Crude protein	29.0	28.9	28.6
Crude fat	18.0	18.3	18.2
Nitrogen-Free Extract	35.6	34.9	34.8
Crude Fiber	1.5	2.2	2.5
Soybean Germ Meal (%)	0	10	10
Total Isoflavones (mg/kg) (Aglucon Units)	70	683	663
Conjugated Linoleic Acid (%)	0	0	1.5
L-Carnitine (mg/kg)	0	0	100
Energy Content			
ME (kJ/g)	4.00	3.91	3.95

Materials and Methods

Dogs and Diets

Forty-five dogs (21 male dogs and 24 female dogs) with the percentage of body fat of less than 17.5% for male dogs and less than 20% for female dogs were recruited in the study. These dogs were between the ages of 2 and 9 years old. The study protocol was approved by the Animal Care Committee of Nestlé Purina PetCare. The dogs were housed in pairs, sharing free access to indoor/outdoor areas together.

The control diet was a commercial premium product for adult dogs. The isoflavone diet was formulated by adding 10% soybean germ meal to provide isoflavones from natural ingredient. The cocktail diet was formulated by adding 10% soybean germ meal, 1.5% CLA and 100 mg L-carnitine per kg diet. All three diets (manufactured by Nestlé Purina PetCare, St. Louis, MO) were isoenergetic and contained the same levels of protein, fat and carbohydrates. Dietary ingredient and chemical composition are provided in Table 1. Diet samples were sent to Nestlé Purina Analytical Laboratories (Nestlé Purina PetCare, St. Louis, MO) for chemical analyses. Ash, crude fat, crude fiber, crude protein and moisture were measured based on Association of Official Agricultural Chemists (AOAC) Methods 942.05, 922.06, 962.09, 990.03, and 930.15, respectively. Isoflavone contents of the diets were also analyzed by Nestlé Purina Analytical Laboratories (Nestlé Purina PetCare, St. Louis, MO). The effects of soybean germ meal, CLA and L-carnitine on the digestibility of diets were evaluated by a standard dog digestion test protocol.

Pretest Maintenance Energy Requirement (MER) Determination and Randomization

The control diet was the only diet for all dogs in this period. The amount of daily diet was adjusted weekly as needed to maintain body weight. The maintenance energy requirement was calculated by the amount of control diet that kept the body weight of the dog from more than 10% change over three weeks.

After the MER for each dog was determined, dual energy X-ray absorptiometry (DEXA) was used to determine the baseline body composition of dogs. The dogs were then randomized into three groups with 15 dogs per group based on MER, percentage of body fat and body weight (Table 2).

Table 2. Baseline Body Weight, Body Composition and Maintenance Energy Requirement (MER)[1]

	Control	Isoflavone	Cocktail
Body Weight, kg	28.87±1.16	28.42±0.92	29.18±1.09
Body Fat, %	13.19±1.13	13.29±1.03	14.65±0.96
MER, kcal/d	1683.01±106.95	1567.16±91.39	1593.69±94.95

[1]Values are mean±SE

Feeding and Key Measurements

During the one-year feeding trial, the dogs were fed once daily for about an hour and were fed 25% more than their MER. Food intake was recorded daily. Dogs had free access to water via a wall-mounted automatic system. Routine blood hematology and biochemistry profile, plasma concentrations of isoflavones and metabolites, DEXA, expanded thyroid profile, and leptin were collected at baseline, every three months after the initiation of the treatments and at the end of the study. Body weight was recorded at baseline, weekly and the end of the study. Insulin sensitivity was measured with intravenous glucose tolerance testing at baseline, every three months after the initiation of the treatments and at the end of the study, and serum samples were sent to Diagnostic Center for Population and Animal Health (Lansing, MI) for glucose and insulin analyses. Serum samples were also collected at baseline, every three months after the initiation of the treatments and at the end of the study for metabonomic analysis. Plasma leptin was determined by radio immuno assay (RIA).[24] Serum samples for expanded thyroid profile were sent to the Diagnostic Center for Population and Animal Health (Lansing, MI) for analysis. Plasma concentrations of isoflavones and metabolites were determined by a liquid chromatography/tandem mass spectrometry method.[25] Samples for blood hematology and biochemistry profile were analyzed by Nestlé Purina Clinical Lab (Gray Summit, MO).

Metabonomic Analysis

Serum samples were collected at baseline and every three months after the initiation of the feeding trial. One-dimensional proton nuclear magnetic resonance (^{1}H NMR) spectra were acquired on a Bruker 600MHz spectrometer. Serum samples diluted in a phosphate buffer (pH 7.4) containing D2O, were measured in 5-mm NMR tubes at 300K using a triple resonance broadband inverse (TBI) probe and a sample changer for automation. For all samples, spectra were recorded using the Carr–Purcell–Meiboom–Gill sequence. All spectra were manually phased and baseline corrected. The full NMR spectra of serum samples were used after removing the water suppression region. The spectra was normalized to total intensity and scaled to unit variance (auto-scaling). The ^{1}H NMR dataset was processed by multivariate tools including principle component analysis (PCA) and orthogonal-partial least squares-discriminant analysis (O-PLS-DA) to determine clustering between the three different groups, i.e., control, isoflavone and cocktail groups, according to a method previously reported.[26] O-PLS can generate improved models particularly where an important source of variance is not related to the metabolic variation of interest. Information based on class (Y matrix — consisting of a single variable relating to dietary intervention) is used to eliminate confounding variance from the X data matrix that is unrelated to the group-specific metabolic differences. PLS models can be validated through internal or external methods. For internal validation purposes, cross validation is often used to estimate the number of model components as well as the predictive ability of the model by calculating the Q^2 value. Q^2 value can vary in the range of -1 to 1, where 1 is optimal. The R^2X and R^2Y parameters, which give the fraction of the variations of X (NMR spectra) and Y (class membership) explained by the model after each component, also were reported. Values of R^2X and R^2Y close to 1.0 indicate a statistically robust model. Multivariate analysis was performed using the software package SIMCA-P+ (version 10.0, Umetrics AB, Umeå, Sweden).

Statistical Analysis

Changes in body weight, percentage of tissue fat, absolute fat mass, lean mass, expanded thyroid profile, blood hematology and biochemistry parameters, serum leptin, IVGTT, and digestibility were analyzed by the analysis of variance.

Results

Effects of 10% soybean germ meal and the cocktail diet on digestibility are summarized in Table 3. Both diets had similar effects and slightly reduced digestibility of protein (less than 3%), fat (less than 1.5%) and carbohydrates (less than 2.5%). Soybean germ meal is enriched with daidzein, the predominant form in the blood was

Table 3. Effects of Soy Germ Meal and Blend on the Digestibility of Diets*

	Control	Isoflavone	Cocktail
Total Digestibility (%)	83.0	81.1	81.6
Protein (%)	88.5	86.2*	86.3
Fat (%)	94.6	93.3*	93.7
Carbohydrate (%)	86.4	84.5	84.4*

*Significantly differed ($p<0.05$) from the control diet

Table 4. Isoflavone Profiles (nM) in Dogs after 3 months of Feeding[1]

	Control	Isoflavone	Cocktail
Daidzein	ND	342.8±102.4	454.0±162.3
DHD	ND	2774.2±1234	4756.3 ± 2360
Equol	ND	21879±3774.7	19439±4463.8
O-DMA	ND	457.7±92.7	639.6±168.9
Genistein	ND	17.9±5.3	42.4±18.1
Total Isoflavones	ND	25472±3960	25330±6099

[1]Values (nM) are mean±SE. ND = not detectable, DHD = dihydro-daidzein, O-DMA = O-desmethylangolensin.

Table 5. Changes in Body Weight (kg) from Baseline in Dogs[1]

	Control	Isoflavone	Cocktail
3 month	1.10±0.98	-0.15±0.93	0.44±0.89
6 month	3.64±0.98	1.43±0.93	2.73±0.89
9 month	4.99±0.98	2.16±0.93*	3.54±0.89
12 month	5.54±0.98	2.68±0.93*	4.31±0.89

[1]Values (kg) are mean±SE. *Significantly differed ($p<0.05$) from the control diet.

Table 6. Changes in Body Fat (kg) from Baseline in Dogs[1]

	Control	Isoflavone	Cocktail
3 month	1.15±0.82	-0.82±0.78	0.81±0.75
6 month	3.56±0.82	0.64±0.78*	2.81±0.75
9 month	4.70±0.82	1.49±0.78*	4.24±0.75
12 month	5.27±0.82	1.97±0.78*	4.25±0.75

[1]Values (kg) are mean±SE. *Significantly differed ($p<0.05$) from the control diet and cocktail diets.

Table 7. Changes in % Body Fat from Baseline in Dogs[1]

	Control	Isoflavone	Cocktail
3 month	2.75±1.98	-2.31±1.88	1.69±1.80
6 month	9.08±1.98	1.76±1.88*	6.79±1.80
9 month	11.45±1.98	4.03±1.88*	10.30±1.80**
12 month	12.75±1.98	5.14±1.88*	9.53±1.80

[1]Values (%) are mean±SE. *Significantly differed ($p<0.05$) from the control diet
** Significantly differed ($p<0.05$) from the isoflavone diet.

equol, a metabolite of daidzein, followed by three other metabolites of daidzein: dihydro-daidzein and O-DMA (Table 4).

Effects of 10% soybean germ meal and the cocktail diet on body weight gain are summarized in Table 5. Compared with the control dogs, isoflavone diet significantly reduced the body weight gain by more than 50% at both 9 and 12 months after the initiation of the feeding trial ($p=0.043$ and $p=0.041$ at 9 and 12 months, respectively). However, to our surprise, the cocktail diet failed to prevent weight gain in dogs. All the dogs were on a weight-reduction program under veterinary supervision at the end of the study until they regained ideal body weight.

Effects of 10% soybean germ meal and the cocktail diet on body fat gain are summarized in Table 6. Compared with the control dogs, isoflavone diet significantly ($p<0.05$) reduced the body fat accumulation by more than 50% at 6, 9 and 12 months after the initiation of the feeding trial. Again, the cocktail diet failed to reduce body fat accumulation in dogs significantly.

Effects of 10% soybean germ meal and the cocktail diet on % body fat gain are summarized in Table 7. Compared with the control dogs, isoflavone diet significantly reduced the % body fat gain by more than 50% at 6, 9 and 12 months after the initiation of the feeding trial ($p<0.05$). The cocktail diet failed to reduce the increase in % body fat in dogs significantly.

There were no significant effects among the three diets on the changes in lean body mass during the study (data not shown). These data indicated that almost all the weight gain in the dogs was due to increased body fat.

Thyroid conditions of the dogs were monitored throughout the study. Expanded thyroid profile, blood hematology, leptin, and blood biochemistry profile were measured in dogs at baseline, 3 months, 6 months, 9 months and 12 months after the initiation of the feeding trial. Neither the isoflavone diet nor the cocktail diet significantly affected total thyroxine (TT4), total triiodothyronine (TT3), free T3 (FT3), free T4 (FT4), T3 autoantibody, T4 autoantibody and thyroid autoantibody, thyroid stimulating hormone (TSH) compared with the control diets (data not shown). In addition, there were no significant differences in blood hematology parameters, leptin and blood biochemistry profiles among the three rations during the feeding trial (data not shown).

Intravenous glucose tolerance test (IVGTT) was performed in the dogs at baseline, 3 months, 6 months, 9 months and 12 months after the initiation of the feeding trial. There were no significant differences in IVGTT test among the three rations at any of the above-mentioned sample collection times (data not shown).

A typical standard ^{1}H-NMR spectrum of dog serum

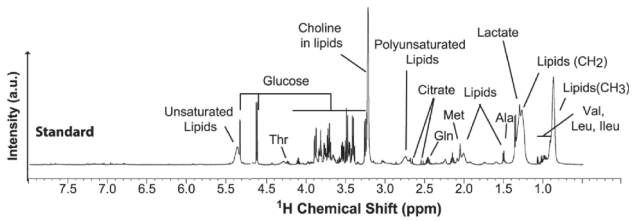

Figure 1: Metabonomic analysis of dog serum samples. A typical Carr–Purcell–Meiboom–Gill sequence (CPMG) 600 MHz ^{1}H NMR spectrum represents a metabolic profile of lipoprotein, amino acids, organic acids (mainly intermediates of tricarboxylic acid cycle), and glucose metabolism in serum of dogs.

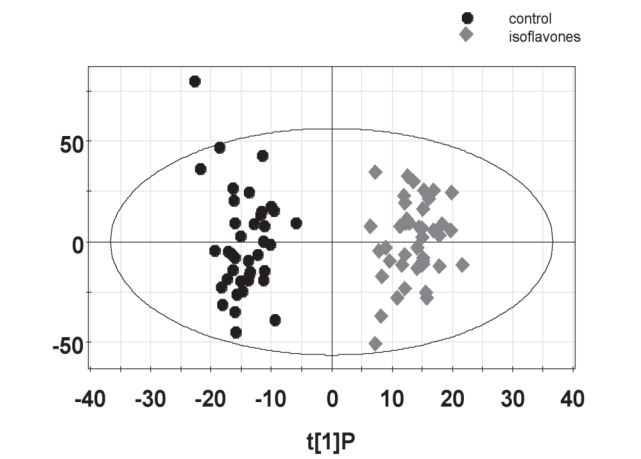

Figure 2: Effects of isoflavones on metabolism in dogs. Serum samples from ^{1}H NMR measurements were analyzed using Orthogonal-Partial Least Squares (O-PLS). Scores plot of O-PLS model showed metabolic difference between control and isoflavone dogs with Q^2Y=0.712, R^2Y=0.941, and R^2X=0.187.

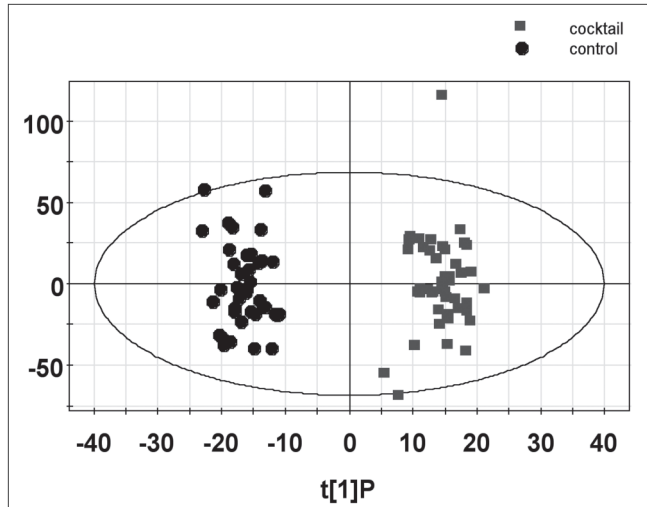

Figure 3: Effects of a blend of isoflavones, CLA and L-carnitine on metabolism in dogs. Serum samples from ^{1}H NMR measurements were analyzed using Orthogonal-Partial Least Squares (O-PLS). Scores plot of O-PLS model showed metabolic difference between control and cocktail dogs with Q^2Y=0.778, R^2Y=0.959, and R^2X=0.205.

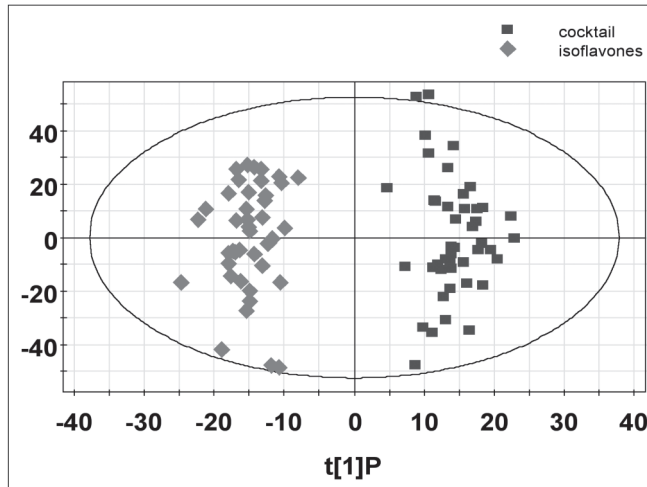

Figure 4: A comparison of metabolic effects between isoflavones and a blend of isoflavones, CLA and L-carnitine in dogs. Serum samples from ^{1}H NMR measurements were analyzed using Orthogonal-Partial Least Squares (O-PLS). Scores plot of O-PLS model showed metabolic difference between control and cocktail dogs with Q^2Y =0.705, R^2Y=0.943, and R^2X=0.180.

is shown in Figure 1. Such a spectrum exhibits a broad set of resonances arising from lipoprotein-bound fatty acyl groups found in triglycerides, phospholipids and cholesteryl esters. Also many sharper peaks arising from the major low molecular weight molecules present in serum are observed. For instance, the NMR spectra of the blood serum contains a number of assignable amino acids (alanine, valine, leucine, isoleucine, glutamine, threonine, methionine), organic acids (citrate and lactate), as well as glucose. Moreover, peaks belonging to saturated and unsaturated fatty acids and choline in phosphatidylcholine and glycerophosphocholine were identified.

Variations between the different serum metabolic phenotypes were investigated using multivariate data analysis, including PCA and O-PLS-DA. Data were visualized by means of component scores plots, where each point represents an individual biochemical profile of a sample (Figures 2, 3, 4). Biochemical components responsible for any detected differences between samples in the scores plot can be extracted from the corresponding loadings plot (not shown), where each coordinates represents a single NMR spectral region. O-PLS-DA analysis of NMR serum profiles maximizes the recovery of metabolic information correlated with sample classes. The obtained scores plots (see Figures 2, 3, 4) show a clear separation of samples according to the nutritional intervention with Q^2 values (0.7 and 0.8) supporting statistical significance of metabolic differences between the control and test groups and between the isoflavone and cocktail group.

Discussion

The isoflavone diet significantly reduced weight gain and body fat buildup by more than 50% in the neutered male and female dogs. This was consistent with the report that soy protein enriched with isoflavones reduced body fat accumulation in postmenopausal women.[17] Even the isoflavone diet slightly reduced the digestibility of dietary protein and fat; the beneficial effects of isoflavone diet on weight gain prevention are more likely due to the effects of isoflavones on energy and fat metabolism in dogs. This was further confirmed by the failure of the cocktail diet to reduce weight gain and body fat accumulation in dogs even though both isoflavone and cocktail diets had similar effects on digestibility. Rachon et al.[27] reported that dietary equol reduced body weight gain and intra-abdominal fat accumulation in ovariectomized rats. Since equol is the predominant form of isoflavone metabolite in the blood of dogs, it is plausible that equol may be the main factor that reduced weight gain and body fat accumulation in neutered dogs fed the isoflavone diet.

Neither soy isoflavones nor the cocktail diets significantly affected total white blood cells, IVGTT or blood biochemical parameters throughout the study. In addition, neither the isoflavone diet nor the cocktail diet had significant effects on TT4, TT3, FT3, FT4, T3 and T4 autontibody, thyroglobulin autoantibody, and TSH compared with the control diets throughout the study. These data indicated that the isoflavone-containing diets had no adverse effects on the health of the dogs. The safety of dietary isoflavones in dogs had been confirmed by an independent safety study in both male and female Beagles. The results did not show any signs of toxicity of soy isoflavones at a dose of 90 mg/kg body weight (NCI, 1996).[28]

Metabonomic analysis of dog serum samples revealed metabolic differences between the control and test groups and between the isoflavone and the cocktail groups. NMR profiling of serum samples reflects the instantaneous homeostatic status of a living organism, providing a simultaneous snapshot on lipoprotein, amino acids, organic acids (mainly intermediates of tricarboxylic acid cycle) and glucose metabolism. Therefore, the obtained statistical results suggest unique metabolic effects of isoflavone and cocktail. A combination of isoflavones, CLA and L-carnitine resulted in a metabolic profile that significantly differed from the metabolic profile induced by isoflavones alone, which may explain why the cocktail diet failed to prevent weight gain. More detailed investigations on the biochemical and metabolic differences between the groups may shed light into the underlying mechanisms of actions in relation to the group-specific weight gain dynamics.

Conclusion

In summary, the soy isoflavone diet was very effective in reducing body fat accumulation in neutered male and female dogs. CLA and/or L-carnitine inhibited the isoflavone benefits.

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