INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

The Effect of Two Levels of Fat and Salmon Oil on Puppies' Growth and Nutrients' Absorption

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Abstract:
An experimental feeding study was conducted to visualize the response of young growing puppies to two levels of fats and salmon oil using iso-nitrogenous equi-caloric diets. Twelve Labrador retriever puppies (6 females and 6males) with 60 days' age and with an average LBW of 6.750 kg were assigned to two groups each of 6 puppies (3 females and 3 males) and housed in individual kennels. Two iso- nitrogenous iso-caloric diets of the same fiber content were prepared by mixing suitable amounts of two commercial dry food brands on the basis of their actual chemical composition. The first diet; high fat and salmon oil diet (HFS) contains 18.5% EE (including 3% salmon oil) and 41.5% NFE. Meanwhile, the second diet;low fat and salmon oil diet (LFS) contains 12% EE (including 0.6% salmon oil) and 48.5% NFE. The feeding trial lasted for 14 weeks. Results revealed that feeding young growing puppies on high levels of fats and salmon oil has a positive significant impact on growth performance parameters including interval and final body weight, body weight gain, actual food amounts and also significantly reduced total amounts of refusal food. In addition, HFS diet exhibited a marked significant increase in serum IGF-1 levels but not affected the serum insulin concentrations HFS diet resulted in a significant elevation of total Cholesterol and LDL levels. Meanwhile, HDL, VLDL and total triglycerides levels and fasting serum glucose were not affected. Finally, HFS diet increased both digestible nitrogen absorption and fat absorption percent.

Keywords: *Labrador retriever, Iso-nitrogenous equi-caloric, high fat, low fat: salmon oil, body weight gain, IGF-1, digestible nitrogen absorption percent, fat absorption percent*

1. Introduction

It is a well-established fact that balanced diets have great impact on health, well-being and performance of dogs especially during puppy stage that considered as a critical period of dog's life. Thus, nutritional-growth performance relationship is one of the most critical concerns during the different stages of puppies' growth. More specifically, energy levels and sources are the main effective nutritional factors that should be carefully considered during pet food formulation and processing. Various energy sources including different lipids (fats and oils) and carbohydrates are considered as the main energy sources in dog food. However, fats and oils are not only highly essential source of energy but also as a source of EFAs that play an important role in dog growth, health and performance. Nutritionists recommended the caloric distribution during growth to be 41% of energy supplied from fat, 27% from protein and 32% from carbohydrate (Case et al., 2002).

The absorption and utilization efficiency of dietary fat is higher than proteins and carbohydrates. Series early studies of commercial dog food brands reported average digestibility coefficients for crude protein, crude fat and NFE of 81%, 85% and 79% respectively (Kendall, Holme and Smith, 1982).

It is well documented that high fat and oil diet is one of the main factors that potentially enhances the growth performance parameters. Research activities indicated that the positive effects of high fat and oil diets on growth may be mediated through one or more of the following ways, via the thermic effects of nutrients, the post-ingestive fuel selection or the role of fat in food intake promotion either by passive overconsumption or weak and insufficient appetite control. Fats have a thermic effect lower than other nutrients, where it was recorded to be 25-30%, 6-8% and 2-3% for proteins, carbohydrates and fats respectively. Thus, the theoretical energy utilization

efficiency for protein is 70-75%, for carbohydrates is 92-94% and for fat is 97-98%. Therefore, dietary fat is utilized more efficiently than protein and carbohydrates (Je´quier, 1995). The post-ingestive fuel selection is highly favorable for carbohydrates and protein oxidation, while dietary fats are preferentially to be stored as triacylglycerol form in adipose tissue. Thus, fatty diet has a tendency to increase the fuel utilization energetic competence(Jequier, 2002). The passive over-consumption is positively related to the improved texture and taste of fatty diets (Prentice et al., 1992; Prentice, 1995; Blundell and Macdiarmid, 1997; McDevitt et al., 2000). Moreover, passive over-consumption seems to be related to energy density of foods, high-fat diets are more energy dense than high-carbohydrate diets, and favour hyperphagia (Prentice, 1998). The appetite control signals are too week or too delayed following fatty diets, thus high fat diets increase energy intake (Jequier, 2002)˙The ingested fat exhibits weak and insufficient ghrelin-suppressing effect compared with carbohydrates and protein (Koliaki et al., 2010). Ghrelin hormone is the only known appetite-stimulating gastrointestinal hormone. High dietary fat intake was proved to be positively correlated with Insulin like growth factor 1 (IGF-1) in dogs, pigs, rats and human (Jahreis et al., 1992; Zhang et al., 1998; Averette et al., 1999; Kaklamani et al., 1999; Zentek et al., 2003). Moreover, fish oil was proved to increase body weight gain in comparison to linseed feeding (Rooke et al., 2000; Schellingerhout et al., 2002). and increased IGF-1 level in weaned piglets (Qizhang et al., 2014).

Nitrogen retention is one of the most important concerns that should be considered while growth performance evaluation. Nitrogen retention is affected by dietary fat, omega-3 and IGF-1 levels. Increasing dietary fat intake was proved to enhance nitrogen retention and utilization in infants (Aerde et al., 1994). Moreover, it increased protein digestibility while decreased nitrogen excretion in young blue foxes' diets (Geng et al., 2012). Nitrogen retention and whole body protein synthesis rate were improved significantly using omega-3 in rats using iso-energetic iso-nitrogenous diets (Hayash et al., 1999). IGF-1 significantly decreased protein oxidation and enhanced nitrogen retention and body weight gain (Lo et al., 1997). Ether extract digestibility and fat absorption percent are improved using high dietary fat levels in blue foxes (Geng et al., 2012; Treuth et al., 2003) and they were improved also using fish oil supplement in infants (Yang et al., 2013).

Female Labrador retriever dogs fed on diets with different fat: carbohydrates (diet A 13:44, diet B 20:33 and diet C 25:26). Diets B and C increased total and LDL cholesterol significantly while diet A increased HDL and triglyceride (Downs et al., 1997). Glucose concentrations were not significantly different between iso-caloric iso-nitrogenous high fat low carbohydrate and low fat high carbohydrate diets, meanwhile, insulin concentrations were significantly higher in LF-HC diet than HF-LC diet (Sunehag et al., 2002).

2. Materials and Methods

2.1. Animals

The experiment was conducted using eight Labrador retriever puppies (6 females and 2 males from the same mother) with 60 days' age and with an average live body weight of 6.750 kg. They were assigned to two experimental groups each of 4 puppies (3 females and only 1 male). Puppies were vaccinated with Vangaurd® vaccine and de-wormed using Drontal plus® before the experiment.

2.2. Housing and Management

Puppies were located each in a separated pet animal research unit of the Internal Medicine Department, Faculty of Veterinary Medicine, Cairo University, Giza -Egypt. Dogs were housed in individual kennels; (2 × 2 m) and had access to an outside kennel (10 × 20 m) for exercise and socialization with each other's for 1 hour daily. Kennels were cleaned twice daily with detergents.

Dogs had access to fresh water ad-libitum throughout the experiment. All puppies were showered once weekly with Betadine® shampoo and Cytéal® antiseptic foaming solution. The experimental feeding study lasted for 14 weeks in addition to one-week preliminary period for acclimatization.

2.3. Diets

Two experimental iso- nitrogenous iso-caloric diets of the same fiber content were prepared by mixing suitable amounts of two commercial dry food brands for puppy stage on the basis of their actual chemical composition. The first diet (HFS diet) was mixed and prepared to contain 18.5% EE (including 3% salmon oil) and 41.5% NFE. Meanwhile, the second diet (LFS diet) was mixed and prepared to contain 12% EE (including 0.6% salmon oil) and 48.5% NFE. Both diets contain 24.6% C.P and 2.5% C.F. Each puppy in both groups was fed separately and the appropriate amount of food was calculated and introduced to each puppy according to its body weight, growth energy requirements and the energy density of the diets using the following equations.

RER (Resting energy requirement) = (30×B.wt) +70 kcal.

MER (Metabolizable energy requirement) = RER×2 kcal.

DER (Daily energy requirement) = MER×2 kcal, then when puppies reached 50% of adult body weight; DER= MER×1.5 kcal (Sandie, 2001).

The food energy density was calculated via the following equation

ME of food = (C.P % × 3.5) + (NFE% × 3.5) + (E.E % × 8.5) kcal/100gm food (Case et al., 2011).

Iso-caloric expression refers to that each puppy was fed its energy requirements depending on its body weight, but it doesn't refer to the same energy density of both diets.

The amount of daily food for each puppy in the two experimental groups was weighed and divided into two equal portions and fed at 9.00 A.M and 5.00 P.M in stainless steel bowl. Each puppy was allowed for 30 minutes to consume the food, then bowls were

removed and any residual food from the previous meal was collected and weighed before the next meal. Dietary composition and chemical analysis of experimental diets were presented in tables (1, 2, and 3).

3. Measurements

3.1. Growth Performance Parameters

Puppies in both experimental groups were weighed individually after an overnight fasting at the onset of the feeding trial then weighed on biweekly basis to calculate body weight gain. The daily consumed and refusal amounts of food were recorded. Results of two dietary fat and salmon oil levels impact on the growth performance parameters are presented in table (4).

3.2. Selected Blood Serum Parameters

Blood samples after an overnight fasting were collectedvia Cephalic vein puncture method after 1 month from the on set of the feeding trial and then after 2 months of the trial. The collected blood samples at the different intervals were centrifuged at 3000 rpm for 10 min and the clear sera were decanted into aseptically treated vials and stored at –20° C untilfurther analysis for determination of serum Glucose level, total Cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and Serum Triglycerides. In addition, some hormonal levels including serum Insulin, Insulin-like Growth Factor one (IGF-1) levels were also determined at the same intervals.All analytical procedures concerning blood seraparameters were carried out in Sigma Laboratory usingdiagnostic kits. The results are illustrated in tables (5, 7).

3.3. Digestible Nitrogen Absorption and Fat Absorption percent

Fecal samples from each puppy in the two experimental groups were collected after 1 month from the onset of the feeding trial and then after 2 months of the trial in tightly closed plastic cuvettes and frozen (−18°C) for subsequent analyses for determination of its nitrogen and fat contents on DM basis, and subsequently the Digestible nitrogen absorption percent, as well as fat absorption percent were calculated according to the method described (Reddy, 2001). Results are presented in table (6).

3.4. Statistical Analysis

The obtained data were analyzed statistically using the statistical package for the social sciences (SPSS). T-test was used to detect the significant variation among all treatments, and the level of significance was set at a minimum at(p<0.05).

4. Discussion

4.1. Impact of Fat and Salmon Oil Levels on Growth Performance Parameters of Puppies

Results indicating the impact of two levels of fats and salmon oil on growth performance parameters are presented in table (4). The obtained data revealed that the average final body weight was (22.125 vs. 16.800 kg) and the average total body weight gain was (15.265 vs. 10.130 kg), the total actual amounts of food intake were (47.041 vs. 37.630 kg) in HFS and LFS diets respectively. Results indicated that the use of highfats and salmon oil level in the food resulted in significant increase in the average final body weight, body weight gain and the actual food intake.

The positive effects of use HFS diet on the growth performance parameters could be explained on the basis that the high fats may have lower thermic effect compared to carbohydrates. Moreover, fats might enhance food consumption either via improved taste and texture of the food or via insufficient appetite control. In addition to the above mentioned modality of actions, the improvements noticed in the growth performance criteria of the growing puppies fed HFS diet might be occurred as result of its stimulating effects on the IGF-1 level that in turn might increase digestive nitrogen absorption percent and fat absorption percent. More or less similar observations were reported; increase body weight gain was recorded in children after the consumption of HF diet compared with LF diet using two iso-energetic iso-nitrogenous meals differ in the fat and carbohydrate contents (Maffeis et al., 2001). Increase in energy utilization efficiency for fat more than carbohydrate was reported (Je´quier, 1995). The high fat content might increase food consumption either via improved taste and texture or via insufficient appetite control because of weak ghrelin-suppressing effect compared with carbohydrate and protein(Prentice et al., 1992; Prentice, 1995; Blundell and Macdiarmid, 1997; McDevitt et al., 2000). High fats and oils in the diet resulted in elevated IGF-1 level which has a positive impact on nutrients' digestibility and in turn on growth (Foster-Schubert et al., 2008; Koliaki et al., 2010).

Our diets' energy distribution is ~ 40.5% from fat, 37.4% from carbohydrates and 22.1% from protein in HFS diet. Meanwhile, energy distribution in LFS diet is ~ 28.5% from fat, 47.5% from carbohydrates and 24% from protein. Thus, high fat and salmon oil diet's energy distribution appears to be close to the caloric distribution recommendations (41% from fat, 32% from carbohydrates and 27% from protein)(Case et al., 2002) So, this caloric distribution might be a main factor for growth performance parameters improvement in puppies fed on HFS diet.

4.2. Impact of Fat and Salmon Oil Levels on Refusal Food Amounts

Results showed the amounts of refusal food are illustrated in table (4). The obtained data revealed that the amounts of refusal food were (0.572 vs. 4.740 kg) in HFS and LFS diets respectively. Data revealed that the amounts of refusal food of puppies fed on LFS diet were significantly higher along the course of the feeding trial.

These results could be attributed to the difference in the amounts of fats and carbohydrate as well as the amount of salmon oil in diets.The HFS diet may have a positive impact on food consumption either by passive overconsumption which seems be related to improved taste and texture of fatty food or weak and insufficient appetite control compared with carbohydrates and protein. The hierarchy of the satiety-inducing capacity is highest for protein, followed by carbohydrates and then fat (protein> carbohydrate>fat). These findings supported the findings of other studies (Prentice, 1998; Jequier, 2002; Foster-Schubert et al., 2008; Koliaki et al., 2010).

4.3. Impact of Fat and Salmon Oil Levels on Some Important Hormones of Puppies

4.3.1. Impact of Fat and Salmon oil Levels on Insulin-like Growth Factor one (IGF-1) Level

Results that illustrate the impact of two fats and salmon oil levels on Insulin-like Growth Factor one (IGF-1) and Insulin levels are presented in table (5). The crude fat content used in this study was composed of both marine and non-marine sources with different amounts. The obtained data revealed that the average Insulin-like Growth Factor one (IGF-1) levels after one month from the onset of the experiment were (443 vs. 324.75 ng/ml) and after two months were (474 vs. 326 ng/ml) in HFS and LFS diets respectively. It was clear that puppies received HFS diet exhibited a marked significant increase in serum concentrations of IGF-1 level along the course of the experimental period. The stimulating effects of HFS diet on Insulin-like Growth Factor one (IGF-1) secretion could be due to one or more of the following possibilities; firstly, it may be due to the fact that increasing dietary fat intake may increase IGF-1 receptor mRNA and protein levels. Secondly, it may be attributed to the glycemic index, as there is a positive relation between IGF-1 level and the hypoglycaemia induced by higher fat intake that increases the level of growth hormone. And finally, it may be mediated via salmon oil anti-inflammatory and immunomodulatory effects.

These findings come in accordance with others' studies. Increasing dietary fat intake can increase IGF-1 receptor mRNA and protein levels (Zhang, Thornton and MacDonald, 1998). Inverse association between carbohydrate intake and serum IGF-1 levels was found; it was attributed to the hyperglycaemia induced by high carbohydrate intake (Kaklamani et al., 1999). Anti-inflammatory and immunomodulatory changes manifested by a decrease in pro-inflammatory cytokines after high fish oil supplementation in dog food were reported (Endres et al., 1989; Caughey et al., 1996; Kew et al., 2003; Luo et al., 2013).

4.3.2. Impact of Fat and Salmon oil levels on Insulin Level

The results of Insulin levels are presented in table (5). The average Insulin levels after one month from the onset of the experiment were (9.73 vs. 9.75 IU/ml) and after two months were (11.48 vs. 11.35 IU/ml) in HFS and LFS diets respectively. It was noticed that serum insulin levels were increased in both experimental diets by increasing the time of administration. However, under the conditions of our experimental trial, the continuous feeding of puppies on either HFS or LFS diets resulted in no significant difference in serum insulin between two groups. Research activities that deal with the impact of feeding of high fats andsalmonoil to growing puppies are scanty and up till now there were no available literatures to justify our findings and this point require further investigations.

4.4. Impact of Fat and Salmon Oil Levels on Digestible Nitrogen Absorption Percent

Nitrogen is one of the metabolic by-products of protein metabolism. Nitrogen balance is defined as the net difference between nitrogen intake and nitrogen excretion, where excretion occurs through different routes like urinary and fecal routes, thus, nitrogen balance measurement requires both urinary and fecal loss measurement. In other words, nitrogen retention or metabolizable nitrogen retention expressions could not be used until both urinary and fecal nitrogen are measured. But if we measured only fecal nitrogen content, we could use digestible nitrogen absorption percent expression.

Both positive and negative nitrogen balance might be predictive tools for growth performance; if intake exceeds excretion, it could be considered as positive nitrogen balance and vice versa.

Results showed the digestible nitrogen absorption as a percentage of gross nitrogen intakes are illustrated in table (6). Data revealed that percent after one month from the onset of dietary treatment was (61.69 vs. 57.21%) and after 2 months of dietary treatment, digestible nitrogen absorption percent was (66.96 vs. 60.21%) in HFS and LFS diets respectively. The significant increase in the digestible nitrogen absorption percent might be due to high fat and /or salmon oil intake and also due to the increased absorption of omega-3 fatty acids.

These results were in agreement with others' studies. Increasing dietary fat level increased protein digestibility (Salas-Salvado et al., 1993; Aerde et al., 1994; Geng et al., 2012). The increase in protein absorption was contributed to omega-3 fatty acids supplementation (Hayash et al., 1999; Smith et al., 2011) a situation which close to our HFS diet formula.

We may also attribute the higher digestible nitrogen absorption percent in puppies fed on HFS diet to either a marked increase in nitrogen absorption or a marked decrease in nitrogen excretion or both. The positive effect on nitrogen fecal excretion in puppies fed on HFS diet might be related to the high fat content itself as it indicated increased fat oxidation, thus decrease in glucose oxidation and utilization (Newsholme and Leech, 1993) and consequently inhibit amino acid oxidation, so decrease protein utilization for energy expenditure and spare it for growth.

The significant increase in digestible nitrogen absorption percent might be due the significant increase in IGF-1 in puppies fed on HFS diet, as it increases the rate of nitrogen absorption on the basic of the fact that IGF-1 level mediates the anabolic effect of GH, as nitrogen absorption might be improved directly or indirectly via IGF-1 and GH. IGF-1 directly stimulates protein synthesis in muscle and other tissues, so it retains more amino acids and nitrogen into cells for protein synthesis process enhancement. In addition to the

role of IGF-1, GH might indirectly decrease protein degradation and nitrogen excretion through alteration in fuel consumption from protein and carbohydrates to fats via increasing lipolysis (Moller and Jorgensen, 2009). Furthermore, omega-3 fatty acids of salmon oil also might have a role in the positive effect of nitrogen fecal excretion in puppies fed on HFS diet, because of the anti-inflammatory effect of omega-3 fatty acids. As reducing the pro-inflammatory cytokines might lead to decreased muscle protein degradation, increased muscle protein synthesis through activation of mTOR-p70s6k signaling pathway and less diverted nutrients to immune system components (Smith et al., 2011), so salmon oil included in HFS diet might decrease the catabolic effect of pro-inflammatory cytokines, thus diverts nutrients as amino acids and nitrogen for growth which in turn increase protein sparing for growth and increase nitrogen absorption percent.

4.5. Impact of Fat and Salmon Oil Levels on Fat Absorption Percent

Results showed the absorbed fat as a percentage of gross fats and oil intakes are illustrated in table (6). Data revealed that the values after one month from the onset of dietary treatment were (95.46 vs. 92 %) and after 2 months of dietary treatment were (95.71 vs. 93.33 %) in HFS and LFS diets respectively.

Our research results showed unexpected increase in fat absorption expressed as a percentage of gross intake with increasing fats and salmon oil content of the puppies' diet. These findings could be explained on the basis that the high fats and salmon oil levels may increase the intestinal absorption capacity due to the fact that dietary fats have a potent trophic effect on intestinal mucosal adaptation, leading to more fat absorption either by increasing the absorptive surface area through mucosal weight, DNA, RNA and protein levels of intestine or by slowing intestinal motility and lengthening the transit time through increasing peptide YY (PYY), or by increasing bile acid synthesis via up-regulation of Cyp7α1 mRNA expression which is a biosynthetic enzyme for bile acid synthesis. These observations come in accordance with some studies. Low fecal fat excretion was recorded by increasing dietary fat level and fish oil supplementation (Yang et al., 2013). The positive impact of high fat intake on fat absorption was attributed to the trophic effect of fat on intestinal mucosal adaptation (Morin et al., 1981; Kollman et al., 1999; Sukhotnik et al., 2003; Sukhotnik et al., 2004). In addition, other authors attributed increase fat absorption with high dietary fish oil supplementation to its positive effect on intestinal absorptive surface area, transit time slowing and bile acid synthesis (Aerde et al., 1994; Yang and Kock 2010).

4.6. Impact of Fat and Salmon Oil Levels on Some Selected Serum Parameters

4.6.1. Impact on Lipid Profile

Results showed impact of two levels of fats and salmon oil on lipid profile are illustrated in table (7). The obtained data revealed that the average total Cholesterol, HDL, LDL, VLDL and triglycerides at the end of the dietary treatments; after 2 months were (389 vs.308.50 mg/dL), (234.25 vs. 203.25 mg/dL), (140.25 vs. 115.50 mg/dL), (14.40 vs. 12.55 mg/dL) and (72 vs. 62.75 mg/dL) in HFS and LFS diets respectively. It was clear that the puppies received HFS diet exhibited a significant higher serum total Cholesterol level, LDL in comparison to the puppies fed LFS diet. However, the levels of HDL, VLDL and total triglycerides were not affected significantly by high fats and salmon oil level. Similar results were recorded in female Labrador retrievers (Downs et al., 1997).

4.6.2. Impact on Fasting Glucose Levels

Results showed the impact of two levels of fats and salmon oil on fasting serum glucose levels are illustrated in table (7). The obtained results revealed that the average fasting serum glucose levels after one month from the onset of the experiment were (90.25 vs. 89.50 mg/dL) and after two months were (100.75 vs. 98.50mg/dL) in HFS and LFS diets respectively. Statistical analysis of the obtained data revealed no significant effects between HFS and LFS diets on fasting serum glucose levels along the course of the experimental feeding study. We may attribute our results to dietary carbohydrates level adaptation. Similar findings were reported in children and adolescents (Sunehag et al., 2002; Treuth et al., 2003).

5. Results

Experimental diets' trade names	HFS diet	LFS diet
Brit®	78 %	---
Proseries®	19 %	---
Miglior®	---	78.7 %
Naxas®	---	20.7 %
Salmon oil (Brit®)	3 %	0.6 %

Table 1: Dietary composition of the experimental diets

Nutrient (%)	HFS diet	LFS diet
Crude protein	24.6	24.6
Ether extract	18.5	12
Salmon oil	3	0.6
Nitrogen free extract	41.5	48.5
Crude fiber	2.5	2.5
Ash	6.7	6.5
Moisture	6.1	5.8

Table 2: Chemical composition of the experimental diets

Nutrient (%)	HFS diet		LFS diet		Salmon oil
	Brit®	Proseries®	Miglior®	Naxas®	
Crude protein	25.6	24.44	25.0	23.776	---
Ether extract	15.87	16.6	11.5	11.619	99.5
Nitrogen free extract	43.23	41.5	48.84	48.663	---
Crude fiber	2.63	2.47	2.2	3.861	---
Ash	6.67	8.0	6.46	6.901	---
Moisture	6.0	7.49	6.0	5.18	---

Table 3: Proximate analysis of the commercial brands used in the experimental diets

	Intervals	HFS diet		HFS diet	
		Mean	SD	Mean	SD
Average initial B. wt		6.860		6.670	
Average final B. wt		22.125		16.800	
Average total B. wt gain		15.265		10.130	
B. wt gain at intervals	1st 4 weeks	4.35 a	0.46	3.18 b	0.63
	2nd 4 weeks	5.35 a	0.69	3.78 b	1.25
	Last 6 weeks	5.56 a	0.72	3.21 b	1.52
Average actual food intake along the experiment		47.041		37.630	
Actual food intake at intervals	1st 4 weeks	9.18 a	0.86	7.31 b	1.46
	2nd 4 weeks	16.65 a	1.57	14.01 b	3.05
	Last 6 weeks	21.22 a	1.98	16.30 b	3
Average refusal food amounts along the experiment		0.572		4.740	
Refusal food amounts at intervals	1st 4 weeks	0.82 a	0.16	1.65 b	1.01
	2nd 4 weeks	0.24 a	0.28	1.53 b	1.56
	Last 6 weeks	0.34 a	0.60	1.57 b	1.35

Table 4: Impact of experimental diets on growth Performance traits of puppies (mean ± SD kg)

(a, b results are Values in the same row with different superscripts are significantly different (P≤ 0.05)

	Hormone	HFS diet		LFS diet	
		Mean	SD	Mean	SD
1st sample After one month from the onset of the experiment	IGF-1 (ng/ml)	443 a	105.80	324.75 b	39.25
	Insulin (IU/ml)	9.73	1.55	9.75	1.14
2nd sample After two months from the onset of the experiment	IGF-1 (ng/ml)	474 a	19.30	326 b	50.74
	Insulin (IU/ml)	11.48	2.29	11.35	3.92

Table 5: Impact of experimental diets on selected serum hormonal levels of puppies at different intervals (Mean ± SD)

a, b Values in the same row with different superscripts are significantly different (P≤ 0.05)

	Item	HFS diet		LFS diet	
		Mean	SD	Mean	SD
1st sample After one month from the onset of the experiment	Digestible nitrogen absorption %	61.69	4.57	57.21	3.02
	Fat absorption %	95.46	1.49	92	3.08
2nd sample After two months from the onset of the experiment	Digestible nitrogen absorption %	66.96 [a]	2.76	60.21 [b]	3.10
	Fat absorption %	95.71 [a]	0. 89	93.33 [b]	1.44

Table 6: Impact of experimental diets on digestible nitrogen absorption percent and fatabsorption percent of puppies (Mean ± SD %)

a, b Values in the same row with different superscripts are significantly different (P≤ 0.05)

	Item	HFS diet		LFS diet	
		Mean	SD	Mean	SD
1st Sample After one month from the onset of the experiment	Cholesterol	348 [a]	31.06	254.75 [b]	53.49
	HDL	225.50	5.32	201.75	22.51
	LDL	110.75 [a]	27.01	41.75 [b]	28.09
	VLDL	11.60	1.13	11.15	4.82
	Triglycerides	58	5.66	55.75	24.10
	Fasting Glucose	90.25	4.99	89.50	2.38
2nd sample After two months from the onset of the experiment	Cholesterol	389 [a]	28.15	308.50 [b]	93.70
	HDL	234.25	11.62	203.25	36.45
	LDL	140.25 [a]	27.17	115.50 [b]	50.47
	VLDL	14.40	2.24	12.55	6.39
	Triglycerides	72	11.22	62.75	31.96
	Fasting Glucose	100.75	4.99	98.50	7.59

Table 7.Impact of experimental diets on selected blood serum parameters (Mean ± SD mg/dL)

a, b Values in the same row with different superscripts are significantly different (P≤ 0.05)

6. Abbreviations

LBW, Life body weight; HFS, high fat and salmon oil; LFS, low fat and salmon oil; EE, ether extract; NFE, nitrogen free extract; IGF-1, Insulin like growth factor 1; LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins;

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