academicJournals

Vol. 7(15), pp. 963-969, 17 April, 2013
DOI 10.5897/JMPR12.1100
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Caryocar brasiliense pulp increases serum HDL and reduces hepatic lipid accumulation in rats fed a high fat diet

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Accepted 7 March, 2013

The fat, fiber and carotenoid composition from *Caryocar brasiliense* pulp points out this exotic fruit as a potential cardio protective food. This study aimed to evaluate the hyoplipidemic effect of *pequi* pulp in rats. Proximate composition, fatty acid profile and carotenoids of *pequi* dehydrated pulp were determined. Next, groups of male rats were fed a standard diet (AIN93M), a control diet (CTRL) added 10% lard, P400 or P600 diets, both similar to CTRL, added *pequi* pulp to provide 400 or 600 mg of *pequi* oil per day, respectively. At the end, serum cholesterol, high-density lipoproteins (HDL), triglycerides and glucose, liver and fecal lipids analysis were performed. *Pequi* pulp had 63.61% of lipids, mainly monounsaturated fatty acids, its fiber content were 17.95% and carotenoids 16,11 mg/100 g. Adding fat to the diets did not increase food intake. There were no changes on serum cholesterol and glucose. The higher level of *pequi* added increased, similarly, HDL and triglycerides in the high fat diets. There was higher liver lipid accumulation in the CTRL group compared to P400 or P600. There was no increase in fecal lipid output in all groups. *Pequi* pulp has a potential hypolipidemic effect, but further studies need to be accomplished because this effect seems related to both dose and duration of the trial.

Key words: *Caryocar brasiliense*, monounsaturated fatty acids, carotenoids, fiber, lipid metabolism.

INTRODUCTION

Among the several exotic fruits available with health promoting benefits, *pequi*, popularly called *piquiá-bravo* or *pequiá* has been quite extensively studied. The *pequizeiro* (*Caryocar brasiliense*) is a native tree from the Brazilian savannah, which belongs to the *Caryocaraceae* family (Oliveira et al., 2008).

The *pequizeiro* is a tree with multiple applications, ranging from the wood industry to regional cuisine) (Oliveira et al., 2010). In addition, it has a potential for fuel and lubricant production. The extract of its leaves shows molluscicidal and antifungal activity in several organisms (Oliveira et al., 2008). The cosmetic industry

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also takes benefits of the high quality oil from its pulp or almond (Oliveira et al., 2010). *Pequi* liqueur has earned national fame and there is a wide variety of confectioner's recipes flavored with it (Lima et al., 2007).

Pequi pulp has a large amount of edible oil, which is rich in carotenoids, and fiber becoming an important food supplement. It should be noted that there is predominance of unsaturated fatty acids in both its pulp (61.35%) and its almond (52.17%) (Lima et al., 2007). Among the unsaturated fats, the highest fatty acid is oleic acid (55.87%) in *pequi* pulp (Lima et al., 2007), which has been related to hypocholesterolemic effects (Pérez-Jiménez et al., 2002).

The amount of fiber found in *pequi* pulp is also noteworthy and accounts for about 18%. The high consumption of these nutrients is associated with cholesterol and other lipid-lowering properties. In addition, its carotenoid content is nearly 16 mg/100g, being significantly higher than other common dietary sources (Jones, 2002). It is believed that these antioxidant compounds interfere with the lipid peroxidation, increasing low density lipoprotein (LDL) resistance to oxidation (Gomes et al., 2005), which also contributes to cardiovascular protection.

Thus, the nutrient composition of *pequi* pulp, especially its oleic acid content, as well fiber and carotenoids, emphasizes its potential as a cardio protective food. The aim of this study was, therefore, to evaluate the hypolipidemic potential of *pequi* pulp in rats. Our hypothesis was that the inclusion of *pequi* pulp in a moderately high fat diet has a beneficial regulatory effect on lipid metabolism (circulating and hepatic levels, fecal output) favoring protective mechanisms against cardiovascular diseases, assuming lipid metabolism disorders are risk factors for such disease development.

MATERIALS AND METHODS

Samples

Ripe *pequi* fruits were collected from previously selected areas of two regions of Minas Gerais state savannah (Brazil), in January 2011. The fruits were gathered manually and taken from the ground above the trees and from all sides (north, south, east and west).

All *pequi* fruits were washed with tap water and subsequently with distilled water. After drying at room temperature, each fruit was cut in half and the pulp was separated from the almond manually. Afterwards, the pulps were placed on trays and dried in an oven at 65°C for 48 h. After drying, the material was grounded, wrapped in a plastic bag, labeled and stored at -18±2°C until the analysis.

Preliminary chemical analysis

Protein content was determined by the semi-micro Kjeldahl method and ashes were quantified by means of sample incineration in a muffle furnace. Total lipids were extracted with ethylic ether, in a Soxhlet apparatus, and moisture was performed in an oven at 105±1°C. Total, soluble and insoluble dietary fibers were quantified by the enzymatic–gravimetric method. These analyses were performed as described by the Association of Official Analytical Chemists (AOAC, 2000). Carbohydrates were obtained by difference, and total energy value (TEV) was estimated using the Atwater factors (Buchholz and Schoeller, 2004).

The fatty acids profile from the pulp oil was determined by gas chromatography, according to Jham et al. (1982).

Total carotenoids were determined by means spectrophotometry, at 410 nm, and expressed in mg/100 g according to the Association of Official Analytical Chemists (AOAC, 2000).

Rat study

Thirty-two male *Wistar* rats (Federal University of Viçosa - UFV), weighing 146.5±2 g, were housed in individual cages and maintained in a room with controlled temperature (22±2°C) and a 12 h light/dark cycle, with free access to food and water. Animal care and animal euthanasia protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA). The study lasted for four weeks.

After 5 days of acclimatization to the conditions, animals were randomly assigned to one of four treatment groups which consisted of four experimental diets. The standard group (SG) received a basal diet as recommended by the *American Institute of Nutrition* – AIN93M (Reeves et al., 1993). The control group (CTRL) received the basal diet added 10% of lard (Table 1). The two *pequi* groups had their diets similar to CTRL, but added pequi pulp in order to provide 400 (P400) (Miranda-Vilela et al., 2009) or 600 mg of pequi pulp (P600) per 25 g of diet, assuming it is the mean daily intake of food per animal (Table 1).

The weight gain was monitored weekly and the food intake monitored every 2 days during the experimental period. The feed efficiency ratio (FER=g weight gain/g food intake) and the food conversion (FC = g food intake/g weight gain) were calculated to evaluate the efficiency of the diet to promote weight gain and the ability of the animal to convert the diet to body weight, respectively (Lima et al., 2002).

Feces were collected for 72 h prior the end of the experiment (28th day), to determine the weight and total lipids (AOAC, 2000). Feces relative weights were also calculated (FeRW = (g wet weight × 100) / g body weight).

At the end of the experiment, all rats were euthanized under light anesthesia with CO_2, and blood samples were collected by means of cardiac puncture. The samples were centrifuged at 1500 rpm for 5 min to remove the serum. Total cholesterol (TC), high-density lipoprotein (HDL), triglyceride (TG) and glucose were determined using commercial kits from LabTest® according to the manufacturer's specifications.

Animal livers were removed, immersed in a saline solution (0.9%), dried with filter paper and weighed in an analytical balance (Shimadzu AX 200), to determine their relative weights (LiRW=(g wet liver weight × 100) / g final body weight). Next, they were oven-dried (60°C±5°C / 72 h), grounded and their lipids were determined (AOAC, 2000).

Visceral fat was also removed, immersed in a saline solution (0.9%), gently dried with filter paper and weighed using an analytical balance (Shimadzu AX 200). The relative weight of visceral fat was calculated (RWVF = (g wet weight of visceral fat × 100) / g final body weight).

Statistics

All chemical analyses, except for fibers, were performed in three

Table 1. Composition (g/Kg) and energy density (Kcal/Kg) of the experimental diets.

Ingredients	Diets*			
	SG	CTRL	P400	P600
Casein	140.0	140.0	140.0	140.0
Cornstarch	465.7	365.7	340.1	327.7
Dextrose	155.0	155.0	155.0	155.0
Sucrose	100.0	100.0	100.0	100.0
Cellulose (fiber)	50.0	50.0	50.0	50.0
Soybean oil	40.0	40.0	40.0	40.0
Mineral mix	35.0	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0	10.0
L-cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5
Pork lard	0.0	100.0	100.0	100.0
Pequi pulp	0.0	0.0	25.6	38.0
Energy density	3601.0	4137.0	4202.0	4233.0

*SG = AIN93-M diet (Reeves et al., 1993); CTRL = SG + 10% pork lard; P400 = CTRL + *pequi* pulp in order to provide 16 g oil/kg of diet (Miranda-Vilela et al., 2009); P600 = CTRL + *pequi* pulp in order to provide 4 g oil/kg of diet.

Table 2. Proximate composition (g/100 g), energy density (Kcal/100 g) and total carotenoids (mg/100 g) of dehydrated *pequi* pulp.

Nutrient	Concentration*
Protein	4.840±0.002
Lipids	63.610±5.590
Soluble fiber	4.850
Insoluble fiber	13.100
Ash	1.210±0.000
Moisture	6.740±0.004
Carbohydrates	5.650±1.120
Energy[b]	614.450±3.360
Total carotenoids	16.110±1.730

*Values expressed as mean ± standard deviation (n=3), except for soluble and insoluble fibers. [b]2570.86±14.06 KJ.

repetitions, and results were expressed as percentage of dry matter. Rat study was conducted in a completely randomized design. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey test, *à posteriori,* using the Statistica® 10.0 (Statsoft, 2010) software at a significance level of $p<0.05$.

RESULTS

Preliminary chemical analysis

Pequi pulp has no significant amount of protein and the lipid content was highlighted. There was an expressive content of total dietary fiber and carotenoids (Table 2).

Among fatty acids, those unsaturated were significant. They accounted for more than 60% of the lipid content. Oleic acid (monounsaturated - MUFA) was highlighted (> 55%), followed by palmitic acid (saturated – SFA) (Table 3).

Rat study

Animals fed P600 diet had their final body weights higher than SG, but they did not differ from CTRL and P400 groups, being the same observed for weight gain. The food intake did not differ among all experimental groups. However, there was a trend of higher intake by CTRL, P400 and P600 groups. FER was higher for P600 and FC was lower compared to SG, but they did not differ from CTRL and P400 (Table 4).

Although *pequi* pulp did not promot significant changes in serum TC and glucose, P600 diet increased HDL when compared to the other groups. Moreover, there was an increase of TG in CTRL, P400 and P600 groups.

Visceral fat weights were higher for CTRL, P400, and P600 groups when compared to SG. The mean values of fecal lipids were similar in all experimental groups. Liver lipids were higher for CTRL group compared to the other groups (Table 5).

Despite the higher concentration of liver lipids for CTRL, there were no significant differences between liver and feces absolute and relative weights for of all experimental groups (Table 6).

DISCUSSION

Pequi pulp can be considered an important food supplement, since it provides an expressive amount of

Table 3. Fatty acid profile (%) of dehydrated *pequi* pulp.

Fatty acid	Number of carbons	Concentration*
Lauric	C12:0	0.04 ± 0.006
Myristic	C14:0	0.11 ± 0.001
Palmitic	C16:0	36.07 ± 0.015
Palmitoleic	C16:1	1.03 ±0.012
Margaric	C17:0	0.06 ± 0.000
Cis-10-heptadecenoic	C17:1	1.19 ± 0.000
Stearic	C18:0	2.10 ± 0.015
Oleic	C18:1	56.76 ± 0.012
Linoleic	C18:2	1.54 ± 0.007
Linolenic	C18:3	0.54 ± 0.001
Arachidonic	C20:0	0.16 ± 0.001
Eicosenoic	C20:1	0.29 ± 0.005
Behenic	C22:0	0.05 ± 0.005
Lignoceric	C24:0	0.08 ± 0.004

*Values expressed as mean ± standard deviation (n=3).

Table 4. Final body weight, weight gain, food intake (g), feed efficiency ratio (%), food conversion (g diet/g body weight) of the experimental groups.

Parameter**	Experimental groups*			
	SG	CTRL	P400	P600
FBW	228.41^{b}±10.23	248.80^{ab}±29.84	255.19^{ab}±35.35	269.84^{a}±29.87
WG	87.35^{b}±9.44	107.16^{a}±27.14	107.05^{a}±38.03	123.46^{a}±31.76
FI	297.88^{a}±10.99	329.79^{a}±24.00	307.62^{a}±12.94	311.50^{a}±52.92
FER	29.00^{b}±3.00	33.00^{ab}±9.00	35.00^{ab} ±11.00	40.00^{a}±7.00
FC	3.45^{a}±0.43	3.33^{ab}±1.19	3.30^{ab}±1.55	2.59^{b}±0.40

*Values expressed as mean ± standard deviation (n=8). Means followed by at least one different superscript letter within a same line are significantly different ($p<0.05$) by one way-ANOVA and Tukey test. SG = AIN93-M diet (Reeves et al., 1993); CTRL = SG + 10% pork lard; P400 = CTRL + *pequi* pulp in order to provide 16 g oil/kg of diet (Miranda-Vilela et al., 2009); P600 = CTRL + *pequi* pulp in order to provide 24 g oil/kg of diet. **FBW = final body weight; WG = weight gain; FI = food intake; FER = feed efficiency ratio; FC = food conversion.

Table 5. Serum glucose, triglycerides, cholesterol and HDL (mg/dL), absolute (g) and relative (%) weights of visceral fat, hepatic and fecal lipids (g/100 g) of the experimental groups.

Variable	Experimental groups*			
	SG	CTRL	P400	P600
Glucose	116.70^{a}±14.57	124.16^{a}±20.57	123.09^{a}±12.04	120.03^{a}±17.05
Triglycerides	90.08^{b} ±7.58	119.43^{a}±12.42	108.91^{a}±8.80	106.34^{a}±13.11
Cholesterol	67.15^{a}±11.61	76.88^{a}±16.03	73.12^{a}±10.36	66.72^{a}±5.85
HDL	43.62^{b}±4.95	44.62^{b}±7.01	44.65^{b}±8.81	92.57^{a}±53.47
AWVF**	9.66^{b}±3.73	15.51^{a}±2.39	16.52^{a}±2.33	14.09^{a}±1.69
RWVF**	4.21^{b}±1.55	6.29^{a}±1.11	6.59^{a}±1.41	5.22^{a}±0.31
Hepatic lipids	15.44^{b} ± 2.80	21.52^{a} ± 5.28	16.80^{b} ± 3.39	15.25^{b} ± 3.29
Fecal lipids	3.31^{a} ± 0.82	4.26^{a} ± 1.60	3.36^{a} ± 0.81	4.23^{a} ± 1.64

*Values expressed as mean ± standard deviation (n=8). Means followed by at least one different superscript letter within a same line are significantly different ($p<0.05$) by one way-ANOVA and Tukey test. SG = AIN93-M diet (Reeves et al., 1993); CTRL = SG + 10% pork lard; P400 = CTRL + *pequi* pulp in order to provide 16 g oil/kg of diet (Miranda-Vilela et al., 2009); P600 = CTRL + *pequi* pulp in order to provide 24 g oil/kg of diet. **AWVF = absolute weight of visceral fat; RWVF = relative weight of visceral fat.

Table 6. Liver and feces absolute (g) and relative (%) weights of the experimental groups.

Parameter**	Experimental groups*			
	SG	CTRL	P400	P600
LiAW	8.19^{a}±1.00	8.12^{a}±1.22	8.32^{a}±1.43	8.93^{a}±0.57
LiRW	3.58^{a}±0.36	3.26^{a}±0.21	3.25^{a}±0.19	3.33^{a}±0.35
FeAW	6.78^{a}±0.98	7.23^{a}±1.40	7.55^{a}±1.42	7.94^{a}±0.89
FeRW	2.98^{a}±0.47	2.90^{a}±0.35	2.96^{a}±0.44	2.96^{a}±0.31

*Values expressed as mean ± standard deviation (n=8). Means followed by at least one different superscript letter within a same line are significantly different ($p<0.05$) by one way-ANOVA and Tukey test. SG = AIN93-M diet (Reeves et al., 1993); CTRL = SG + 10% pork lard; P400 = CTRL + *pequi* pulp in order to provide 16 g oil/kg of diet (Miranda-Vilela et al., 2009); P600 = CTRL + *pequi* pulp in order to provide 24 g oil/kg of diet. **LiAW = liver absolute weight; LiRW = liver relative weight; FeAW – fecal absolute weight; FeRW = fecal relative weight.

energy. The protein content is consistent with results from other studies (Oliveira et al., 2010; Santos et al., 2010), in which it ranged from 2.0 to 4.9 g/100 g, depending on its processing. The ash content was higher than other studies (Lima et al., 2007; Santos et al., 2010), probably because samples were from different regions to those used in our research. Moisture classified the pulp as a dehydrated product in accordance to Brazilian law (Brasil, 2005) since it was lower than 12%.

Lipid content was expressive, especially for unsaturated fatty acids, being oleic acid (monounsaturated fatty acid - MUFA) the highest. It has been postulated that the intake of dietary MUFA's promotes healthy blood lipid profiles, mediates blood pressure, improves insulin sensitivity and regulates glucose levels. It has also been suggested to play a role in preferential oxidation and metabolism of dietary MUFA´s, influencing body composition and ameliorating the risk of obesity (Gillingham et al., 2011).

According to Brazilian *Agência Nacional de Vigilância Sanitária* (ANVISA - Brasil, 1998), a solid food must have at least 3 g per 100 g of fibers to be considered as a source. Thus, *pequi* pulp can be considered a good source of dietary fiber and its intake can bring health benefits. Soluble fibers, for example, are capable to link bile acids and to reduce their absorption, to promote water retention, and to increase viscosity. Insoluble fibers increase feces formation and bile acid excretion, and stimulate intestinal motility (Rodríguez et al., 2003). *Pequi* pulp also had a higher proportion of insoluble fibers which could be related to an increased intestinal transit and prevention of gastrointestinal diseases (Rodríguez et al., 2003).

Our results also have indicated considerable levels of total carotenoids in pequi pulp, higher than 7.25 mg/100 g as indicated in a study performed by Lima et al. (2007) (7.25 mg/100 g). It is important to highlight that the carotenoid amount was also higher compared to some fruits and vegetables commonly consumed and considered sources of these nutrients, such as corn (0.88 mg/100 g), papaya (0.86 mg/100 g), and peach (0.65 mg/100 g) (Godoy and Rodriguez-Amaya, 1994).

Epidemiological evidence suggests that carotenoid-rich diets are associated with lower risk of cardiovascular diseases, especially due to their antioxidant action. Additionally, the α, β, γ-carotene, and cryptoxanthin show pro-vitamin A activity, which is essential for the visual cycle, normal growth, development, and maintenance of the immune response, especially in relation to lymphocyte and macrophages activation (Brasil, 2007). Thus, the addition of *pequi* pulp at food preparations, may contribute to the provision of antioxidants and vitamin A, whose deficiency is still considered a public health problem.

Adding fat to diets did not promote a higher food intake possibly indicating that rats tended to remain their energy intake relatively fixed (Behnke, 1996). However, there was a trend towards higher intake by rats from the high fat groups when compared to the SG group. This can be attributed to the higher palatability of these diets, given by the addition of lard. In addition, despite no differences for food intake, diets with more fat, promoted higher weight gain, which points out that the relationship between energy intake and body weight does not increase linearly and also depends on the constituents of the diet (Behnke, 1996). In fact, animals feed P400 and P600 diets were more efficient in gaining weight as well as food consumption per g of weight gained.

In our study, we added a quantity of *pequi* pulp to provide 400 or 600 mg of oil per each 25 g of diet, considering that this was the mean daily intake for rats. This choice was due to the fact that the oil extracted from *pequi* pulp is high in unsaturated fatty acids, especially oleic acid (Table 3). Thus, an additional 36 and 54 mg/day of oleic acid were added to P400 and P600 diets (15.65 and 23.48%, respectively).

According to Gillingham et al. (2011), MUFA´s have potential cardioprotective properties by modulating serum lipids, blood pressure, and insulin sensitivity. In fact, several studies indicate that there is a modulatory effect,

especially for TC, low density lipoprotein (LDL) and HDL (Mensink et al., 2003). On the other hand, some authors postulated that evidences for these effects are still weak (Degirolamo and Rudel, 2010; Vafeiadou et al., 2012). Degirolamo and Rudel (2010) also stated that the dietary source of MUFA's might influence the cardioprotective effects by interacting with other food constituents.

The modulating effects, related to MUFAs, on glycemia are still unclear. It seems that these fatty acids could improve insulin sensitivity, especially in individuals with insulin resistance phenotype (Risérus et al., 2009), which explains, at least in part, the lack of clear results in relation to this parameter in our study.

The higher proportion of fiber from *pequi* pulp is insoluble, a fraction that is more associated with increased intestinal transit and feces formation and not with the reduction of serum lipids or glycemia (Fardet, 2010). The cardioprotective effects of carotenoids are complex and not due to a single carotenoid in particular. It is also hypothesized that there is a correlation between their serum levels and their protective effects, indicating a possible dose-dependent effect (Bhupathiraju and Tucker, 2011).

We observed few or no changes in fecal lipids, which points out no increase in fecal excretion of lipids when lard or *pequi* pulp was added to the diets.

Regarding liver lipids, they were higher for the CTRL group, probably due to the addition of lard. Studies suggest that the fatty acid composition of foods, especially saturated fatty acids, correlates with its tissue deposition in animal models (Perona et al., 2000). In addition, *pequi* pulp added to diets, promoted liver lipid levels similar to SG group, indicating a probable protective effect of *pequi* pulp against liver steatosis. In this case, the unsaturated fatty acids, especially the monounsaturated, may show a significant impact against liver steatosis, as well as the carotenoid content.

Carotenoids may also have had some impact on liver lipid deposition. These compounds can act synergistically with other antioxidants, providing a stronger protection against reactive oxygen species. This action is important, since lipoproteins, when oxidized, release their lipid content which accumulates in the endothelium of blood vessels or into cells such as liver (Stahl and Sies, 1996), resulting in fatty infiltration, with changes in hepatic metabolism of lipids.

We also inferred an increase in visceral fat indicative of lipid deposition for all groups fed high fat diets, as shown by their higher weights. According to Terada et al. (2011), diets high in fat, especially saturated fatty acids, are considered the main factor for visceral fat deposition and insulin resistance development. The increased amount of adipose tissue in experimental animals observed in our study has been well documented (Caton et al., 2012). Bernardes et al. (2004) also observed a significant increase in visceral adipose tissue weight of rats fed a high fat diet. However, in relative terms, there was a lower visceral fat weight for the P600 group, which is indicative of a protective effect of *pequi* pulp.

Our findings also reinforces that no significant interference in the intestinal absorption of lipids in animals fed with the high fat diets was evident. The excessive intake of these nutrients increased the triglyceride levels and did not affect serum levels of total cholesterol and glucose.

We can therefore infer that *pequi* pulp presented a hypolipidemic potential, since it may have accounted for the lower liver fat deposition and the increased serum HDL levels, which appears to be dose dependent. Its fatty acid and carotenoid composition may be involved in these effects. However, it should be considered that the experimental time for feeding and the lipid composition/ quantity in the diets may have influenced the results. Long and medium term studies with higher doses, should be considered to have a better understanding of the possible metabolic effects of this exotic fruit, considering it is an additional source of nutrients and bioactive compounds when added to the diet.

ACKNOWLEDGMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

ABBREVIATIONS

AIN93M, American Institute of Nutrition Maintenance Diet for Rodents; **ANVISA,** *Agência Nacional de Vigilância Sanitária;* **AWVF,** absolute weight of visceral fat; **FBW,** final body weight; **FC,** food conversion; **FeAW,** feces absolute weight; **FER,** feed efficiency ratio; **FeRW,** feces relative weight; **FI,** food intake; **FRW,** feces relative weight; **HDL,** high-density lipoprotein; **LDL,** low density lipoprotein; **LiAW,** liver absolute weight; **LiRW,** liver relative weight; **MUFA,** monounsaturated fatty acid; **RWVF,** relative weight of visceral fat; **SFA,** saturated fatty acid; **TC,** total cholesterol; **TEV,** total energy value; **TG,** triglycerides; **WG,** weight gain.

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