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### RESEARCH ARTICLE

#### IMPACT OF FOUR FAT-RICH INFANT FLOURS ON LIPID AND ATHEROGENIC PROFILE OF WISTAR RATS

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#### Abstract

Dietary fat content influences serum lipid parameters and the incidence of cardiovascular and coronary heart disease. The point of this study was to find out what happened to Wistar rats' serum lipid profiles and atherogenic indices when they ate a high-fat PASLoc meal after they had been on a restricted diet. Forty-two male rats,  $80 \pm 5$  days of age, were subjected to 19 days of food restriction and then fed PASLoc infant formula for 14 days. Blood samples from the rats were taken at the end of the periods of not eating and eating so that serum lipid constants (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) could be found and predictive parameters for atherosclerotic and coronary heart disease could be calculated. The results showed that dietary restriction led to an imbalance in serum parameters, with LDL cholesterol ( $0.11 \pm 0.01$ ), HDL cholesterol ( $0.35 \pm 0.08$ ), total cholesterol ( $0.60 \pm 0.07$ ), and triglycerides ( $0.44 \pm 0.08$ ) all decreasing. At the end of the dietary experiment, these constants improved according to the standards recommended for rats. Consumption of PASLoc flours had no negative effect on the Castelli indices (CRI-I and CRI-II), the atherogenic coefficient (CA), or the atherogenic plasma index (AIP). The fats contained in these flours do not represent an atherogenic health risk for children.

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#### Introduction:-

Despite global efforts, malnutrition persists as a significant challenge in developing countries, representing a primary risk factor for illness and mortality among millions of young children (Dalvi et al., 2018). As indicated by the FAO/STAT data for the year 2023, 9.1% of children—representing a total of more than 733.4 million children—are affected by undernutrition on a global scale. In Côte d'Ivoire, more than 900,000 children are affected by stunting and over 300,000 by wasting (FAO/STAT, 2023).

To address this problem, various ready-to-use therapeutic foods (RUTFs) are increasingly being utilized for the prevention and treatment of different forms of malnutrition (Prudhon et al., 2006). The consumption of these

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therapeutic foods has contributed significantly to a reduction in infant morbidity and mortality (Prudhon et al., 2006; UNICEF, 2023).

Additionally, in the pursuit of a solution to child malnutrition, local resources are employed to create infant flours that serve as a food source for the nutritional rehabilitation of children suffering from moderate acute malnutrition. Studies by Kra et al. (2022), Assoumou et al. (2022), and Kouadio et al. (2024) have shown that these flours are nutritionally valuable, especially when it comes to helping rats that have been on a food restriction period get back to normal. However, three of the flours exhibited markedly elevated fat contents in their biochemical compositions. The fat content of the flours was found to be  $21.53 \pm 0.11\%$ ,  $23.86 \pm 0.11\%$ , and  $21.66 \pm 0.24\%$ , respectively, for Pasloc1B, Pasloc2A, and Pasloc2B (Kra et al., 2022; Assoumou et al., 2022). These high fat contents present a potential for disruption to the serum lipid profile, which could in turn lead to the onset of numerous metabolic diseases (Matos et al., 2005). When you eat these high-fat flours, your serum levels of total cholesterol, triglycerides, low-density lipoprotein (LDL-C), and high-density lipoprotein (HDL-C) will go up. This is especially true after a period of dieting restrictions (Vafa et al., 2011). Hyperlipidemia is a risk factor for the development of atherosclerosis, which in turn is a major cause of cardiovascular disease, including hypertension, coronary heart disease, and stroke. It is thus imperative to assess the lipid profile of PASLoc flours utilized for the nutritional rehabilitation of moderately malnourished children. The objective of this study was to analyze the lipid profile of PASLoc flours consumed by Wistar rats subjected to a period of food restriction.

## Materials and Methods:-

### Raw materials

The local flours were produced using a variety of grains, including millet (*Panicum miliaceum*), soybean (*Glycine max*), maize (*Zea mays*), rice (*Oriza sativa*), and groundnut (*Arachis hypogea L.*). The aforementioned grains were procured from the Forum market in Adjamé, Abidjan. Ready-to-use products, including brown sugar and palm oil fortified with vitamin A, were procured from a commercial establishment in the Abidjan district. The fish meal of the *Clupea harengus* species was procured from the Autonomous Port of Abidjan (Côte d'Ivoire).

### Raw Material Processing

The diverse range of raw materials was processed into flour in accordance with the methodology outlined by Kra et al. (2022). The flour was produced from millet, soybean, maize, rice, groundnut, and fish and was subsequently stored in glass bottles at room temperature.

### Feed formulation

The various infant foods were formulated in accordance with the recommendations set forth in the Ivorian National Nutrition Policy guide, which was established in 2017 (GNR, 2017). Consequently, four distinct types of flour, each based on local products, were formulated by combining the flours that had been previously obtained (Kra et al., 2022).

### Batch Composition

The study utilized 35 male rats (*Rattus norvegicus*) of Wistar strains,  $80 \pm 5$  days old, with an average weight of  $103.46 \pm 5.10$  g, sourced from the vivarium of the Ecole Normale Supérieure (Abidjan, Ivory Coast). The animals were placed in individual metal cages under a photoperiod of 12 hours of light and 12 hours of darkness, with an average temperature of  $25 \pm 2^\circ\text{C}$ , in the biological research laboratory of the aforementioned educational institution (Abidjan, Côte d'Ivoire). Five experimental batches, each comprising seven rats, were formed: a control batch (LTC) and four experimental batches, each fed with one of the previously formulated PASLoc flours. The control batch was fed a diet containing 16% casein, while the food distributed during the food restriction period consisted of 4% casein.

### Experimental Procedure

A 38-day experiment was done with three separate parts: a first phase of adaptation, a 19-day phase of food restriction, and a final phase of 14 days where the nutritional effect on lipid constants was measured after eating PASLoc meals (Kouadio et al., 2024a). The adaptation phase entailed the provision of the control diet (LTC) to all batches of rats for a period of five days. The dietary restriction phase entailed the provision of a restricted diet containing 4% protein to five batches of rats. In the part that looked at how nutrition affected lipid constants, PASLoc flours were given to four groups of rats, along with the LTC control diet to one group and the LIM restriction diet to one group. Throughout all phases, rats were provided with 50 g of the corresponding diet each

morning between 8:00 and 9:00 a.m. Food scraps were then collected the following day before any further distribution.

### Evaluate the influence of nutritional factors on the lipid profile

Following completion of the dietary restriction phase, three rats from each cohort were anesthetized with urethane ether and subsequently euthanized (Kouadio et al., 2024b). Whole blood was collected from the rats following the jugular vein incision. At the conclusion of the study, the remaining rats from each batch were euthanized. The blood was collected in hemolysis tubes containing an anticoagulant (EDTA) for the determination of lipid parameters, including triglycerides, cholesterol, HDL, and LDL. The tubes containing blood were placed in a cooler with ice and transported to the Institut Pasteur de Côte d'Ivoire laboratory for multiparameter assays. The lipid profile indices were calculated as follows:

Castelli risk index (IRC-I) = TC/HDLc

Castelli risk index (CRI-II) = LDLc/HDLc

Atherogenic plasma index (AIP) =  $\log(TG/HDLc)$

Atherogenic coefficient (AC) =  $(TC-HDLc)/HDLc$ .

### Statistical Analysis

The data were analyzed using XL STAT V.2019.2.2 software. The values are expressed as means  $\pm$  standard deviation, and the means were compared using Duncan's test. To find a connection between PASLoc flours, lipid profiles, and atherogenic indices, principal component analysis (PCA) and hierarchical ascending classification (HAC) were used. The overall significant difference was set at a P value  $< 0.05$  for all tests.

## Results:-

### Effect of Infant Formula Consumption on Lipid Constants in Rats

Table 1 depicts the serum lipid profile of rats that were fed the various infant meals following the food restriction period and at the end of the nutritional experiment. The results demonstrate a notable discrepancy in lipid constituents at the conclusion of the study, subsequent to the consumption of the infant meals, in comparison to the values obtained following the food restriction period. Additionally, the levels of HDL cholesterol were observed to exceed those of LDL cholesterol for each cohort of rats. The rats that were fed Pasloc1b and Pasloc2b had the highest levels of LDL cholesterol, while those that were fed Pasloc1a and Pasloc2b had the highest levels of HDL cholesterol. The rats that were fed Pasloc1b flour and the control rats exhibited the highest serum total cholesterol levels. In terms of triglycerides, the rats from the control batch had the highest serum levels, followed by the rats that were fed Pasloc1b flour.

**Table 1:-** Lipid profile of rats fed infant formula.

Batch of rat		Pasloc1a	Pasloc1b	Pasloc2a	Pasloc2b	Control
LDL-c	RP	0.11 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>a</sup>
	NP	0.09 $\pm$ 0.01 <sup>b</sup>	0.14 $\pm$ 0.02 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>ab</sup>	0.14 $\pm$ 0.02 <sup>a</sup>	0.16 $\pm$ 0.03 <sup>ab</sup>
HDL-c	RP	0.35 $\pm$ 0.08 <sup>a</sup>	0.35 $\pm$ 0.08 <sup>a</sup>	0.35 $\pm$ 0.08 <sup>a</sup>	0.35 $\pm$ 0.08 <sup>a</sup>	0.40 $\pm$ 0.08 <sup>b</sup>
	NP	0.46 $\pm$ 0.06 <sup>a</sup>	0.34 $\pm$ 0.00 <sup>b</sup>	0.37 $\pm$ 0.03 <sup>ab</sup>	0.44 $\pm$ 0.04 <sup>a</sup>	0.42 $\pm$ 0.07 <sup>ab</sup>
TC	RP	0.60 $\pm$ 0.07 <sup>a</sup>	0.60 $\pm$ 0.07 <sup>a</sup>	0.60 $\pm$ 0.07 <sup>a</sup>	0.60 $\pm$ 0.07 <sup>a</sup>	0.52 $\pm$ 0.08 <sup>a</sup>
	NP	0.60 $\pm$ 0.06 <sup>ab</sup>	0.62 $\pm$ 0.04 <sup>a</sup>	0.50 $\pm$ 0.05 <sup>b</sup>	0.59 $\pm$ 0.05 <sup>ab</sup>	0.68 $\pm$ 0.08 <sup>a</sup>
TG	RP	0.44 $\pm$ 0.08 <sup>b</sup>	0.44 $\pm$ 0.08 <sup>b</sup>	0.44 $\pm$ 0.08 <sup>b</sup>	0.44 $\pm$ 0.08 <sup>b</sup>	0.70 $\pm$ 0.10 <sup>a</sup>
	NP	0.88 $\pm$ 0.10 <sup>c</sup>	1.23 $\pm$ 0.04 <sup>b</sup>	0.77 $\pm$ 0.11 <sup>c</sup>	0.75 $\pm$ 0.01 <sup>c</sup>	1.55 $\pm$ 0.08 <sup>a</sup>

The values shown in the table are the means  $\pm$  standard deviations of tests carried out in triplicate. In the same row, values with the same exponent do not differ significantly at threshold ( $\alpha > 0.05$ ). TC: Total cholesterol, HDL: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: Triglycerides, RP: Restriction phase, NP: Nutritional phase.

### The Effect of Lipid Parameters on Atherogenic Indices in Rats

Table 2 illustrates the atherogenic profile of rats fed the various flours. The statistical analysis showed that the CRI-I, CRI-II, and AC indices of rats that were fed Pasloc1a, Pasloc1b, Pasloc2a, and Pasloc2b flours were not statistically different from those of rats that were fed the control diet. However, the atherogenic plasma index (AIP) of the different batches of rats at the conclusion of the dietary restriction and nutritional experimentation period exhibited a negative value. At the end of the experiment, there was a statistically significant difference ( $P < 5\%$ ) in

the rats' atherogenic indices (CRI-I, CRI-II, AIP, and AC). The rats that were fed Pasloc1B flour had the highest Castelli index CRI-I value of  $1.81 \pm 0.10$ , the lowest Castelli index CRI-II value of  $0.40 \pm 0.07$ , the highest atherogenic coefficient (AC) value of  $0.81 \pm 0.10$ , and the lowest atherogenic plasma index (AIP) value of  $-0.690 \pm 0.01$ .

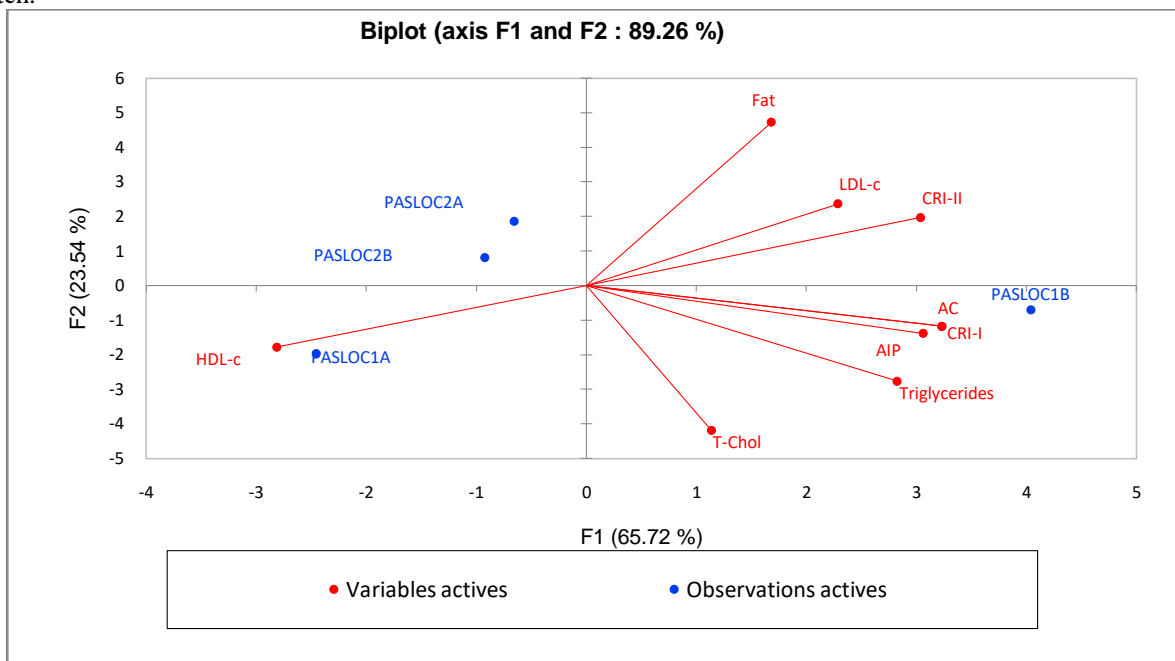
**Table 2:-** Atherogenic profile of rats fed different flours.

Lot de rats		Pasloc1a	Pasloc1b	Pasloc2a	Pasloc2b	Control
CRI-I	RP	$1.23 \pm 0.06^a$	$1.23 \pm 0.06^a$	$1.23 \pm 0.06^a$	$1.23 \pm 0.06^a$	$1.28 \pm 0.41^a$
	NP	$1.31 \pm 0.04^c$	$1.81 \pm 0.10^a$	$1.35 \pm 0.03^c$	$1.34 \pm 0.01^c$	$1.62 \pm 0.09^b$
CRI-II	RP	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.24 \pm 0.04^a$
	NP	$0.19 \pm 0.01^b$	$0.40 \pm 0.07^a$	$0.30 \pm 0.02^{ab}$	$0.31 \pm 0.01^{ab}$	$0.26 \pm 0.13^b$
AIP	RP	$-0.41 \pm 0.00^b$	$-0.41 \pm 0.00^b$	$-0.41 \pm 0.00^b$	$-0.41 \pm 0.00^b$	$-0.14 \pm 0.01^a$
	NP	$-0.08 \pm 0.00^b$	$-0.690 \pm 0.01^c$	$-0.04 \pm 0.03^a$	$-0.13 \pm 0.04^c$	$-0.21 \pm 0.05^d$
AC	RP	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.23 \pm 0.06^a$	$0.28 \pm 0.41^a$
	NP	$0.31 \pm 0.04^c$	$0.81 \pm 0.10^a$	$0.35 \pm 0.03^c$	$0.34 \pm 0.01^c$	$0.62 \pm 0.09^b$

The values shown in the table are the means  $\pm$  standard deviations of tests carried out in triplicate. In the same row, values with the same exponent do not differ significantly at the threshold ( $\alpha > 0.05$ ). CRI 1: Castelli risk index I, CRI II: Castelli risk index II, AIP: Atherogenic index of plasma: AC: Atherogenic coefficient, RP: Restriction phase, NP: Nutritional phase.

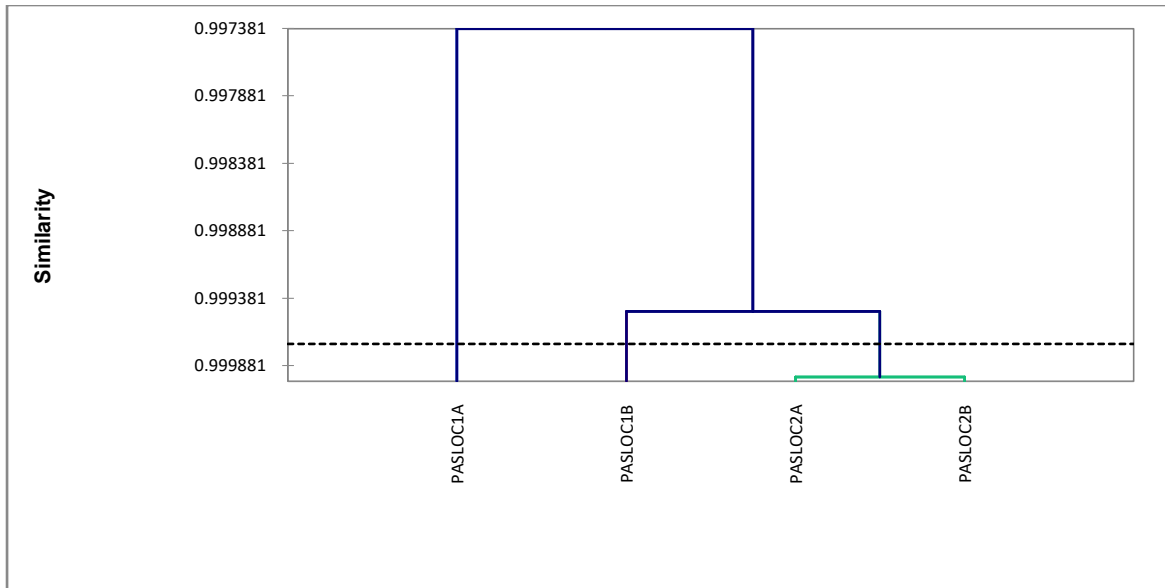
**Establishing the correlation between lipid and atherogenic profiles and the fat content of flours**

Figure 1 illustrates the correlation between the lipid and atherogenic profiles of rats fed infant formula. The projection in the plane demonstrates that there is no correlation between the PASloc1A rat batch and the HDL cholesterol parameter on either the F1 or F2 axis. The PASloc2A and PASloc2B rat batches are positively correlated with the LDL cholesterol and CRI-II parameters on the F2 axis. On the other hand, the PASloc1B rat batch is positively related to most of the parameters on axis 1, such as total cholesterol, LDL cholesterol, CRI-II, AC, CRI-I, AIP, and triglycerides. The aforementioned breakdown of rat batches according to parameters allows for their classification into three distinct batches: batch 1, which is comprised solely of the Pasloc1A rat batch; batch 2, which is comprised of the PASLoc 2A and 2B rat batches; and batch 3, which is comprised of the Pasloc1B rat batch.



**Figure 1:-** Principal component analysis showing the link between lipid and atherogenic profiles and the fat content of flours.

Figure 2 illustrates the hierarchical ascending classification of flours according to their lipid and atherogenic profiles. An analysis of the figure reveals a division of the rat batches into three groups based on similarities between lipid and atherogenic parameters. The first group, PASLoc 1A, comprises the first batch, while the second group, PASLoc 1B, comprises the second batch. The third group, PASLoc 2A and PASLoc 2B, comprises the third batch.



**Figure 2:-** Hierarchical ascending classification of flours by lipid and atherogenic profile.

### Discussion:-

Dyslipidemia represents a significant risk factor for the development of a range of cardiovascular diseases, including stroke, coronary heart disease, myocardial infarction, and vascular disease (Rached et al., 2014). Previously, dyslipidemia was regarded as a disease prevalent in economically advanced societies and the elderly. However, recent evidence suggests that these consequences are affecting individuals from all social strata, including children, who are increasingly at risk of developing cardiovascular disease due to the foods they consume (Vafa et al., 2011; Okafor, 2012).

The goal of this study was to find out how eating lipids and the atherogenic markers that go with them affected the heart health of Wistar strain rats so that the risk of getting cardiovascular disease (CVD) could be predicted. The findings showed that the lipoprotein levels (HDLc, LDLc, triglycerides, and total cholesterol) returned to the normal levels suggested by Vigneshwar et al. (2021) after the PASLoc flours were eaten. This recovery indicates that the flours provide the body with beneficial lipoproteins (Linton et al., 2000). It is known that these lipoproteins help move fat-soluble vitamins to the right parts of the body (Assoumou et al., 2022; Kouadio et al., 2017), keep skin and cell membranes safe, and make the chemicals the body needs to work properly (Meijaard et al., 2022; Kra et al., 2022). The elevated HDL cholesterol levels in the serum of PASLoc1a and PASLoc2b rats indicate that the consumption of these foods may confer a more favorable impact on the children's well-being. However, it is important to note that these values do not necessarily guarantee optimal well-being, as the atherogenic and lipid parameters are more closely correlated with fluctuations in the Castelli indices, the atherogenic coefficient, and the atherogenic plasma index.

The Castelli CRI-I and CRI-II indices serve as predictive indicators of exposure to the risk of developing cardiovascular and coronary heart disease (Lumu et al., 2023). The Castelli CRI-I indices in our study are below 3.5, which is the maximum value recommended by Olamoyegun et al. (2016) and Agu et al. (2024). Similarly, the Castelli CRI-II is also below 3.0, the maximum value recommended by Olamoyegun et al. (2016) and Agu et al. (2024). This adherence to the recommended values indicates that the consumption of the various flours did not pose an increased risk of cardiovascular disease. With regard to the atherogenic coefficient (AC), the results of our study are all below 3.0, which is the maximum value recommended by Olamoyegun et al. (2016) and Agu et al. (2024).

These findings indicate a minimal risk of atherogenic disease following the consumption of PASLoc flours. With regard to the atherogenic plasma index (AIP), the results demonstrate compliance with the recommendation of Agu et al. (2024). Indeed, the indices are found to be below 0.1. This allows for the classification of the consumed foods as having a low atherogenic index. These findings indicate a reduced likelihood of developing coronary heart disease as a result of the accumulation of LDL cholesterol and triglycerides within the arteries and veins. This would prevent the genesis of numerous anomalies, including arterial hypertension, venous thrombosis, and obesity (Huffman, 2003). These low AIP values indicate that the fats present in these foods are of exceptional quality. The low levels of the atherogenic plasma index (AIP) may also be due to the fats' quality, especially the amounts of saturated and unsaturated fatty acids in the different baby flours. The amount of these acids is linked to the different indices in a positive way.

The principal component analysis (PCA) and canonical correlation analysis (CCA) showed that the amount of fat in the food and the levels of CRI-I, CRI-II, AIP, AC, and LDL cholesterol were all positively related in rats that were fed PASLoc1b flour. This positive correlation indicates that consumption of this food could have a beneficial impact.

### **Conclusion:**

The objective of this study was to evaluate the influence of PASLoc flours, high-fat content into the diet of rats that had undergone a period of dietary restriction. The results demonstrate a gradual recovery of lipid parameters that had been previously unbalanced by dietary restriction. An analysis of the Castelli indices (CRI-I and CRI-II), the atherogenic coefficient (AC), and the atherogenic plasma index (AIP) following meal ingestion revealed no significant impact of the different meals' consumption. Collectively, the results indicate that the high fat content of PASLoc flours has no adverse effect on lipid parameters and atherogenic constants.

### **Statement on the Welfare of Animals**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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### **Conflicts of Interest**

The authors declare no conflict of interest concerning this study.

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