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Effect of consumption of cookies made with Andean crops on biochemical indicators in recently weaned Wistar rats

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Abstract: Andean crops such as quinoa, cañihua and tarwi have high nutritional value; however, their consumption has lower demand compared to traditional cereals such as wheat, rice, barley and soybean. Therefore, the aim is to evaluate the effect of its incorporation in the diet on biochemical indicators in Wistar rats. The research had a quantitative approach, descriptive type, using an experimental design and establishing 24 rats as sample size. There was a control treatment composed of wheat only, and three that contained 50% of wheat flour and the remaining quinoa, cañihua and tarwi flour. With the Andean crops, HDL levels were increased, while LDL levels tended to decrease concerning the wheat treatment; likewise, with the Andean cereals, the glucose level was maintained at optimal standards, and there was also an increase in hemoglobin and hematocrit. It concludes that the intake of products made from Andean crops has a positive effect on the body, increasing HDL levels, hemoglobin and hematocrit levels and decreasing or attenuating glucose concentrations in the blood, which is essential for proper health.
Keywords: *Andean crops, Biochemical indicators, Cholesterol, Glucose, Hemoglobin.*

1. Introduction

Andean crops have been part of the diet of indigenous populations since ancient times and, among their characteristics, are considered rustic, resistant to drought, frost and salinity, in addition, to have a great nutritional value; however, little work has been done to improve it. In addition, there are crops with great potential in the Andes to be transformed into processed food of high nutritional value, such as grains, tubers, roots, fruits, and aromatic and medical plants. It should be noted that nutrition is one of the main determinants of health and the physical-mental performance of humans, fundamental for personal and social development (Jacobsen et al., 2003).

In this same orientation, we can mention that among the main Andean grains include quinoa (*Chenopodium quinoa* Will), cañihua (*Chenopodium pallidicaule* Aellen) and tarwi (*Lupinus mutabilis* Sweet) which, although they are less in demand than traditional grains such as wheat, rice, barley and soybean, have a very high nutritional value. (Apaza, 2019). In effect, scientific studies show that quinoa is the only vegetable-based food in which are present all the essential amino acids, trace elements and vitamins. Also, it has a high capacity for edaphoclimatic adaptation as well as resistance to drought and salinity (Ramírez et al, 2017).

Similarly, cañihua presents a high nutritional contribution, evidenced by the important content of essential amino acids like lysine, tryptophan and arginine, also including iron and magnesium in its constitution, a considerable protein content, 60% of carbohydrates, about 8% of oils (Indecopi, 2018), as well as Vitamin E, B complex and gluten-free grains (National Institute of Agricultural Innovation

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(INIA), 2008). Likewise, tarwi is mentioned as an Andean grain with a high percentage of proteins such as globulin and albumin, even higher than those of other leguminous plants such as soybean, and when compared with soybean and bean, we can see a clear difference in protein content which can be increased in tarwi from 47 to 64% if the lipids and alkaloids are extracted (Tapia, 2015). Although is deficient in tryptophan, they highlight a considerable amount of sulfur amino acids and fatty acid content compared to most legumes (Chirinos-Arias, 2015).

In this context, research was conducted to highlight the nutritional importance of Andean grains and their possible incorporation into the daily diet, as well as their effects on the organism. For that purpose, laboratory rats were used as test subjects, due to their genetic similarity to humans, which were fed with the aforementioned grains. Moreover, the researchers analyzed the lipid levels as well as the growth and weight of the rodents, finding high dietary fiber contents (Porras et al., 2006) Similarly, studies have shown that wheat flour can be substituted by Andean crops flours, improving the nutritional value in terms of protein composition and bioactive components (Rosell, 2009).

In view of the above, due to the potential nutritional value of Andean crops and their impact on physiological aspects of the organism, as well as their possible agronomic development and their historical-cultural importance, the following question is stated: What nutritional impact does a diet based on products made from Andean crops generate? Therefore, the aim of the research was to evaluate the effect of incorporating quinoa, cañihua and tarwi flour in cookies on biochemical indicators using recently weaned Wistar rats as test subjects.

2. Materials and Methods

The research was developed from a quantitative approach, being descriptive level, using an experimental design with random assignment of the units. In relation to the sample size used, this was established with 24 laboratory rats of the Wistar strain, whose characteristics are shown in table 1.

Table 1.
Characteristics of the laboratory rats used for the study.

Order	**Rodentia**
Suborder	Myomorpha
Family	Muridae
Genus	Rattus
Specie	Norvegicus
Strain	Wistar
Weaning age	21 days
Sex	Male
Life expectancy	2-3, 5 years
Body weight/Age	60 grams
Standardized genetic line	Outbred stock

Source: Own elaboration.

2.1. Location of the Study Area

The study in question was carried out at the Biotherium of the Faculty of pharmaceutical, Biochemical and Biotechnological Sciences of the Catholic University of Santa Maria, and at the clinical analysis laboratory of the Faculty of Human Medicine of the National University of San Agustin, both located in the city of Arequipa, Peru (Figure 1)

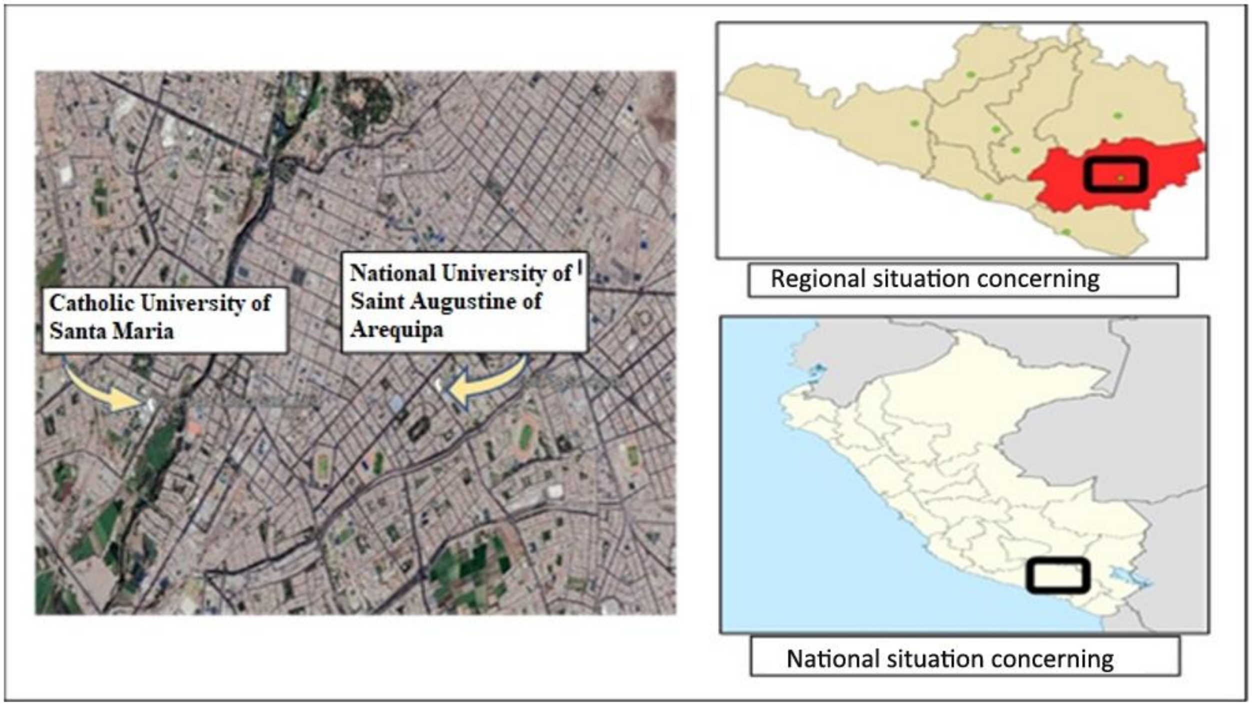

Figure 1.
Location of the study area.
Source: Modified from google earth.

2.2. Characteristics of Study

Maintenance conditions: Individual polycarbonate cages, water ad libitum, feed as experimental and control formulations.

Environmental conditions: Temperature 20 ± 5 ° C, relative humidity between 50-70 % and light/dark cycles of 12 hours light/12 hours dark.

Experimental characteristics: General model in biomedical research (Sengupta, 2013)

2.3. Proceeding

For the biological tests, 24 male Wistar rats, 21 days old at weaning, from the Vivarium of the Catholic University of Santa Maria, were randomly distributed into four study groups with six study units in each one. For the determination of the biochemical profile, a fasting blood sample was taken using venous puncture to the orbital sinus or internal ocular commissure (Fernández et al., 2007). Then we carried out the biochemical determinations of: Total cholesterol, LDL, HDL, triglyceride, glucose, hemoglobin and hematocrit which were measured using an Ohaus semi-automatic biochemical analyzer, with standard reagents, in addition to the following materials; 70% ethyl alcohol, cotton or gauze, lancets, 1- or 5-ml syringes, 23 G needles and heparinized eppendorf tube. Likewise, the duration of the treatments provided was six weeks by allocating the groups as follows: 1 control (GC) and 3 experimental (GE1, GE2 y GE3) (Figure 2).

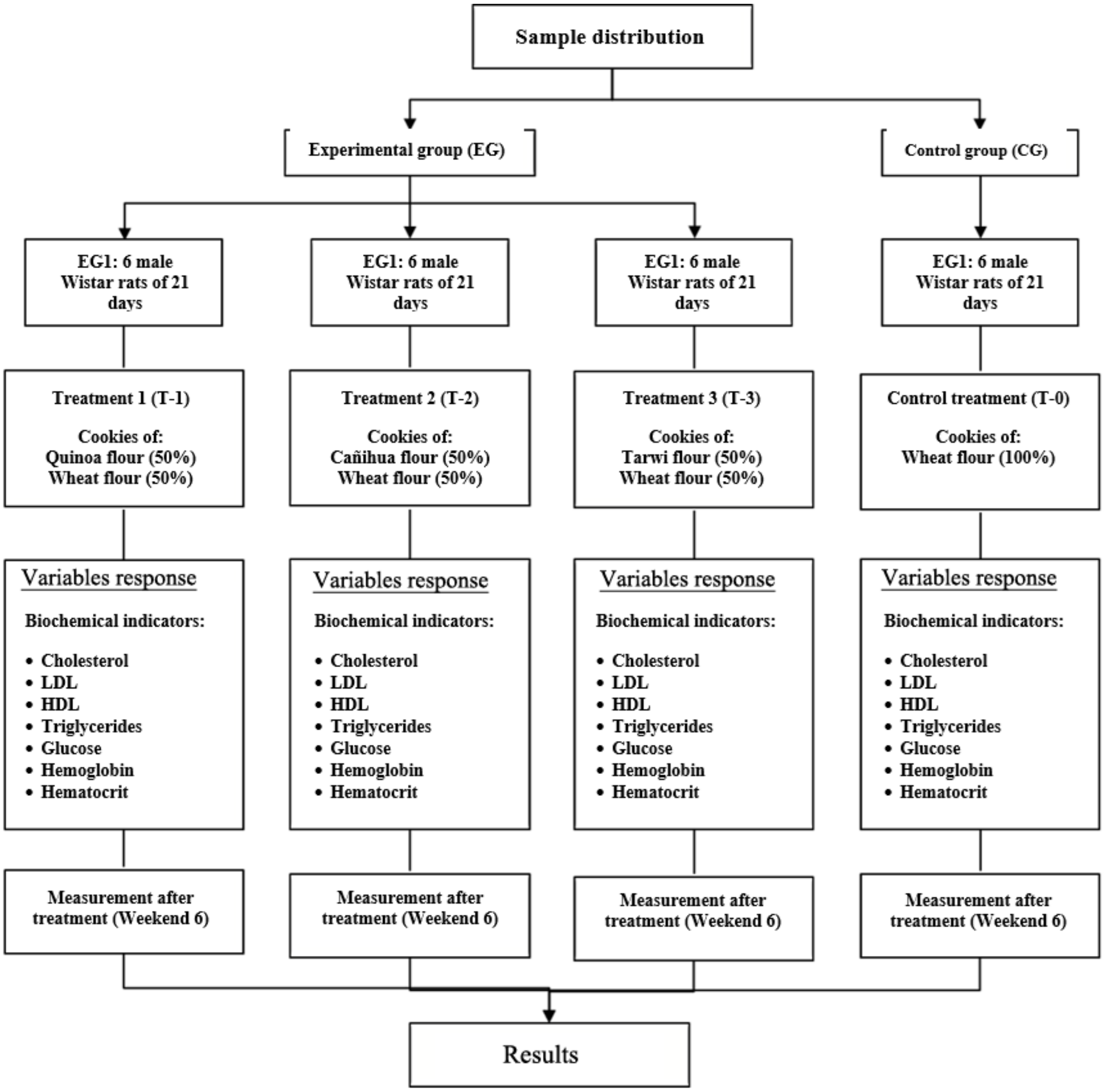

Figure 2.
Experimental design used.
Source: Own elaboration.

Food processing: Cookies were prepared with Andean grains using white quinoa of the INIA variety, cañihua of the Cupi variety, tarwi of the INIA variety and commercial wheat, with a partial substitution based on the following formulations:

- Treatment 0 (T-0): 100% wheat flour cookie.
- Treatment 1 (T-1): Cookie with 50% of quinoa flour + 50% of wheat flour.
- Treatment 2 (T-2): Cookie with 50% of cañihua flour + 50% of wheat flour.
- Treatment 3 (T-3): Cookie with 50% of tarwi flour + 50% of wheat flour.

Food (cookies) and water were administered ad libitum. The feeding method commonly used in experimental nutrition according to Fernandez et al. (2007), is to offer the animals a higher amount of diet than they can consume and the voluntary intake is determined by remaining weight. Treatment was administered based on the following considerations:

- Control group 0 (GC) = Treatment 0 (T-0).
- Experimental group 1 (GE1) = Treatment 1 (T-1).
- Experimental group 2 (GE2) = Treatment 2 (T-2).
- Experimental group 3 (GE3) = Treatment 3 (T-3).

2.4. Statistical Analysis

For the processing of the data obtained, they were first stored in Microsoft Excel spreadsheets and then develop, based on the completely random design (CRD) with six replications for each dependent variable, an ANOVA analysis with Tukey's test where the observable cases presented significant differences with $\alpha = 0{,}05$. The SPSS V.22. was used as the statistical software. ANOVA analysis is based on the linear additive model:

$$y_{ij} = \mu_{..} + \tau_i + \varepsilon_{ij}$$

Where:

y_{ij}: It is the value of the response variable.

$\mu_{..}$: Effect of the average.

τ_i: Effect of treatments.

ε_{ij}: Effect of experimental error.

The responsible variables are: *y1* = lipid profile, *y2* = Glucose and *y3* = Hemoglobin and Hematocrit.

3. Results and Discussion

3.1. Lipid Profile

On analysing the lipid profile of the test subjects, the results shown in Table 2 were obtained.

Table 2.
Lipid profile according to consumption of wheat, quinoa, cañihua and tarwi.

Treatment	N	Total cholesterol	LDL	HDL	Triglyceride
		mg/dl	mg/dl	mg/dl	%
Wheat (T-0)	6	91.50 (±8,74)	46,40[a] (±31,55)	62,97[a] (±7,79)	41.2 (±12,45)
Quinoa (T-1)	6	108.83 (±9,87)	15,96[b] (±6,38)	84,42[b] (±8,57)	50.5 (±16,28)
Cañihua (T-2)	6	106.33 (±10,27)	21,17[b] (±8,12)	79,37[b] (±13,42)	41.8 (±14,08)
Tarwi (T-3)	6	110.33 (±18,97)	16,98[b] (±12,29)	84,18[b] (±17,98)	45.8 (±7,63)
p-value		p=0,065	p=0,023	p=0,025	p=0,580

Differents letters indicates significatives differences with α = 0,05

Source: Own elaboration.

Table 2 shows that the treatment that caused the lowest total cholesterol level in Wistar rats is the control group (CG) that was administered the wheat cookies T-0 (91,50 mg/dl), the other experimental groups that consumed Tarwi cookies T-3, quinoa cookies T-1 and cañihua cookies T-2 have high values. The ANOVA analysis indicates that, despite differences between the values obtained for each treatment, these are not significantly different, since the p-value = 0.065 is greater than 0.05, therefore, the differences were not significant between the control group and experimental groups.

In relation to LDL, it is observed that the experimental group GE1, which was administered the quinoa cookie treatment T-1, presents the lowest value with 15,96 mg/dl, and in the experimental groups that were administered the tarwi cookie T-3, cañihua cookie T-2 and the control group (CG) that received the wheat cookie T-0, it is observed higher values where the CG stands out with the highest value of 46,40 mg/dl. The ANOVA indicates that there is a significant difference between the results obtained, with a p-value = 0.0231 ($p<0,05$). The Tukey test indicates (according to the letters) that there are no significant differences between the treatments but there are significant differences between the treatments and the control group.

In the case of HDL, the experimental group GE1, which received the quinoa cookie treatment T-1, had the highest value of 84,42 mg/dl, followed by the groups that received tarwi cookie T-3 and cañihua cookie T-2, which were similar. However, the CG showed the lowest value of 62,97 mg/dl, compared to the experimental groups. The ANOVA indicates that there is a significant difference between the treatments with a p-value = 0,025 which is less than 0,05. The Tukey test indicates that there is a difference between CG and experimental groups, which in turn showed no difference between them.

Regarding triglyceride values, no significant differences were observed between treatments, since the p-value of the ANOVA analysis was higher than 0,05 (0,580), indicating that the consumption of cookies with Andean grain flours had no significant effect on triglycerides, when compared with cookies made only with wheat flour.

3.2. Level of Glucose

The results obtained from the glucose levels of the test individuals after the application of the treatments are shown in Table 3.

Table 3.
Level of glucose according to consumption of wheat, quinoa, cañihua and tarwi.

Treatment	N	Glucose mg/dl
Wheat (T-0)	6	$75{,}00^{b}$ (±11,05)
Quinoa (T-1)	6	$61{,}17^{a}$ (±8,93)
Cañihua (T-2)	6	$62{,}67^{a}$ (±9,14)
Tarwi (T-3)	6	$60{,}83^{a}$ (±8,04)
p-value		p=0,048
Differents letters indicates significatives differences with α = 0,05		

Source: Own elaboration.

In this context, it is observed in Table 3 that the diet that causes the highest level of glucose in Wistar rats is the one received by the experimental group CG to which the wheat cookie T-0 with 75 mg/dl was administered. The other groups that consumed cañihua cookie T-2, quinoa cookie T-1 and tarwi cookie T-3 have similar values. The ANOVA test indicates that there is a significant difference between treatments with a p-value = 0,048 which is less than 0,05, and Tukey's test; therefore, the only treatment that differs from the others is T-0.

3.3. Determination of Hemoglobin and Hematocrit

Table 4 below shows the results obtained in the determination of hemoglobin and hematocrit of the test subjects submitted to different treatments.

Table 4.
Level of hemoglobin and hematocrit according to the consumption of wheat, quinoa, cañihua and tarwi cookies.

Treatment	N	Hemoglobin, mg/dl	Hematocrit, %
Wheat (T-0)	6	$16{,}00^{a}$ (±0,71)	$48{,}5^{a}$ (±2,2)
Quinoa (T-1)	6	$16{,}50^{a}$ (±0,84)	$49{,}8^{a}$ (±2,6)
Cañihua (T-2)	6	$15{,}33^{a}$ (±0,52)	$48{,}0^{a}$ (±1,4)
Tarwi (T-3)	6	$17{,}83^{b}$ (±1,13)	$53{,}8^{b}$ (±3,4)
p-value		p=0,002	p=0,003

Differents letters indicates significatives differences with α = 0,05
Source: Own elaboration.

As we can see in Table 4, the highest hemoglobin and hematocrit values were obtained in the experimental group GE3 which was administered the tarwi cookie treatment T-3 with 17,83 mg/dl and 53,8% respectively, on the contrary, the lowest values were obtained in the rats fed with cañihua cookies T-2. The ANOVA analysis indicates that there is a significant difference between different treatments; likewise, the Turkey test indicates that the only treatment that reported significant differences was T-3, which in turn was the one that reported the highest values.

3.4. Discussion

The data obtained in the study show that there are no differences between the treatments in total cholesterol and triglycerides, but the same is not happening for LDL and HDL, which do show significant differences. In this regard, Paśko et al. (2010) in their study, found that rats whose diet included quinoa, showed a significant reduction of LDL (57%) and an increase in HDL, in contrast to a control group. On the other hand, similar to the previous author, Hejazi (2016) reported a significant decrease in total cholesterol, and LDL triglycerides, stressing a significant increase in HDL in groups whose diet was based on quinoa and dietary fiber.

In addition, other relevant results and related to quinoa were those Farinazzi-Machado (2012) who administered this cereal, as food bars, to students, in which a considerable decrease in LDL (67,5%) and triglyceride (55,9%) was evidenced, in relation to the lipid profile. Similarly, a study of the effects of quinoa consumption on biochemical parameters and bowel pattern measurements in obese rats revealed low small intestine weight, low abdominal circumference of 0,56 cm and the presence of Peyer's plaques (lymphatic tissue in this region that constitutes the mucosa), although total cholesterol, HDL and triglyceride levels showed no significant differences (Bernuy-Osorio et al., 2018).

Based on the above, it is evident that, although the results of this research did not show significant differences in total cholesterol between quinoa treatments, in contrast to the authors and research mentioned above, there is agreement that LDL levels decreased and HDL levels increased the most. This could be explained, as indicated by FAO (2011), because, among the benefits of quinoa consumption, are the reduction of LDL, commonly called bad cholesterol, and the elevation of HDL, called good cholesterol, in response to the action of omega 3 and omega 6, which are contained in this cereal.

Regarding cañihua, the results also show a positive effect on increasing HDL and decreasing LDL; this can be compared with the reported by Porras et al. (2016) who analysed the effect of a diet based on toasted cañihua flour on the lipid profile in weaned albino rats, finding that the probability of increasing HDL is five times higher than if casein is consumed. Likewise, Mujica et al. (2006) indicate that treatment based on cañihua manages to reduce cholesterol levels from 281,2 to 232,8 mg/dl in hypercholesterolemic patients, asserting that the positive effect corresponds to linoleic, oleic and linolenic acid, which are found in high amounts in cañihua resulting beneficial for the lipid profile by promoting the increase of HDL, as well as reducing the cholesterolemia associated with LDL. On the other hand, Chirinos-Arias (2015) refers that the presence of triglycerides, total cholesterol, high LDL and HDL content in low amounts, are predisposing factors of cardiovascular diseases and, to reduce this risk, cañihua is presented as an ideal option due to its content of isoflavones or isoprenoids that are suppressors of the action of free radicals and confer antioxidant protection to reduce the lipid profile. Also, Ligarda et al. (2012), reports that cañihua has a high content of total dietary fiber that goes between 9,29 and 20,0% (soluble and insoluble fiber), whose components are beta-glucans and pentosans, promoters of blood cholesterol reduction.

In reference to tarwi, which also showed positive effects on the lipid profile, we can mention Fornasini et al. (2007) who concluded that by managing tarwi with a dose of 500 mg/kg for 30 days to laboratory rats, it is evident that, although there was perceived an increase in the triglycerides value from 45,9 to 76,55, the values of total cholesterol and HDL were significantly elevated. These results would lie in the controlling effect of the aforementioned cereal on cholesterol; this effect is linked to the high content of unsaturated fatty acids it possesses, including (FA) linoleic acid (omega 6) (Salvatierra-Pajuelo et al., 2019).

In relation to the consumption of cookies supplemented with Andean grains flour on the glucose level, the wheat-based treatment was the one that presented the highest glycemic index, in contrast to the quinoa, cañihua and tarwi treatments, which showed the lowest levels. These results are similar to those reported by Farinazzi-Machado et al. (2012), who refers to the low glycemic index of quinoa, supporting this statement in a study where it was observed that 56,7% of the male subjects showed a reduction in initial glucose (88,33 mg/dl) with respect to post-treatment (81,40 mg/dl); in turn, the authors mention that this effect would be related to antioxidants such as polyphenols, phytosterols and flavonoids. Hejazi (2016) states that there is evidence of quinoa and its content of antioxidant peptides, which have a hypoglycemic action due to its dietary fiber content, which could reduce the risk of diabetes.

In this same orientation, Pasko (2010) indicates that quinoa seeds significantly reduce the glucose level and assumed that compounds such as tocopherols and polyphenols present in quinoa seeds could be the cause of this reduction. Likewise, the results obtained by Mercado (2018) show that Andean grains consumption specifically quinoa and tarwi, present a low glycemic profile in relation to other similar grains, contributing to the prevention of diabetes.

Regarding the results in tarwi, it is appropiate to highlight the study carried out by Burgos et al. (2004), where it was established to determine the glucose level in people from 18 to 30 years old, with an administration of 200 grams, obtaining as a result that the pre-cooked tarwi without peel has a low glycemic index and, therefore, is recommended for diabetic patients and people with intense activity. Similarly, analogous studies were conducted, where a decrease in glucose was observable as a consequence of tarwi consumption; in relation to this, it is assumed that it is due to the action of chemical compounds present in this grain such as phytoestrogens, among which are isoflavones, which have antioxidant properties, (Tapia, 2015).

Regarding the influence of the consumption of Andean grains on hemoglobin and hematocrit levels in the blood, the highest values were obtained with the diet based on tarwi cookies compared to those containing wheat, quinoa and cañihua. In this context, we mention studies undertaken by Ligarda (2012), which focused on evaluating the effect of tarwi flour on hemoglobin levels, in which laboratory rats were administered an aqueous preparation of tarwi flour at a dose of 500 ml/kg for 30 days, achieving a significant increase in hemoglobin. For its part, Fornasini et al. (2019) also report improvements in blood hemoglobin values following consumption of snacks supplemented with tarwi flour.

4. Conclusions

From the research previously carried out on recently weaned Wistar rats, it can be affirmed that the Andean crops studied have functional components with beneficial properties for the organism and can be effective in reducing the levels of total cholesterol, LDL and triglycerides, and also incorporate compounds in charge of suppressing the action of free radicals, which generate cellular imbalance and, consequently, trigger various pathologies. Likewise, the presence of oleic (Omega 9), linoleic (Omega 6) and linolenic (Omega 3) acids, part of its composition, acts as a stimulating agent of HDL and cholesterol reducer at the expense of LDL, so that the lipid profile, resulting from the consumption of cookies supplemented with Andean grains, is much more beneficial and healthy.

Similarly, within the framework of the glycemic analysis, the diet based on cookies made with wheat contains a higher level of glucose compared to that which included Andean crops in its composition. Thus, a diet whose composition includes tarwi, quinoa and/or cañihua, is beneficial for maintaining a balance in the level of sugars in the body; therefore, it is presented as a very attractive alternative for the development of a diet focused on diabetic patients and those with high physical activity. Additionally, the consumption of these crops incorporates phytoestrogens to the body, such as isoflavones, with very beneficial antioxidant properties.

In this same guideline, on the levels of hemoglobin and hematocrit in the blood, a diet whose composition is associated with tarwi would result in a significant increase of the compounds described in the first line, compared to a diet that includes wheat, quinoa or cañihua, which would have an indistinct effect between them; this would be ideal to constitute a food plan that seeks to reduce the incidence of anemia in people, by including this cereal in the diet.

Finally, it is concluded that, when evaluating the effect of incorporating quinoa, cañihua and tarwi flour in cookies, on biochemical indicators in recently weaned Wistar rats, with the formulations previously made, the characteristics of the lipid profile, glucose level, hemoglobin and hematocrit in blood show optimal values, being important to elaborate nutritional regimens focused on improving the physiological conditions of people, especially those with cholesterol, diabetes and anemia problems.

Abbreviations: HDL (High-Density Lipoprotein), LDL (Low-Density Lipoproteins)

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