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Study of digestibility and effect of Spirulina on weight growth in Wistar rats

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Abstract

In order to evaluate the digestibility of spirulina, a nutritional supplement, a study was carried out in wistar rats which received spirulina as a supplement in addition to industrial pellets. A reference group receiving only the therapeutic food, the plumpy nut, was formed.

Five homogeneous batches of six rats each, three males and three females, were formed and placed in metabolism cages. Batch 1(CPn) received the therapeutic feed and distilled water, batch 2 (CG) the conventional feed (pellets) and distilled water. In addition to the pellets, batches 2, 3 and 4 received spirulina at rate of 10 mL/kg (batch 2), 50 (batch 3) and 100 mg/kg (batch 4) of body weight. The ingesta and excreta were determined over a period of 5 days from daily measurements of the feed distributed and from the collection of faeces and wasted feed.

The analysis of the results obtained indicated a harmonious weight growth in all animals with a preponderance for the batch receiving 100 mg/kg of spirulina for a Mean Daily Gain (MDG) of 1.94 g/d. The results also revealed that the digestibility of the proteins contained in spirulina is important with a protein efficiency coefficient of 2.03 ± 0.04. With regard to lipid and fiber digestibility, no significant difference was recorded between the Spirulina supplemented batches and the controls.

This study has thus demonstrated that Spirulina supplementation has very remarkable nutritional advantages with regard to its impact on nutritional parameters and the degree of digestibility of the organic matter it contains.

Keywords: Digestibility; Spirulina; Weight; Growth; Wistar rats

1. Introduction

Many developing countries suffer from malnutrition in various forms, the most obvious cases being undernutrition. According to the report on the State of Food Security and Nutrition in the World in 2018, the number of people suffering from hunger has increased to 821 million people in 2017, or one person in nine [1]. The proportions in sub-Saharan Africa are rising, with an estimated 22.7 per cent in 2016 compared to 20.8 per cent in 2015 [2]. To remedy this, international bodies such as WHO (World Health Organization) and FAO (Food and Agriculture Organization), respectively in charge of health and food policies, have recommended that researchers around the world re-examine humanity's food potential. It is in response to this call that some researchers have taken an interest in spirulina, which is a micro-alga with nutritional and therapeutic virtues [3,4].

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In view of its role in the fight against malnutrition, spirulina is one of the unconventional nutritional and therapeutic sources that is rich in nutritional information. In addition, it has been proposed in the human diet by several scientists and nutritionists thanks to its exceptional qualities, ease of cultivation, high productivity and low cost of production [5]. Although this seaweed has undeniable nutritional virtues [6], it should be verified with certain nutritional parameters.

The aim of this work is therefore to evaluate in vivo the nutritional parameters of spirulina (*Spirulina platensis*) in rats based on the study of digestibility.

2. Material and methods

2.1. Material

2.1.1. Spirulina

It consists of *Spirulina platensis*. This cyanobacterium was supplied by the Spirulina Laboratory of SAP de la Mé (south of Côte d'Ivoire) in powder form; the obtained amount was 400 grams.

2.1.2. Rats

Albino rats of the *Rattus norvegicus* species and strain Wistar, male and female weighing from 45.7 ± 5.51 to 47.8 ± 4.07 g and eight weeks old were used.

These rats come from the vivarium of the Ecole Normale Supérieure (ENS) (Abidjan, Côte d'Ivoire). They were acclimatised in metabolism cages ten days before the beginning of experiment. During this period, they received FACI® brand granules and had unlimited access to water. They were subjected daily to an ambient temperature of 28°C, 12 hours of light and 12 hours of darkness. The different experimental protocols were followed in accordance with the experimental animal protection protocols of the European Council on Legislation [7].

2.2. Methods

2.2.1. Administration of Spirulina doses and determination of blood parameters

Spirulina powder (*Spirulina platensis*) was used to prepare different doses which should be administered to rats orally. Thus, we used distilled water as a solvent in which we added spirulina powder. The preparation method has been described by Ouédraogo *et al*. [8].

The method of administration used during this study consisted of daily treatment of the animals by giving them all conventional FACI® food at a rate of 10% of their average body mass always at the same time, ie between 7 am 30 and 8:30 am and Spirulina as a food supplement for four weeks.

For this, five homogeneous groups of six (6) rats each, three (3) males and three (3) females were formed. Group 1 (control 1) received FACI® granules and distilled water at a rate of 10 mL/kg of body weight. Group 2 (control 2) received only therapeutic food plumpy nut. As for groups 3, 4 and 5 they respectively received FACI® and spirulina at the rate of 10; 50 and 100 mg/kg body weight. Food intake was evaluated daily by determining the difference between the quantity of food distributed and refusals using a precision digital scale (SF-400 and S-234 Neo Tech SA, Belgium).

2.2.2. Growth phase

To estimate the nutritional parameters we carried out a digestibility experiment over 15 days. Also, the weight gains of animals were compared to those observed during the same experiment with a reference food composed only of therapeutic food, plumpy nut.

2.2.3. Digestibility

Digestibility is a criterion that defines the degree to which organic matter is digested by an animal. It has been determined following the guidelines of European Reference Method for measuring *in vivo* digestibility established for rabbits [9]. It reflects the level of potential protein that can be used by the body. Its determination involves the identification of nutritional parameters that group together all the indices related to food intake.

$$A.D. = \frac{I - Fe}{I} \text{ or } DUC\ (MS)\ =\ (I\ -\ F) \times 100 \times I - 1$$

A.D: Apparent digestibility or DUC: Apparent digestible utilisation coefficient
I: ingested protein (same for lipids and fibers)
Fe: protein excreted from faeces (also for lipids and fibers)

Digestibility allows the evaluation of ingestion, feed and protein efficiency coefficients.

2.2.4. Measurement of ingestion

The quantities of food distributed and refusals were weighed every day. To calculate the quantities of food consumed effectively by the animals, food wastage was duly noted, the wasted food being present on the faeces collection grid placed under the cages.

The wasted food was often wetted by water and urine so it was necessary to oven dry this portion to be able to determine the amount of DM wasted. For each batch we collected the wasted food every day, accumulated it over 5 days and determined the DM level on this accumulation.

Weighing food distributed, refusals and feeds allowed us to determine the ingestion over the experimental period. The consumption index (CI) was also calculated from the values obtained.

2.2.5. Weight gain (W.G) and ingested dry matter (I.D.M)

With growing animal, weight gain is the variation of weight due to ingested food. It is determined by weighing over a period of 15 days by the following relationship:

$$\text{W.G. (g)} = \text{Final weight} - \text{Initial weight}$$

Ingested dry matter (I.D.M.) is the product of total amount of fresh ingested food by the animal and the dry matter content. It is expressed in g of dry matter according to the relationship below:

$$D.\mathrm{M.I}\left(\frac{\mathrm{g}}{\mathrm{d}}\right) = \frac{\sum \mathrm{FS \ x \ DMi} - \sum \mathrm{FR \ x \ MDr}}{\mathrm{N}}$$

D.M.I.: Dry matter ingested
DMi: Dry matter of the food served (g/d)
MSr: Dry matter of the rejected feed (g/d)
N: Number of days of experience
FS: Food weight served per day
FR: Refused food mass per day

2.2.6. Food efficiency coefficients and protein efficiency coefficient

Food efficiency coefficient reflects the efficiency with which food is assimilated. Otherwise, it is the weight gain obtained per unit of food consumed. It is calculated as follows:

$$\mathrm{FEC} = \frac{\mathrm{DWG\ (g/d)}}{\mathrm{DMI\ (g/d)}}$$

The protein efficiency ratio (PER) demonstrates the protein utilization efficiency of the ingested substance [10], and is calculated according to the following equation:

$$\mathrm{PER} = \frac{\mathrm{DWG\ (g/d)}}{\mathrm{PTI\ (g/d)}}$$

FEC: food efficiency coefficient
PER: protein efficiency ratio

2.3. Statistical analyses

The results were expressed as means followed by the standard error (M ± SEM) and proportions (%). The evolution of the weight of the rats during the growth was evaluated by one way analyzes of variances (ANOVA1). This statistical test was combined with the Bonferroni test as a post hoc test. Statistical analysis of data in this context was performed using GraphPad Prism 5.01 software (San Diego, California, USA). In addition, the obtained proportions according to reference values for each chosen period of growth of the rats on the one hand and on the other hand, the proportions of variation of the main blood parameters during the growth of the rats, were compared by the test G. This test was carried out with the Windows R version 2.0.1 computer program [11]. The significance threshold was set at a probability threshold p of less than 0.05 for the expression of results.

3. Results

3.1. Weight change

A progressive increase in weight was recorded in the rats in proportion to the dose of spirulina received. The values obtained ranged from 63.8 ± 2.53 g (TG control) to 74.3 ± 3.66 g for the rats supplemented with 50 mg/kg (Table 1). However, no significant difference (P > 0.05) between the weights of control and treated rats was recorded.

Weight gains and mean daily gains (MDG) showed a significant increase (P < 0.05) in rats given spirulina at 50 and 100 mg/kg of bw compared to the control values. The results obtained were 26.5 ± 2.49 g (50 mg/kg) and 27.2 ± 2.01 g (100 mg/kg) for weight gains and 1.89 ± 0.17 g/d (50 mg/kg) and 1.94 ± 0.15 g/d (100 mg/kg) for DMDs, respectively (Table 1).

Table 1 Changes in mean weight of young growing rats in metabolic studies

Growth Factors	**Controls**		**Controls Spirulina Doses (mg/kg)**			**P**
	CPn	**CG**	**10**	**50**	**100**	
Initial weight (g)	47.8±4.07	47.6±1.78	47.6±1.72	47.8±4.65	45.7±5.51	> 0.05
Final weight (g)	65.38±3.26	63.8±2.53	66.4±1.43	74.3±3.66	72.9±4.96	> 0.05
Weight Gain (g)	17.58±1.81	16.2±2.59	18.8±2.33	26.5±2.49*	27.2±2.01*	< 0.05
DWG (g/d)	1.26±0.13	1.16±0.24	1.33±0.21	1.89±0.17*	1.94±0.14*	< 0.05

CPn: Controls fed with plumpy nut, CG: Controls fed with granules. *: p < 0,05. ADG: Average daily gain.

3.2. Digestibility characterization

The average values of the nutritional parameters are shown in Table 2. In view of the results obtained, the rates of ingested dry matter (IDM), the consumption index (I) and all apparent digestibilities (DUC, ADL, ADP, ADF, NDF, ADF and ADL) of the experimental batches were not significantly different (P > 0.05) from those of the control batches for all doses. Furthermore, the consumption indices (CI) obtained were as follows: 0.56 ± 0.04 (controls), 0.46 ± 0.03 (10 mg/kg), 0.34 ± 0.02 (50 mg/kg) and 0.26 ± 0.02 (100 mg/kg). The highest value was recorded with the control group, while the lowest value was recorded with the rats supplemented at 100 mg/kg of bw. Compared to the control batch, the CI decreased very significantly (P < 0.001) at doses ranging from 10 to 100 mg/kg of bw.

As for the food efficiency coefficients (FEC), at the end of the experiments, values ranging from 0.20 ± 0.08 (controls) to 0.40 ± 0.03 (100 mg/kg) were recorded. The highest value was recorded with the batch supplemented at the 100 mg/kg dose and the lowest value was obtained with the control batch. The FEC obtained at 10 mg/kg dose was not significantly different from the control batch. On the other hand, at 50 and 100 mg/kg of bw doses, a highly significant difference (P < 0.001) was reported compared to the control lot.

With regard to protein values including total ingested protein (TIP), protein efficiency ratio (PER) and apparent protein digestibility (ADP), there were increases in protein values in spirulina-treated rats. Thus, the ITP assessment showed a non-significant (P > 0.05) increase at 10 and 50 mg/kg of bw and a highly significant (P < 0.001) increase at 100 mg/kg of bw compared to the control lot. The highest ITP value was recorded at 100 mg/kg of bw. (15.53 ± 0.56 g) and the lowest (8.69 ± 0.4 g) was obtained with the control lot.

PER also showed a non-significant (P > 0.05) increase at the spirulina doses of 10 and 50 mg/kg bw and a significant (P < 0.05) increase at the 100 mg/kg of bw dose compared to controls. The highest PER value (2.03 ± 0.04) was noted with the batch of rats that ingested spirulina at 100 mg/kg of bw and the lowest value (1.82 ± 0.03) with the control rats.

Finally for ADP, the increase was non-significant (P > 0.05) at 50 mg/kg of bw, significant (P < 0.05) at 10 mg/kg of bw and highly significant (P < 0.01) at the 100 mg/kg of bw dose of spirulina. The highest ADP was recorded in rats supplemented at 100 mg/kg with a value of 89.02 ± 0.1% and the lowest in controls at 81.47 ± 0.31%.

Table 2 Mean values of some nutritional parameters

Nutritional parameters	Controls		Spirulina dose (mg/kg bw)		
	CPn	CG	10	50	100
IDM (g/d)	8.4±0.12###	16.02±0.64	16.14±1.10	16.12±1	15.8±0.82
FEC	0.46±0.01###	0.20±0.08	0.23±0.04	0.31±0.04***	0.40±0,03***
I (gDM)	2.29±0.17###	8.98±0.62	8.49±0,59	8.62±0.74	8.25±0.6
DUC (%)	76.45±2.2	74.15±2.3	75.09±2.5	70.47±4.7	73.42±4.08
CI (gDM/d)	0.21±0.03###	0,56±0.04	0.46±0.03***	0.34±0.02***	0.26±0.02***
PTI (g)	9.02±0.18	8.69±0.4	9.94±0.22	12.81±0.71	15.53±0,56***
PER	1.9±0.01	1.82±0.03	1.84±0.02	1.96±0.04	2.03±0.04*
ADp (%)	78.77 ± 0.53	81.47 ± 0.31	85.18 ±0.15*	84,2 ± 0.11	89.02 ± 0.10**
ADl (%)	64.12±3.4###	46.86±2.2	44.04±2.8	45.44±3	47.29±1.9
ADf (%)	10.89±1.13	12.28±1.05	11.94±1.22	12±0.84	12.17±2
NDF (%)	34.5±1.48	36.25±2.64	35.95±3.75	36.44±3.38	35.23±2.97
ADF (%)	9.74±0.87	10.51±1.44	10.06±0.94	10±1.17	10.28±1.39
ADL (%)	0.0±0	0.01±0.01	0.02±0.01	0.0±0	0.03±0.01

: p < 0.001: Comparison between the two types of controls. *: p < 0.05; **: p < 0.01; ***: p < 0.001. IDM: Ingested dry matter, FEC: Food efficiency coefficient, I: Food intake, DUC: Apparent digestible utilisation coefficient, CI: Consumption index; TPI: Total protein ingested, PEC: Protein efficiency coefficient, ADp: Apparent protein digestibility, ADL: Apparent lipid digestibility, ADF: Apparent total fibre digestibility; CPn: Controls fed with plumpy nut, CG: Controls fed with granules.

4. Discussion

Weight values are part of indicators of the nutritional efficiency of feed [12]. All treated rats and controls groups, showed weight growth that would be due by the levels of ingested dry matter (IDM) and especially the levels of ingested protein (influenced by the quantity administered). Furthermore, the high growth of rats which received spirulina would confirm the impact and influence of the nutritional value of this algae on rats growing [1314]. Ijarotimi *et al*. reported that weight gain is influenced by the composition and quality of food ingested by rats [15].

The nutritional efficiency of food is assessed by determining the food efficiency coefficient (FEC) and protein efficiency coefficient (PEC or PER). Food efficiency coefficient (F.E.C.) reflects the efficiency with which animals assimilate the dry matter of feed consumed. An improvement of F.E.C. according to spirulina doses received was observed during this study. Moreover, F.E.C. obtained with rats supplemented with spirulina at doses of 50 and 100 mg/kg of b.w. showed a very highly significant increase with preponderance at 100 mg/kg dose compared to controls. The recorded results show that spirulina has nutritional benefits capable of influencing general well-being of pups. This nutritional efficiency is much more felt at dose of 100 mg/kg of bw. As for PEC, it reflects good quality of the proteins derived from spirulina, especially at dose of 100 mg/kg of bw. Indeed, the quality of a protein is poor if the PEF is less than 1.5 and good if it is between 1.5 and 2 [16, 17]. All treated rats had a PEF greater than 1.5, demonstrating the benefit of using spirulina, especially at dose of 100 mg/kg of bw.

The digestibility rates in different groups of rats show that more than 80% of the proteins contained in spirulina were assimilated by the rats. Indeed, according to FAO/WHO [18], an apparent digestibility (AD) greater than 70% is desired in foods desired for children of weaning age. These results are similar to the rates between 87.82 and 97.57% obtained by Kamau *et al.* [19]. The use of ingested proteins is determined by digestibility, i.e. the proportion of protein nitrogen absorbed, as well as by amino acid composition [16]. This fact explains the very good digestibility of spirulina proteins from 83 to 90% (pure casein 95.1%) [20]. Thus, spirulina does not require any cooking or special treatments to make its proteins accessible. This is a considerable advantage both from the point of view of simplicity of production and for the preservation of high-value constituents such as vitamins and polyunsaturated fatty acids.

Concerning lipids, only rats fed the therapeutic food have a significantly higher apparent digestibility compared to that of the controls TG. This can be explained by the fat content in the reference food, plumpy nut (Pn), compared to the low-fat pellets. Indeed, the high presence of lipids in a ration leads to metabolic reactions aimed at limiting food intake as suggested by Gaillard *et al*. [21].

Moreover, the measurement of apparent digestibility of different fibers presents almost identical results in all rats including the two types of controls (TG and TPn). Indeed, the digestive tract of animals is colonised by several types of microorganisms whose enzymatic activity adds to that of the body's own enzymes and influences physiological and biochemical processes [22]. In most monogastric species (pig, rat and pony), carbohydrate digestion by the intestinal microflora is only important in the terminal part of digestive tract (large intestine, cecum). The carbohydrate constituents that reach the large intestine and then subjected to bacterial digestion are essentially "dietary fiber" because they cannot be completely broken down by the digestive enzymes of these animals. Therefore, the digestibility of fiber is strongly dependent on the type and compartment of the digestive tract (small intestine, cecum, large intestine).

The results of this work do not reveal any significant difference in the digestibility of fiber types between controls and batches treated with spirulina. The main factor of variation in the digestion of fibers is first of all their nature. Indeed, the fibers characterised are insoluble fibers (NDF, ADF and ADL) theoretically not digestible by monogastric organisms except herbivores. However, they have an important role in food digestion and the protection of digestive tract. Thus, NDF or Neutral Detergent Fibre is relatively digestible because of the intestinal microbiota. This fraction causes caecal microbial activity, moderately affects food digestion and does not slow down digestive transit. It has an estimated digestibility of more than 30% in all rats. In fact, in rats, digestibility of insoluble fiber (pectin and NDF) starts in the small intestine and continues in the cecum and large intestine as revealed by Gidenne *et al*. [23], and is due to the fact that these organisms do not possess specific enzymes capable of hydrolysing fiber. It should be noted that fiber degradation is dependent on the bacterial fibrolytic activities of the cecum [24].

ADF (Acid Detergent Fiber), which is poorly degradable in all rats, slows down the digestibility of food and stimulates digestive transit. Its intake appears to be effective in reducing digestive disorders. Indeed, this fraction is mainly degraded by microbial digestion in the cecum and large intestine, which do not have any specific enzyme according to Gidenne, [25]. As for ADL (Lignin Sulphuric), it is almost indigestible in the monogastric tract because neither digestive enzymes nor microbial interactions are able to provoke its digestion. However, it is especially important in rabbits and aulacodes where it contributes to healthy digestive system. ADF and ADL fibers are inert to digestive enzymes in rats. However, their presence facilitates intestinal transit and defecation. They are essential for the proper functioning of intestinal transit. In addition, the presence of probiotic fibers in the formulations has the role of delaying gastric emptying and helping to reduce cholesterol and glycaemia levels. In addition, the presence of ADF in a ration delays digestion and the intake of NDF has a neutral effect (role of diluent) on digestion (but does not indicate a zero digestibility of NDF), as previously described for pigs [26]. Indeed, compared to ADF, the NDF residue contains in addition hemicelluloses, which are better digested by rabbits than cellulose, resulting in a lower digestibility of the ration. Similar results were obtained by Gidenne ***et al*.** [23]

5. Conclusion

Ultimately, this digestibility study revealed that rats subjected to spirulina compared to controls who received nothing, experienced good growth. In addition, evaluations of the nutritional parameters of spirulina in growing rats showed a better dry matter intake. Also, the dietary and protein efficiency coefficients of spirulina showed very good levels especially for proteins which resulted in a weight gain in spirulina fed rats superior to that of rats fed only pellets and plumpy nut. The coefficient of effectiveness is less important with spirulina at 100 mg/kg bw compared to plumpy nut.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no interest conflict detrimental to the results of this research and its publication.

Statement of ethical approval

All experiments in this study were conducted in accordance with the international standards of animal welfare as recommended by the European Union on animal care (CE Council 86/609).

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