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Reproductive Characteristics of Male Rabbits (*Oryctolagus cuniculus*) Fed on Spirulina (*Spirulina platensis*) Hydro-Ethanolic Extract

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Research Article

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Abstract

Spirulina, a filamentous cyanobacterium, possesses diverse biological activities and nutritional significance due to high concentration of natural nutrients. This work aimed to evaluate the effects of hydro-ethanolic extract (HEE) of *Spirulina platensis* on organ weights, sperm characteristics, reproductive hormones and fertility index in male rabbits. 30 male rabbits were randomly distributed into 5 groups (6 rabbits/group). For 120 days, group 1 animals (control) were orally given distilled water, those of group 2 were treated with 100 mg/kg bw of vitamin E, while groups 3, 4 and 5 received respectively 100, 200 and 400 mg/kg bw of spirulina extract. The results showed that the relative weights of the vas deferens and sexual glands increased significantly ($p<0.05$) in the HEE groups compared to the control, whereas no significant ($p>0.05$) difference was noticed in the weights of testes and epididymis, sperm mobility and membrane integrity. Sperm count was significantly ($p<0.05$) higher in animals given 400 mg/kg bw of HEE of spirulina with reference to all other treatments. The sperm morphological anomalies declined significantly ($p<0.05$) in HEE-treated rabbits compared to distilled water and vitamin E-given animals. The serum concentration of FSH decreased significantly ($p<0.05$) in rabbits receiving 400 mg/kg bw of HEE of spirulina compared to those of other groups. Meanwhile, that of LH did not show any significant ($p>0.05$) difference among treatments. The serum content in testosterone increased significantly ($p<0.05$) in spirulina extract-treated and vitamin E groups with reference to the control group. The litter size and the sex ratio did not present any significant ($p>0.05$) difference among treatments. The kid weight at birth and the litter size at weaning increased significantly ($p<0.05$) in spirulina extract-treated and vitamin E groups with the reference

to the control group. The viability rates at weaning were significantly ($p<0.05$) higher in spirulina extract-treated groups referring to the vitamin E and distilled water groups. In conclusion, the administration of hydro-ethanolic extract of spirulina enhanced the sexual organ weights, sperm characteristics, reproductive hormone levels and performances in male rabbit.

Keywords: Reproductive Characteristics; Hydro-Ethanolic Extract; Male Rabbit; *Spirulina platensis*

Introduction

Male reproduction is a multifaceted process very important in animal breeding [1]. In fact, it contributes not only in the perpetuation of animal species, but also in the improvement of animal productivity. In mammals, the reproductive ability of an animal is closely associated to the production of semen containing a high proportion of normal spermatozoa. However, many factors like stress (heat, oxidative, nutritional…), aging, and drugs could act on the production and/or transport of semen and reproductive hormones with consequences on the fertility [2-4]. To neutralize the effects of these factors, breeders tend to intensively use antibiotics so as to improve animal performances. Nonetheless, these products lead not only to the development of antimicrobial resistance, but have also been suspected to be mutagenic, carcinogenic, hormonal pertubators, haematotoxic and even reprotoxic [5]. So, several countries regulated and even banned the use of antibiotics as growth promoters in farm animals all over the world [6]. Thus, much attention has been focused in finding plant extracts, since they are cheaper, biodegradable and safer products than antibiotics. Among much used natural substances is *Spirulina platensis*.

Spirulina is considered as an important nutrient with high nutritive value notably high level of proteins (60-70%), carbohydrates (15-25%) and lipids (5.6-11%) [7-9]. In addition, spirulina is a very good source of vitamins, essential amino acids, minerals, essential fatty acids [10]. Moreover, it possesses interesting antioxidative potentials [8,11-13]. Besides, studies have shown its role in the prevention of pathologies like cancer, cardiovascular diseases and premature aging [14,15]. Also, this pharmaceutical plant has been shown to exhibit anti-viral, antibacterial, antifungal, anti-parasites activities [16,17] and are great growth promoters [18-20]. Furthermore, Rudic, et al. [21] and Kistanova, et al. [22] reported that the administration of spirulina extract to bull and boar led to an increase in spermatozoa concentration, mobility and viability. Yet, the effects of spirulina on male reproduction remain scarce, therefore, this study aimed to determine the effects of hydro-ethanolic extract of spirulina on sperm characteristics and reproductive performances in male rabbits.

Material and Methods

Study Area

The study was conducted from February to April 2018 at the Teaching and Research Farm of the University of Dschang, located in the Sudano-Guinean zone (5°36' - 5°44' North Latitude and 9°85' - 10°06' East Longitude; altitude 1500 m). The average temperature is 20°C and the relative humidity is generally greater than 60%. The average annual rainfall is 2000 mm.

Plant Material and Preparation of Hydroethanolic Extract of *Spirulina platensis*

Six kilograms (6 kg) of dried granules of spirulina collected from Lake Chad were ground. The obtained powder was macerated in 30 liters of hydro-ethanol at ambient temperature for 72 hours. The mixture was then filtered with the help of the filter paper Whatman number 3. The filtrate was evaporated in a rotary evaporator (60°C) under reduced pressure, and then went through freeze-drying so as to have a black paste.

Animal Material

A total of 30 New zealand adult male rabbits, aged 4.5-5 months, weighing 2-2.5 kg reared at the Teaching and Research Farm of the University of Dschang were used. Throughout the experimental period, compounded feed and water were provided *ad libitum* to animals in adapted equipment.

Experimental Design

At the beginning of the experiment, animals were weighed and then assigned randomly into 5 groups of 6 rabbits each, comparable in terms of body weight and age. For 120 days, animals of group 1 (control) daily received orally 1 ml of distilled water per kg of body weight (bw),

those of group 2 were given 100 mg/kg bw of vitamin E, while the three other groups (3, 4 and 5) were respectively treated with 100, 200 and 400 mg/kg bw of hydro-ethanolic extract of spirulina. The animal body weight was recorded weekly and the doses of solutions adjusted accordingly.

Data Collection

Organ Weights and Sperm Characteristics

At the end of treatment period, rabbits were sacrificed by overdose of ether vapour. The abdominal cavity and inguinal sac were opened, and organs like testes, epididymis, vas deferens and sexual accessory glands were carefully removed and weighed separately using a scale of 160 g and 10^{-3} precision. The relative organ weight was calculated according to the following formula:

$$\text{Relative organ weight } (\%) = \frac{\text{Organ weight } (g)}{\text{Live body weight } (g)} \times 100$$

The cauda epididymides of each rabbit were isolated, weighed and minced in a petri dish containing 10 ml of 0.9% NaCl previously incubated in a water bath at 37°C. Sperm mobility was assessed by direct examination of 20 µl of this solution at 40X magnification and the mobility score was attributed according to Baril, et al. [23], using a scale from 0 to 5. The sperm count was done using the Thoma haemocytometer, while sperm morphological anomalies (small and big heads, tails winding) and the plasma membrane integrity were evaluated using an eosin-nigrosin solution and the hypo-osmotic test respectively.

Reproductive Hormones

Before slaughtering, blood samples were collected from the jugular vein in non-heparinized tubes and then centrifuged at 3000 rpm for 15 min. The serum was stored at -20 ˚C for measurement of testosterone, FSH and LH concentrations, according to the instructions of commercial kits Omega Diagnostics (Scotland, England).

Reproductive Performances

At birth and weaning (4 weeks), kids were counted and weight to obtain the litter size, the number of living kids (the viability) and the weight of the young. The sex ratio was obtained by expressing the number of young males over that of young females.

Statistical Analysis

Data were submitted to one-way analysis of variance. When differences were significant between means, they were separated using the Duncan test. The limit of significance was 5%. The analyses were performed using SPSS 20.0.

Results

Sexual Organs Weight

The effects of hydro-ethanolic extract of spirulina on sexual organs weight in rabbit are summarized in table 1. The weight of sexual accessory glands increased significantly ($p<0.05$) in rabbits receiving 400 mg/kg bw of hydro-ethanolic extract of spirulina compared to control, vitamin E and other extract-treated groups.

The weight of vas deferens augmented in animals submitted to 100 or 200 mg/kg bw of extract, meanwhile, the difference was significant ($p<0.05$) only when compared to those treated with the highest dose of extract (400 mg/kg bw). No significant ($p>0.05$) difference was noticed among treatments for the weights of testes and epididymis.

Sexual organs weight (g/100 g of bw)	Controls		Doses of spirulina extract mg/kg bw)			p
	DW (n=6)	Vit E100 (n=6)	HEE100 (n=6)	HEE200 (n=6)	HEE400 (n=6)	
Testes	0.21±0.006	0.201±0.007	0.202±0.015	0.207±0.007	0.206±0.009	0.75
Epididymides	0.034±0.003	0.034±0.002	0.033±0.002	0.034±0.002	0.032±0.004	0.85
Vas deferens	$0.043±0.004^{ab}$	$0.042±0.004^{ab}$	$0.045±0.004^{a}$	$0.045±0.005^{a}$	$0.037±0.003^{b}$	0.03
Sexual glands	$0.103±0.002^{b}$	$0.103±0.005^{b}$	$0.103±0.005^{b}$	$0.101±0.008^{b}$	$0.112±0.005^{a}$	0.04

a, b: on the same row, values affected with the same letter are not significantly different ($p>0.05$). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: hydro-ethanolic extract of spirulina at doses 100, 200 and 400 mg/kg body weight.

Table 1: Effects of hydro-ethanolic extract of spirulina on sexual organs weight in rabbit.

Sperm Characteristics

The sperm mobility and membrane integrity (Table 2) generally increased in spirulina extract-treated groups than in control groups, but no significant ($p>0.05$) difference was observed among treatments. The administration of 400 mg/kg bw of hydro-ethanolic extract of spirulina led to a significant ($p<0.05$) increase in sperm count with reference to other treatments. The sperm morphological anomalies declined significantly ($p<0.05$) in spirulina extract-treated rabbits compared to distilled water and vitamin E-given animals.

Epididymis sperm characteristics	Controls		Doses of spirulina extract (mg/kg bw)			p
	DW (n=6)	Vit E100 (n=6)	HEE100 (n=6)	HEE200 (n=6)	HEE400 (n=6)	
Individual mobility (%)	82.00±8.37	80.00±7.07	82,00±8,37	84,00±5,48	86.00±5.48	0.72
Conc/cauda epididymis ($x10^8$)	$3.10±0.49^{c}$	$4.25±0.48^{ab}$	$4.00±0.73^{ab}$	$3.60±0.72^{bc}$	$4.70±0.21^{a}$	0
Conc/g of cauda epididymis ($x10^8$)	$5.97±1.13^{c}$	$8.04±1,43^{b}$	$7.75±1.41^{bc}$	$7.36±1.33^{bc}$	$11.05±1.16^{a}$	0
Sperm membrane integrity (%)	74.40±8.85	71,40±7.98	70.20±5.93	75.40±8.35	81.80±11.58	0.28
Big and small head spermatozoa (%)	$1.00±0.00^{a}$	0.90±0.22a	$0.60±0.22^{b}$	$0.30±0.12^{c}$	$0.20±0.12^{c}$	0
Coiled tail spermatozoa (%)	$3.40±0.42^{a}$	$2.70±0.27^{b}$	$1.80±0.45^{c}$	$1.00±0.50^{d}$	$2.50±0.35^{b}$	0
Total anomalies (%)	$4.40±0.42^{a}$	$3.60±0.22^{b}$	$2.40±0.65^{c}$	$1.30±0.49^{d}$	$2.70±0.34^{c}$	0

a, b, c, d: on the same row, values affected with the same letter are not significantly different ($p>0.05$). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: hydro-ethanolic extract of spirulina at doses 100, 200 and 400 mg/kg body weight. Conc: sperm concentration (sperm count).

Table 2: Effects of hydro-ethanolic extract of spirulina on epididymis sperm characteristics in rabbit.

Reproductive Hormones

Table 3 presents the effects of hydro-ethanolic extract of spirulina on some reproductive hormones in rabbit. The serum concentration of FSH decreased significantly ($p<0.05$) in rabbits receiving 400 mg/kg bw of hydro-ethanolic extract of spirulina compared to those of other groups. That of LH did not show any significant ($p>0.05$) difference among treatments. Whereas, serum concentration of testosterone increased significantly ($p>0.05$) in spirulina extract-treated and vitamin E groups with reference to the control group.

Reproductive hormones	Controls		Doses of spirulina extract (mg/kg bw)			p
	DW (n=6)	Vit E200 (n=6)	HEE100 (n=6)	HEE200 (n=6)	HEE400 (n=6)	
FSH (mUI/ml)	$95.60±3.65^{a}$	$94.80±3.11^{a}$	$94.00±6.52^{a}$	$94.80±5.40^{a}$	$77.80±6.06^{b}$	0
LH (mUI/ml)	19.53±1.18	19.96±1.58	16.36±2.74	19.79±4.52	16.23±3.23	0.11
Testosterone (ng/ml)	$35.04±1.92^{b}$	$37.24±1.38^{a}$	$36.58±0.74^{a}$	$36.61±0.30^{a}$	$36.87±0.11^{a}$	0.04

a, b: on the same row, values affected with the same letter are not significantly different ($p>0.05$). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: hydro-ethanolic extract of spirulina at doses 100, 200 and 400 mg/kg body weight.

Table 3: Effects of hydro-ethanolic extract of spirulina on some reproductive hormones in rabbit.

Reproductive Performances and Viability Rate

The litter size at birth and the sex ratio males/females (Table 4) did not reveal any significant ($p>0.05$) difference among treatments. Meanwhile, the number of males was almost equal to that of females in different groups, apart from the group receiving the highest dose of extract, where the number of males was more than 2 times that of females.

The kid weight at birth and the litter size at weaning augmented significantly ($p>0.05$) in spirulina extract-

treated and vitamin E groups compared to the control group. All kids were viable at birth in all treatments. But at weaning, the viability rates were significantly ($p>0.05$) higher in spirulina extract-treated groups referring to the vitamin E and distilled water groups.

Parameters	Controls		Doses of spirulina extract (mg/kg bw)			p
	DW (n=6)	Vit E200 (n=6)	HEE100 (n=6)	HEE200 (n=6)	HEE400 (n=6)	
Reproductive characteristics						
Litter size at birth	7.20±0.84	8.00±2.00	6.40±1.52	7.00±1.00	6.80±1.92	0.57
Litter size at weaning	3.80±0.84^{b}	5.80±1.30^{a}	6.00±1.58^{a}	6.20±1.10a	5.80±1.64^{a}	0.04
Weight at birth (g)	50.08±7.53^{c}	63.96±7.82^{ab}	67.33±7.60^{a}	57.73±3.69^{bc}	59.98±8.60^{ab}	0.01
Sex ratio ♂/♀	1.00±0.52	1.18±0.45	1.10±0.74	0.98±0.49	2.60±1.04	0.2
Viability rate (%)						
At birth	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1
At weaning	52.50±7.53^{c}	73.17±5.55^{b}	93.50±8.52^{a}	89.64±16.39^{a}	86.23±10.32^{a}	0

a, b: on the same row, values affected with the same letter are not significantly different ($p>0.05$). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: hydro-ethanolic extract of spirulina at doses 100, 200 and 400 mg/kg body weight.

Table 4: Effects of hydro-ethanolic extract of spirulina on some reproductive performances and viability rate in rabbit.

Discussion

Spirulina is a freshwater microalga traditionally used by some populations for its food properties. It is characterized by its high digestibility and high protein content (70%) [24]. It also contains carbohydrates (15 to 25%), lipids (5.6 to 11%), vitamins, β-carotene, oligo-elements and minerals [8,9,25]. These molecules possess pharmacological activities that can improve the characteristics of reproduction in animals.

In the current study, the sexual organs weight (testes, epididymides, vas deferens and accessory glands) increased in rabbits receiving hydro-ethanolic extract of spirulina compared to control. The increased weight of reproductive organs hydro-ethanolic extract-treated animals could be due to the androgenic activity of bioactive molecules such as flavonoids and saponins contained in spirulina; since the development and functioning of reproductive organs is under androgenic control [26]. On the other hand, the increase in reproductive organs could be the result of spirulina molecules with antioxidant properties. These molecules protect cells from reactive oxygen species attack and consequently increase the thickness of cell membrane, thereafter increase the organ weights. This results are in agreement with those of Tchoffo, et al. [27] which revealed that the oral administration of ginger essential oil to animals increase their reproductive organ weights. The author linked these effects to the protection of animal cells by ginger bioactive molecules riche in antioxidant properties.

Sperm characteristics such as count, mobility and morphology are key indices of male fertility, as these are the prime markers in testicular spermatogenesis and epididymal maturation [28]. The oral administration of hydro-ethanolic extract of *Spirulina platensis* in male rabbits increases the sperm count, the mobility, the plasma membrane integrity and decrease the percentage of abnormal spermatozoa. This effect might be explained by the increase in testosterone level on one hand and the antioxidant effects of the hydro-ethanolic extract of *Spirulina platensis* on the other hand. As a matter of fact, antioxidant compounds might protect spermatozoa against free radical attacks and enhance sperm characteristics [29].

With reference to the control, the serum content in testosterone increased in animals treated with hydro-ethanological extracts of spirulina. Conversely, the levels of LH and FSH decreased. Testosterone, a major testicular androgen, is synthesized in Leydig cells that depend directly on luteotrophic hormone (LH) stimulation. It stimulates muscle development, sexual activity and inhibits the secretion of LH by negative feedback [30]. The effect of spirulina hydro-ethanolic extracts on these hormones suggests a possible androgenic and estrogenic action. Bioactive molecules of the hydro-ethanolic extracts of spirulina would have increased the serum levels of androgens and estrogens, which acted negatively on the hypothalamic-pituitary axis leading to underproduction of LH and FSH.

Results of this study revealed the increase of litter size and viability rate at weaning. These results corroborate those of Azafack, [31,] in rabbit does treated with 10, 20 and 30 mg/kg bw of aqueous extract of guava leaves. This can be explaining by the stimulation and transfer of maternal immunity to the pups via the placenta and the colostrum. Besides, the immunological properties of spirulina might have reduced the mortality rate, thereby increasing litter size at weaning. Additionally, the bodies of young rabbits at birth are very rich in lipids which render them susceptible to free radicals. The chemical screening of the hydro-ethanolic extract of spirulina revealed the presence of polyphenols and phycocyanin which have antioxidant activity [32,13], hence protect against free radicals. The increasing of the weight at birth could be attributed to the better availability and mobilization of nutrients for development during gestation.

Conclusion

The hydro-ethanolic extract of spirulina at doses of 200 and 400 mg/kg bw improve the sperm characteristics in rabbit. This effect subsequently ameliorated the male fertility traits and reproductive performance.

Significance Statement

This study showed the positive effect of spirulina on rabbit reproductive characteristics. Due to their bioactive molecules, *S. plantesis* can be used in animal production as feed additive to reduce the effects of endogenous and exogenous factors in favor of growth and reproductive performance.

Ethical Issue

Experimental protocol used in the present study was in accordance with recommendations of institutional guidelines for the care and use of laboratory animals. Rabbits were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Competing Interests

The authors certify that no competing interests exist.

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