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Valorization ESsay of *Arthrospira platensis* in Alimentation of Broilers

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Abstract: This experience contributes to the knowledge of the influence of an algae species of Spirulina (*Arthrospira platensis*) about the health and quality of the meat in chicken. 160 male broilers of Hubbard-ISA strainat 11 day-old are divided into 4 batches of 40 subjects which each one were nourished during 35 days by diets added by Spirulina produced locally by a firm of culture. In the first the *Spirulina* is ground and added in the diet at various rates 0, 1, 3 and 5 %, respectively. Measurements during the expérimentation, related to a growth performances and a carcasses parameters as well as the quality of the meats of the animals. The rates of incorporation of 1 and 3% of *Spirulina* in the diets improved significantly a growth performances (body weight, gain of weight and consumption index) and a carcasses parameters (weight carcasses, viscerated carcasses weight and carcass outputs) of broilers chicken compared to control group. These rations supplemented of *Spirulina* developed nevertheless the abdominal fat and raised more the weight of the heart as well as liver of animals. The advantageous effect of the *Spirulina* on the health of the animal during the experimental period appeared particulary by remarkable decreases of the glycemia and appreciable rises in the plasmatic HDL-c levels. The alimentation of chickens supplemented with *Spirulina* also induced an enrichment in polyinsaturated fatty acids of the meat comparatively to standard diet. The acid arachidonic and the α-linoleic acid found with significant proportions undoubtedly play an important role in the value health of meat. In addition, the various pigments contained in the *Spirulina* conferred to chickens meat after cooking a characteristic colour, very appreciated by the panelists and similar to that of farm poultry.

Key words: *Spirulina* · Broilers Chicken · Incorporation · Performances · Plasmatic Analyses · Meat · Fatty Acids

INTRODUCTION

The *Spirulina* is regarded as a food energetic, since it contains high percentages of glucides (14 to 24%) and in lipids (6 to 8%), but also constructor thanks to his content of proteins (60 to 70%) and protective because, it is rich in vitamins, trace elements, salt, pigments and various bioactive elements not yet identified at this days [1, 2]. Thus, the *Spirulina* food is very concentrated into principal nutriments that the majority of the nutritionists qualify like the richest food of our planet. However, it must be used with prudence. It is regarded as a food complement rather than a basic food [3].

The *Spirulina* belongs neither to vegetables nor animals micro-organisms; it is classified among the cyanobacteria [4, 5]. It is an oldest form of "photosynthetic" life which appeared on the ground since approximately three billion and half years [6]. It belongs to

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the Monera reign, the group or under group of Procaryotes, the class of Cyanophyceae, the order of Oscillatoriales, the family of Oscillatoriaceae and comprises two genera of which *Spirulina* and the *Arthrospira* [7]. In practice, it is well known that the *Spirulina* name corresponds to a species of cyanobacteria always belonging to *Arthrospira* genus and in generally rolled up in spirals from where its commercial name [8]. This microscopic blue alga develops well in the tropical and subtropical areas [9-11].

In Algeria the *Arthrospira* species of which *Arthrospira platensis* (known as the most answered) grow naturally especially in Tamanrasset in the South of the country [12]. Nowadays, the *Spirulina* draws much the attention of the scientists. Many studies showed positive effects of its consumption on human and animal health: reinforce the immune system, struggle against proteinic malnutrition as well as obesity, treat the nutritional anaemia, the xerophthalmia and the endemic goitre, improve the growth and fertility… etc. [13-18]. it seems like an alga of the hope which can be used especially as remedy for certain diseases related to malnutrition and which touch in particular the poor countries [19].

As for the world production of the *Spirulina*, it regularly increased from 1400 Tons in 1995 to more than 4000 Tons in 2008 [20].

It is well established that the nature of the food lipids constitutes the principal factor able to influence the nutritional quality of the meat of the broiler chickens. Indeed, the animal fats (tallow and lard) enrich the lipidic deposits of animal in C16:0 and C18:0; while the vegetable oils rich in poly unsaturated fatty acids (colza, soya, flax) increase the proportions of poly unsaturated fatty acids with 18 atoms of carbon. In addition, the copra and palm oils increase the proportions of fatty acids with short and saturated chain. Also, marine oils concentrate in the meat of chicken a significant quantities in fatty acids with long and poly unsaturated chains such as C22:5 and C22:6 of the series n-3 [21-23].

Although several authors such as Santillan [24], Challem [25] and Earthrise [26] reported a values in total lipids generally variable from 6 to 8% of the dry weight of the *Spirulina*, other studies revealed a higher concentration in this alga being able to reach 13% [1, 27, 28]. The *Spirulina* is considered among the best known sources of Gamma-Linoleic Acid (GLA) as well as milk and some rare vegetable oils such as (oil of onager, borage, pip of blackcurrant and hemp) [9, 29, 30]. This fatty acid (GLA) constitutes from 10 to 20% of the fatty acids at *Siprulina maximum* and up to 40% at *Spirulina platensis*. Its importance is to be underlined because it is a precursor of chemical mediators (prostaglandins, leukotriens and tromboxans) of the inflammatory and immunizing reactions [3]. Other fatty acids are also present in the *Spirulina* such as the essential linoleic acid (C18:2 ω -6) which represents from 13 to 40% of the fatty acids, the palmitic acid (C16:0) which is found with variable proportions from 25% at *Spirulina platensis* to 63% at *Siprulina maximum*, as well as the low levels of palmitoleic acid (C16:1, ω-6), of stearic acid (C18:0) and of oleic acid (C18:1, ω -6) [31]. In addition, some sulfolipids constitutive of the Spirulina such as the sulfoquinovosyl diglycérides, revealed a protective activity against several viral infections. Also, the sulfoquinovosyl diacylglycérol lipidic compound showed its capacity in vivo to inhibit the hiv-1 and hiv-2 transcriptase [32, 33].

This study contributes to the knowledge of the influence of the *Arthrospira platensis* produced locally on the growth performances, the carcasses characteristics, as well as the health and the nutritional quality of the meat in male Hubbard-ISA broiler chickens.

MATERIALS AND METHODS

Animals and Experimental Diets: The specie of Spirulina (*Arthrospira platensis*) used in this study has been provided by an enterprise of production situated in Sidi Fellague township, located in Mostaganem town - Algeria. Once delivered in twig form of 0.5 cm length on 0.5 mm in diameter, the microalgae of very marked greenish color underwent a meticulous crushing to powder via a manual crusher (Pestle- Mortar) and in finally incorporated in a homogeneous way at various rates namely 0, 1, 3 and 5%, respectively in growth diet worked out by a Unit of Cattle Food (UCF) located in Mostaganem, Algeria. The Ingredients and nutrient composition of the experimental diets are shown in Table 1.

The experimentation was carried out in a room with 400 m2 of surface, carefully cleaned and conceived to receive four hundred Hubbard-ISA broilers All chicks were alimented from the 1st to 10th days of age on a standard growth diet (2.802 kcal/kg, 21.53 % protein) and were allowed free access to water and food. At the 11^{th} day, 160 male chickens having an identical body weight (303.34±2.22 g) were chosen and divided into four batches (04 batches) of 40 subjects each one. The birds of each batch were nourished then during 35 days of the life cycle of the animals (4 weeks of the growth phase and 1 week of

Table 1: Composition of experimental diets

Composition of diets	0% *Spirulina*	1% *Spirulina*	3% *Spirulina*	5% *Spirulina*
Ingredients (%)				
Spirulina	0	1	3	5
Corn	60, 5	59, 895	58, 685	57, 475
Wheat Bran	8	7, 92	7, 76	7, 6
Soya bean meal	28, 5	28, 215	27, 645	27, 075
Calcium	1	0, 99	0, 97	0, 95
Phosphorus	1	0, 99	0, 97	0, 95
Vit-min premix	1	0, 99	0, 97	0, 95
Calculated composition				
ME (Kcal/kg)	2.802	2.865	2.991	3.117
* Methionine (%)	0.32	0.34	0.39	0.43
* Lysine (%)	1.13	1.17	1.24	1.31
* Cysteine (%)	0.45	0.45	0.46	0.47
Analysed composition (%)				
Crud protein (%)	21.53	22	22.98	23.95
Lipids %	3.53	3.54	3.58	3.61
Calcium (%)	0.95	0.95	0.95	0.95
Phosphorus (%)	0.75	0.75	0.75	0.75
Ash (%)	5.60	5.65	5.75	5.83
FA analysis (% of the identified FA)				
C14:0	0.10	0.10	0.09	0.09
C16: 0	13.15	13.66	14.65	15.58
C18: 0	2.55	2.53	2.50	2.47
C20:0	0.32	0.32	0.31	0.31
C14:1	0.13	0.13	0.12	0.12
C16: 1 (n 7)	0.12	0.21	0.39	0.56
C18: 1 (n 6)	46.36	45.70	44.44	43.26
C20:1 (n-9)	0.46	0.46	0.45	0.45
C18: 2 (n 6)	33.96	33.73	33.30	32.90
C18: 3 (n 6)	0	0.020	0.05	0.09
C18: 3 (n 3)	2.84	3.09	3.56	4.02
PUFA	36.80	36.84	36.93	37.02
MUFA	47.07	46.51	45.43	44.40
SFA	16.12	16.62	17.57	18.48

Vit-min premix (provided in mg per kg of diet): vitamin E= 6, vitamin K3= 0.80, vitamin B1= 1, vitamin B2= 3, Pantothenate of Ca= 6, vitamin B6= 1.5, vitamin B12= 0.006, folic acid= 0.2, nicotinic acid= 12, copper= 5, cobalt= 0.65, manganese= 65, zinc= 65, selenium= 0.25, iron= 50; iode= 0.8, magnesium= 100; EM: Metabolisable energy, determined according to the formula of Sibald (1980) [34]; FA: Fatty Acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; *:The contents of principal amino acids are given while referring in the tables of food composition of Blum (1984) [35]

the Finishing phase) with the control diet and the test diets added with *Spirulina* at various rates. During the two experimental periods (Growth and Finish), the animals of batch 1 were nourished with the growth diet without *Spirulina* (Pilot), the chickens of the second batch (Batch 2) received the standard ration added with 1% of *Sirulina*, those of Batch 3 were nourished with the diet added at 3% of *Spirulina* and finally, the subjects of the fourth batch (Batch 4) were fed with the diet supplemented with 5% of *Sirulina*.

Measurements: The body weights of the birds, feed consumption and the levels of mortalities for each experimental batch were recorded on days 10, 17, 24, 31, 38 and 45 of age. On day 45, fifteen (15) chickens of each batch (taken randomly) were selected and sacrificed after 24 hours of fasting in a local commercial slaughter-house. After the plucking, the takeoff of head and legs and the evisceration of the animals, several ponderal measurements were established such as carcasses weight, heart weight, liver weight and abdominal adipose tissue weight. Samples of 100 g from the chickens breast fillets (*Pectoralis major*) were obtained. Meat samples from 10 birds per batch were placed in plastic bags and frozen at -20°C until analysis. Also, an organoleptic test was established on 15 chicken carcasses nourished with the control diet and the diet added with 5% of *Spirulina*. The carcasses in this study were whitewashed slightly with butter without addition of any other condiment and were cooked in a furnace at 150°C. Thus, a tasting cards was drawn up and distributed individually to 20 panelists in order to appreciate certain sensory criteria of cooked meats such: Bitterness, flavour, colour, juiciness and tenderness. Blood samples were likewise taken from 5 subjects of each experimental batch during the growth phase, at the 17, 31 and 38 days. The blood samples recovered periodically from left wing vein of birds were deposited in heparin tubes and centrifuged at 8000 turns/minute during 10 minutes. The recuperated plasmas after centrifugation were orientated for biochemical analyses.

Analyses

Analyses of the Diets: The chemical composition of diets was measured in accordance with the recommendations of AFNOR [36]. The dry matter was determined after a drying of samples at 105°C during 24 hours until constant mass (NF V03-903). The dosage of proteins was established by Kjeldahl's method (protein Concentration = N X 6.25) [37] which consists to transform organic nitrogen of diets into mineral nitrogen [$(NH3)_2$ SO4] under the action of the concentrated sulphuric acid after heating in the presence of a suitable catalyst. The nitrogen of ammonium sulphate was moved under the action of a soda; the formed NH3 was then displaced by distillation and collected in an aqueous solution of boric acid. The ammoniacal boric solution was finally titrated by a hydrochloric solution. Cellulosic matters constitute the organic residue obtained after two successive hydrolyses in an acid (H2SO4 = 0.255 N) and an alkaline solutions (NaOH = 0.313 N) (NF Vo3-040). The fat content of the samples was extracted with hexane in Soxhlet apparatus. After each extraction, the solvent was recovered by a vacuum distillation and the lipid residue was finally weighed (NF V18-101). Content of mineral matter of a diet is conventionally the residue of the substance after destruction of the organic matter by incineration in a muffle furnace during 3 to 4 hours at 550°C (NF V18-101). Concerning total phosphorus, the sample was mineralized then treated by vanadomolybdic reagent and in the end the optical density of the yellow solution formed was measured by spectrophotometry at 430 nm (NF V18-106). As for calcium, the analysis begin with an incineration from a sampling of diet, afterwards the ashes are treated by a pure solution of hydrochloric acid (d = 1.14) and the calcium was then precipitated into calcium oxalate form. After dissolution of the precipitate in sulphuric acid (d = 1.13), the formed oxalic acid was finally treated by a potassium permanganate solution (0.1N). 01 ml of potassium permanganat (0.1N) correspond to 2.004 mg of calcium. The result was expressed in % of sample (1st directive of CEE).

Analyses of Meats: The total lipids of each sample of the chickens breast fillets of *Pectoralis major* and experimental diets were extracted with the mixture chloroform-methanol (2:1) according to the method of Folch *et al.* [38]. The lipidic extracts are saponified by soda (Na OH) and then methylated according to the methanol-trifluoride method of boron (methanol-BF3) [39]. The methyl esters of fatty acids were analyzed by chromatography of gas (Perkin-Elmer Auto system XL) equipped with flame ionization detector and a capillary column (30 m X 0.25 mm interior diameter). The operating conditions of the chromatograph were as follows: injector and detectorthe temperature is fixed at 220 and 280°C, respectively; the temperature of the furnace was programmed to increase from 45 to 240°C and from 20 to 35°C per minute; aliquot of 1 µl were injected with bicyanopropyl phenylic silicone as a stationary phase; hydrogen was employed as conducting gas; fatty acids peaks were identified by comparison with a retention times of methyl fatty acids standards; quantification was made by reference to an internal standard (C17:0).

Plasmatic Biochemical Analyses: The Blood samples were drawn for determination of plasmatic levels of glucose, total cholesterol (Tot-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), proteins (P), creatinin (C) and urea by enzymatic methods using Merck reagent kits and Elan 2000 autoanalyser. High density lipoprotein cholesterol (HDL-c) was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. LDL cholesterol was calculated with the Friedewald formula [LDL-c = Tot-C-(TG/5 + HDL-c)] when triglycerides concentrations were <5.0 mmol/L. Subjects with triglycerides levels >5.0 mmol/L were excluded [40].

Statistical Analyses: The Parametric data obtained were expressed as mean and Standard Erreur of the mean (SEM) and were statistically analyzed by one way Analysis of Variance (ANOVA) and Newman et Keuls test to determine significant difference between groups. Furthermore, the data relating to the organoleptic test were treated by the nonparametric test of Friedman using the StatBox software (version 6.4). The level ($p < 0.05$) was considered significant.

RESULTS

Growth Performances: The influence of the experimental diets on growth performances of broilers is presented in Table 2. The supplementations at 1 and 3% of S*pirulina* in the diets have not affected the mortalities levels of animals which observed comparables results than standard group. However, during the growth phase the subjects having consumed the diet at 5% of *Spirulina* showed an elevated mortality compared to control group; 3 vs. 1%. Apparently, during both experimental periods from 17th to 45th days of breeding, food consumption was inversely proportional to the variable rates of 0, 1 and 3% of *Spirulina* in the diets. On the contrary, body weights and gain weight of chickens recorded a proportional performances ($p<0.05$) with increase in incorporation rates of *Arthrospira platensis* algae species in the diet. The consumption indices were also clearly improved ($p<0.05$) in the animals with the rise in *Spirulina* levels in the diets. However, in particularly at 38th and 45th day, the *Spirulina* added at 5% in the diet seems to cause a lower ($p<0.05$) gain weight and consumption indices in animals group than those on the other diets.

Carcasses Parameters: The eviscerated weights, as well as the carcasses outputs of chickens alimented with diets prepared at 1 and 3% of *Spirulina* were significantly better (p< 0, 01) than those on the diets added at 0 and 5% of *Spirulina*. Concerning the ponderal measurements of animals organs, after evisceration, it appears that the highest weights of the heart were observed with diets at 3 and 1% of *Spirulina*; whereas the lower values were shown at those of control group and those on diet prepared at 5% of *Spirulina*. At 45th day, the weights liver of chicken fed with diet at 1% of *Spirulina* revealed higher results ($p<0.01$) than those on control diet and those nourished at 3 and 5% of *Spirulina*; 43, 64 vs. 35, 81 vs. 36, 43 vs. 36, 38 g. The enrichment of the alimentary rations with *Spirulina* allowed a more significant development of the abdominal adipose tissue weight in the chickens ($p<0.01$); the lipidic quantities thus deposited in the abdomen were clearly increased ($p<0.01$) from 9, 21 to 16 g/ subject with increase from 0 to 5% of *Spirulina* in the diets (Table 3).

Organoleptic Tests: At day 45, the panelists appreciated the colour of the breast fillets of chickens alimented with diet at 5% of *Spirulina* of more marked pink ($p<0.05$) than those on control diet; 27 vs. 36 sum of the ranks. Also, the sensory test showed that the drumstick meat resulting from the diet at 5% of *Spirulina* was significantly ($p<0.05$) less bitter than that the control diet; 29 vs. 34 sum of the ranks. It seems, that the supplementation of *Spirulina* in diet did not modify significantly ($p<0.05$) the other organoleptic criteria of meats such as (tenderness, juiciness and flavour) (Table 4).

Biochemical Parameters: During the growth period, from the 17th to the 38th day, the glucose and the cholesterol rates did not varied significantly ($P>0.05$) in male broilers chickens according to the changes of incorporation rates of *Spirulina* in the diets. Nevertheless, the values of glycaemia recorded in animals on *Spirulina* diet was relatively ($P>0.05$) lower than those on control diet. At 17th day, the levels of plasmatic triglycerides are remained stable ($P>0.05$) in all experimental animals. On the contrary, during the other periods, the rise from 0 to 5% of the incorporation rates of this microalgae in the ration seems to increase proportionally ($P<0.05$) the plasmatic triglycerides concentrations. The addition of *Spirulina* from 0 to 5 % in the diet improved significantly ($P<0.05$) the HDL-c levels whose plasmatic values raised clearly from 0.03 to 0.09 g/l. Besides, the *Spirulina* added in diet did not exert any influence on the LDL-c rates. Finally, the tests diets did not c h a nge significantly the proteins values, uraemia and creatinin levels in animals. However, at the 3rd growth week, the broilers which consumed the diets at 1 and 5% of *Spirulina* marked exceptionally higher plasmatic urea values ($p<0.05$) than those on diets prepared at 0 and 3% of *Spirulina*; 0.11 vs. 0.09 vs. 0.08 vs. 0.06 g/l (Table 5).

Total Lipids and Fatty Acid Muscular: At 45th day, after slaughtering of the experimental animals, it was recorded no significant variation (from 1.12 to 1.20 %; $P>0.05$) in proportions of intramuscular total lipids of the breast fillets of the carcasses.

The fraction of saturated fatty acids (SFA) expressed in % total fatty acids identified (% TFAI) did not vary significantly ($P>0.05$) in chicken breast fillets of experimental groups; from 34.31 to 35.95%. The meats were rich particularly in palmitic acid (23.85 to 24.35%) and stearic acid (08.56 to 09.78%). However, no significant effect was exercised by the diets on the variations of both fatty acids types.

Table 2: Influence of diets added with *Spirulina* on growth performances of broilers

Measurements	Breeding Phases	Weeks (W)	Days	*Spirulina* rates incorporated in the diets 0%	1%	3%	5%	SEM	Diets effect
Mortality (%)	Growing	-	-	01	01	01	03	-	-
	Finishing	-	-	02	00	01	01		
Consumption of diets (g/chicken)	Growth	W_1	17	216.25	248.70	183.30	157.07	-	-
		W_2	24	637.00	644.70	634.15	814.20	-	-
		W_3	31	898.90	782.75	809.00	941.20	-	-
		W_4	38	623.32	622.63	606.26	993.33	-	-
	Finishing	W_5	45	785.31	773.50	716.00	866.82	-	-
Body weights (g)	End of starting	W_0	11	298.00	297.35	304.80	313.20	02.22	NS
	Growth	W_1	17	435.00 ^{b}	428.85 ^{b}	466.65 ^{a}	465.13 ^{a}	02.50	**
		W_2	24	747.65 ^{b}	712.50 ^{c}	794.35 ^{a}	784.47 ^{a}	04.12	**
		W_3	31	1126.40 ^{c}	1097.40 ^{d}	1158.00 ^{b}	1212.07 ^{a}	07.32	**
		W_4	38	1442.47 ^{b}	1399.74 ^{c}	1515.16 ^{a}	1479.75 ^{ab}	09.64	**
	Finishing	W_5	45	1644.94 ^{b}	1658.25 ^{b}	1721.31 ^{a}	1676.91 ^{b}	08.69	**
Weight gains (g)	Growth	W_1	17	137.00 ^{b}	131.50 ^{b}	161.85 ^{a}	151.93 ^{a}	02.04	**
		W_2	24	312.65 ^{b}	283.65 ^{c}	327.70 ^{a}	319.33 ^{ab}	03.73	**
		W_3	31	378.75 ^{b}	384.90 ^{b}	363.65 ^{b}	427.60 ^{a}	05.98	*
		W_4	38	316.07 ^{b}	302.34 ^{b}	357.16 ^{b}	267.68 ^{c}	09.64	*
	Finishing	W_5	45	202.47 ^{a}	258.51 ^{b}	206.15 ^{a}	197.16 ^{c}	07.62	*
Consumption index	Growth	W_1	17	01.58 ^{b}	01.89 ^{a}	01.13 ^{c}	01.03 ^{c}	00.04	* *
		W_2	24	02.04 ^{c}	02.27 ^{a}	01.94 ^{c}	02.55 ^{b}	00.03	* *
		W_3	31	02.37 ^{a}	02.03 ^{c}	02.22 ^{b}	02.20 ^{b}	00.03	* *
		W_4	38	01.97 ^{c}	02.06 ^{b}	01.70 ^{d}	03.71 ^{a}	00.01	* *
	Finishing	W_5	45	03.88 ^{b}	02.99 ^{d}	03.47 ^{c}	04.40 ^{a}	00.36	* *

For each group the animals number is 40 (n = 40); results expressed as mean and standard error of the mean (SEM); NS = Not significant effect; * = significant effect; ** = highly significant effect; means in the same column with different superscripts are significantly different.

Table 3: Influence of diets added with *Spirulina* on carcass parameters of broilers

Measurements	*Spirulina* rates incorporated in the diets 0%	1%	3%	5%	SEM	Diets effect
Carcasses body weight (g)	1547.8 ^{b}	1687.00 ^{a}	1698.88 ^{a}	1537.25 ^{b}	28.47	* *
Eviscerated weights (g)	1218.8 ^{b}	1326.38 ^{a}	1323.00 ^{a}	1221.88 ^{b}	23.60	* *
Carcasses output (%)	74 ^{abc}	80 ^{a}	78 ^{a}	77 ^{a}	1.08	**
Heart weight (g)	07.48 ^{b}	08.44 ^{a}	08.88 ^{a}	06.85 ^{b}	0.29	* *
Liver weight (g)	35.81 ^{b}	43.64 ^{a}	36.43 ^{b}	36.38 ^{b}	1.61	* *
Abdominal adipose tissue weight (g)	09.21 ^{acd}	10.38 ^{abc}	14.25 ^{a}	16.00 ^{a}	1.38	**

For each group the animals number is 15 (n = 15); results expressed as mean and standard error of the mean (SEM); **: highly significant effect; Means of each category followed by different latters are significantly different at 1% level of probabilities

Table 4: Sensory evaluation of the meat of broilers chicken nourished with the *Spirulina* diets

Measurements	Parts of the carcass	*Spirulina* rates incorporated in the diets 0%	5%	Diets effect
Pink colour	Drumstick	34.50	28.50	N.S
	Breast Fillet	36.00 ^{a}	27.00 ^{b}	*
Tenderness	Drumstick	30.50	32.50	N.S
	Breast Fillet	31.50	31.50	N.S
Juiciness	Drumstick	30.00	32.00	N.S
	Breast Fillet	30.50	32.50	N.S
Bitterness	Drumstick	34.00 ^{a}	29.00 ^{b}	*
	Breast Fillet	30.50	32.50	N.S
Flavour	Drumstick	31.00	32.00	N.S
	Breast Fillet	31.00	32.00	N.S

For each group the animals number is 10 (n = 10); results were expressed in sum of the ranks; NS = Not significant effect; * = significant effect; the sum of the ranks of each category followed by different latters are significantly different at 5% level of probabilities.

Table 5: Influence of diets supplemented of *Spirulina* on the variations of certain plasmatic biochemical parameters in broilers chicken during growth period.

			Spirulina rates incorporated in the diets					
Measurements	Weeks (W)	Days	0%	1%	3%	5%	SEM	Diets effect
Glucose level (g/L)	W1	17 J	01.34	01.01	01.05	01.15	00.12	NS
	W3	13 J	01.49	01.18	01.16	01.21	00.14	NS
	W4	38 J	02.34	01.98	02.31	02.28	00.08	NS
Total cholesterol (g/l)	W1	17 J	01.15	01.11	01.13	01.08	00, 02	NS
	W3	13 J	01.02	01.14	01.11	01.17	00, 03	NS
	W4	38 J	01.31	01.39	01.38	01.37	00, 02	NS
Triglyceride (g/l)	W1	17 J	00.25	00.30	00.30	00.35	00, 02	NS
	W3	13 J	00.39 ^{c}	00.55 ^{b}	00.88 ^{a}	00.92 ^{a}	00, 13	*
	W4	38 J	00.35 ^{c}	00.58 ^{b}	00.89 ^{a}	00.93 ^{a}	00, 14	*
HDL cholesterol (g/l)	W1	17 J	00.03 ^{c}	00.05 ^{b}	00.05 ^{b}	00.09 ^{a}	00.05	*
	W3	13 J	00.04 ^{b}	00.04 ^{b}	00.06 ^{b}	00.09 ^{a}	00.05	*
	W4	38 J	00.04 ^{c}	00.04 ^{c}	00.07 ^{b}	00.09 ^{a}	00.11	*
LDL cholesterol (g/l)	W1	17 J	01.01	00.99	01.03	01.00	00.04	NS
	W3	13 J	00.96	00.9	00.99	00.87	00.06	NS
	W4	38 J	01.15	01.22	01.17	01.16	00.04	NS
Protein levels (g/l)	W1	17 J	27.10	29.96	28.94	30.68	00.98	NS
	W3	13 J	27.78	30.58	28.53	32.18	00.87	NS
	W4	38 J	27.52	30.36	30.5	31.66	00.96	NS
Urea levels (g/l)	W1	17 J	01.18	00.17	01.16	00.16	00.30	NS
	W3	13 J	00.08 ^{b}	00.11 ^{a}	00.06 ^{b}	00.09 ^{a}	00.04	*
	W4	38 J	00.30	00.34	00.67	00.35	00.08	NS
Creatinin levels (g/l)	W1	17 J	05.92	06.42	05.92	05.83	00.49	NS
	W3	13 J	04.6	07.21	05.47	06.42	00.57	NS
	W4	38 J	10.6	08.42	10.16	07.13	00.72	NS

For each group the animals number is 5 (n = 5); results expressed as mean and standard error of the mean (SEM); W: week; NS: Not significant effect; *: significant effect; Means of each category followed by different latters are significantly different at 5% level of probabilities

Table 6: Total lipid content (%) and fatty acid composition (% of identified FA) of *Pectoralis major* muscle of broilers chickens according to the diets enriched with or without *Spirulina*

Diets	0% *Spirulina*	1% *Spirulina*	3% *Spirulina*	SEM	Diets effect
Total lipids (g/100g)	01.12	01.20	01.20	0.38	NS
C16:0	24.35	23.85	24.17	0.27	NS
C18:0	09.78	08.56	08.89	0.65	NS
C16:1 (n-7)	04.61	05.47	05.13	0.33	NS
C18:1 (n-9)	30.23 $^{a\,b}$	33.36 ^{a}	32.09 ^{a}	1.54	*
C18:2 (n-6)	15.85	15.14	16.64	0.85	NS
C20:4 (n-6)	02.67 $^{c\,b}$	03.67 ^{a}	04.02 ^{a}	0.75	*
C18:3 (n-3)	00.59 $^{c\,b}$	00.71 ^{a}	00.69 ^{a}	0.06	*
C20:5 (n-3)	00.29	00.32	00.30	0.20	NS
C22:6 (n-3)	00.17	00.19	00.18	0.01	NS
SFA	35.95	34.31	34.96	0.83	NS
MUFA	35.48 $^{a\,c}$	39.47 ^{a}	37.86 $^{a\,b}$	1.85	*
PUFA	19.68 ^{b}	20.16 ^{a}	21.95 ^{a}	1.18	*
PUFA/SFA	00.55 ^{b}	00.59 ^{ab}	00.63 ^{a}	0.05	*
n-6	18.52 ^{b}	18.81 ^{b}	20.66 ^{a}	1.28	*
n-3	01.16 $^{b\,c}$	01.35 ^{a}	01.29 ^{a}	0.10	*
n-6: n-3	15.97 ^{a}	13.93 ^{b}	16.02 ^{a}	2.19	*
LA/ALA	26.86 ^{a}	21.32 ^{c}	24.11 ^{b}	2.98	*

For each group the animals number is 10 (n = 10). Results expressed as mean and standard error of the mean (SEM). Means in the same line with different superscripts are significantly different at 5% level of probabilities.; NS: Not significant effect; *: significant effect; SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids; LA: Linoleic acid; ALA: α linoleic acid

Among the Fatty Acids, the percentages of Monounsaturated Fatty Acids (MUFA) recorded in muscle chickens on *Spirulina* diets were higher ($P<0.05$) than those on control diet. The oleic acid was found especially at higher concentrations ($P<0.05$) in the meat of chickens on diets prepared at 1 and 3% of *Spirulina* than those on control diet; 33.36 vs. 32.09 vs. 30.23%. The meat fillets of chickens nourished with the *Spirulina* were also relatively richer ($P>0.05$) in palmitoleic acid compared with the control diet; 5.3 vs. 04.61%, on average.

The polyunsaturated fatty acids values (PUFA) were remarkably more important ($P<0.05$) in the meat of broilers fed the diets added with *Spirulina* than in those fed the control diet; 20.16 to 21.95 vs. 19.68%. Indeed, PUFA of the ω 3 series were significantly ($P<0.05$) higher in the meat of animals on both diets prepared at 1 and 3% of *Spirulina* compared with the control diet; 1.35 vs. 1.29 vs. 1.16%. The meats of chickens on diets added of *Spirulina* were marked by higher proportions ($P<0.05$) in α linoleic acid (ALA) and lower values in acid eicosapentaenoic (EPA) as well as in acid docosahexaenoic (DHA) than those on the contol diet. The Polyunsaturated Fatty Acids (PUFA) of the ω6 series were also found in higher proportions ($P<0.05$) in chickens nourished with *Spirulina* diets compared to the control diet; 18.52 to 20.66%. The linoleic acid (LA) being the predominant constituent of this last series of the PUFA has not changed significantly ($P>0.05$) in the meats; 15.14 to 16.64%. However, the proportions of arachidonic acid concentrated in the breast fillets were increased proportionally ($P<0.05$) with the augmentation from 0 to 3% of *Spirulina* in alimentation; 2.67 to 4.02%.

The n-6:n-3 ratio of meats was lower ($p<0.05$) in animals fed with enriched diet at 1% of Spirulina compared to the control diet; 13.93 vs. 15.97%. Furthermore it appears that the PUFA:SFA ratio was higher ($p<0.001$) in the meats fillets of chickens alimented with diets added of Spirulina at 1 and 3% than in those fed with the standard diet; 1.32 vs. 1.16%. The LA:ALA ratio was also higher ($p<0.001$) in broilers fed Spirulina diets (Table 6).

DISCUSSION

Growth Performances: The death rates recorded in chickens during the growth and finishing periods are acceptable and did not exceed the standard rate of 10%. These observations were confirmed by Manafi *et al.* [41] who reported that *Spirulina* administered at low levels in the diet (0.1%) improves singnificativement health, fertility and the level of embryonic mortality in the laying hens during breeding period. Undoubtedly, the test diets with and without *Spirulina* did not exert a harmful influence in animals whose weak mortalities observed were certainly induced by a bad control of environment temperature in experimental room. Indeed, at the end of the experimental period, from 38th to 45th day, because of the heat waves of the summer period, the degrees of temperatures were increased remarkably inside the experimental building compared to reference temperature recommended in chickens [42].

The diet prepared at 3% of *Spirulina* was weakly consumed compared to the other diets in particular prepared at 0 and 1% of *Spirulina*. Being rich in proteins (from 60 to 70% M.S) as well as in principal alimentary compounds (glucidic and lipidic) [12, 43], the rise in incorporation level of *Spirulina* up to 3% in the diets seems to control best food consumption in chickens. At this subject, Okwuosa *et al.* [44] reported that the food consumption in generally is decreased if the levels of proteins and energy are increased in the diets distributed to the animals. A rise of 1% of the content of nitrogenous matter of diet provokes an ineluctable reduction from approximately 3% of the alimentary ingestion in chickens. At the 4th week, the substantial falls of the food consumption in chickens is exceptional and certainly due to the strong fluctuations of the temperature of experimental ambiance having exceeded the normal tolerated by the animals during this period and which should be maintained from 19 to 20°C [42]. In addition, the diet at 5% of *Spirulina* although it is richest in the principal alimentary compounds, it recorded the higher level of the food ingestion in chickens compared to those on the other test diets. Except the difference in the chemical consistence of the diets, other parameters can also explain these variations in the food consumption in poultry such as: the density of animals, the granulation of foods, the fluctuations of environment temperature, the stress of animals, a bad control of light… etc. [45].

The body weight tends to develop according to age of the animals independently of the nature of consumed diets. During his development, the broiler chicken passes by a starting phase where he accumulates a weak weights, then by a growth phase during which he believes remarkably and complete his physiological cycle by a Finishing phase where his growth stops, all the while his weight performances continue to increase in particular by an accumulation of fat in abdomen [46]. At the end of the growth and finishing periods, the best body weights were observed in chickens on the diet added with 3% of *Spirulina*, followed by those on diets prepared respectively at 1 and 0% of *Spirulina*. Apparently, the ponderal performances of chicken are clearly improved with increase in incorporation rate of *Spirulina* in the standard ration. It is well established that the experimented microalgae (*Arthrospira platensis*) is rich in essential nutritional components to growth of animals such as: protids, glucides, lipids, amino acids essential and fatty acids essential [3, 12, 28, 43, 47]. These results are in line with those of Razafindrajaona *et al.* [46] who reported also an evolution proportional of the body weight according to rise in the incorporation rates from 0.5 to 1% of *Spirulina* in the diet of broiler chickens. Nevertheless, the experimental diet enriched with the *Spirulina* microalgae at a high rate of 5% showed controversial body weights in chickens. According to Falquet and Hurni [3], the *Spirulina* can accumulate during its culture a many toxic substances such heavy metals able to exert an appreciable reduction of the growth and a harmful effects on health of animals. This last hypothesis is rejected in our study because of the respect of the good hygiene and manufacturing practices by the producer of the experimental microalgae. These results may be explained by the slimming effect that the *Spirulina* can exert when it is employed with high quantities. This has been confirmed by Vidalo [48] who suggests that the *Spirulina* improves the body weight among obese patients which presented certain metabolic diseases.

During the growth period, the gains of weights obtained in chickens were marked by remarkable increases from the 1st to the 3rd week. However, at 4th and 5th week, the gains of weight were relatively decreased in all experimental subjects on diets with and without *Spirulina*. According to Castello [42], this weight reduction in animals at the end of growth and finishing periods is probably in relation with the bad controls of the ambiance conditions of raising chickens. In fact, during the summer period of August, the augmentations of temperatures from 25 to 28°C inside the building of chickens can explain clearly the decreases of growth and plumage in animals. Apparently, at the end of both periods (growth and finish), the subjects on diet prepared at 5% of *Spirulina* recorded a low gains of weights compared to those on other diets. It is probable that the *Spirulina* incorporated in the alimentary diet at the weak doses can improve the weight and the other performances of the animals, whereas its addition with strong quantities in the food seems to exert a slimming effect in the chickens. These results are in line with those of Vidalo [48] who reported that this microalgae is able to reduce the overweight and improve the problems of the alimentary behavior among undoubtedly the patients with certain metabolic diseases such as (obesity, diabetes, bulimia...etc.). However, in general, during experimental period, the gains of weights of the chickens were remarkably improved according to increasing from 0 to 3% of the *Spirulina* in diets. These results were in agreement with those reported by Razafindrajaona *et al.* [46] in broilers. Thus, the enrichment of the diets by the nutriments contained in the *Spirulina* microalgae such as proteins and the lipids [12, 28, 47, 49] can contribute undoubtedly to the improvement of the performances of raising chickens. Indeed, the *Spirulina* contains the majority of the essential amino acids such as (isoleucine, leucine, lysin, méthionine… etc), as well as a strong concentration in essential fatty acids including in particularly the omega-3 and the omega-6 which are essential to the good development of poultry [12, 31].

During this experimentation, the best consumption indexes were obtained with the diet prepared at 3% of *Spirulina* followed with those to 1 and 0% of *Spirulina*, respectively. The richness of this microalgae with some nutriments essential (proteins, glucides and lipids) [3, 12, 31] is certainly at the origin of these results. It appears that the chickens are able to increase the conversion of food into meat and can also reduce the alimentary ingestion according to the rise in the levels of proteins and energy resulting from the addition of the *Spirulina* in the ration [44]. According to Costa *et al.* [50], the digestibility of this micro-organism varies from 75 to 83% and requires no special treatment for a better assimilation of the principal nutritional elements constituting it in animal. The values of consumption indexes indicated more mediocre results in chickens on diet prepared at 5% of *Spirulina* compared to those on other experimental diets. These results were not in agreement with those reported by Ross and Dominy [51] which observed no variation of the consumption indexes in chickens on diet added at 12% of *Spirulina*. In fact, the *Spirulina* is considered in pharmacy like a drug rather than an alimentary ingredient. Nowadays, any case of overdose of *Spirulina* was documented in the literature. It appeared that the consumptions of more than 10 g/jour of this microalgae during several years do not report any negative effects in man [52]. However, like all drugs an overdose of *Spirulina* can exert a very toxic effect which can to deteriorate the performances of growths and to affect the health of the animals. Indeed, a potentially toxic amino acid, Beta-N-Methylamino-L-Alanine5 (BMAA), would be present in 97 at 98% of the stumps and species of cyanobacteria [53]. The neurotoxicity of this molecule has made object of several publications reported by Cruz-Aguado *et al.* [54] and Lobner *et al.* [55]. Finally, it would be extremely interesting to carry out a toxicological study on the *Spirulina* species studied (*Arthrospira platensis*) to know exactly if it contains molecules able to affect the animal health.

Carcasses Parameters: The most interesting values of the carcasses body weight, eviscerated weights and Carcasses output were noticed with the diets supplemented at 1 and 3% of *Spirulina*, whereas the carcasses of chickens nourished with the diets prepared at 0 and 5% of *Spirulina* were marked the weakest results. This does not corroborate with the study of Ross and Dominy [51] undertaken in broilers chicken alimented during 41 days with rations containing 0, 1.5, 3, 6 and until 12% of *Spirulina* and which revealed any significant variation in the carcasses parameters in studied animals. According to Fox [12], Clément [43], Jacquet [47] and Borowitzka & borowitzka [56], the improvement of the carcasses weights and eviscerated weights during the experimental period of chickens alimented with diets added at 1 and 3% of Spirulina is correlated with the great richness of this microalgae in proteins whose contents can reach 70% of dry matter and who also have a good digestibility which can vary from 75 to 83% in the poultry [50]. However, the diet at 5% of *Spirulina* indicated similar values in chickens than those on control diet. It is probable that this microalgae presents a toxic effect when it is incorporated at the high doses in the ration. Indeed, according to Falquet & Hurni [3] and Razafindrajaona *et al.* [46], under the bad conditions of culture, the *Spirulina* can accumulate certain heavy metals such as lead, cadmium and mercury which can affect the carcasses parameters as well as the animals health. Also, other authors such as Santillan [24], Charpy *et al.* [52], Cox *et al.* [53], Cruz-Aguado *et al.* [54], Lobner *et al.* [55] and Weiss & Choi [57] reported that certain toxic substances contained in cyanobacteria such as (nucleic acids, beta-N-methylamino-L-alanine5) can damage significantly the performances and health of the animals. For lack of material of laboratory, these chemical molecules were not identified in this studied. Concerning the organs weights of the carcasses, it seems that the heart weight levels are directly proportional to the concentration of *Spirulina* in the consumed diets during experimental period. In addition, the highest weights values of the liver were found in the animals group which were alimented with the ration prepared at 1% of *Spirulina*. As seen in this study, some authors reported also an abnormal rise in the liver weights in chickens alimented especially with diets rich in certain nuriments such as proteins and lipids of which exaggerated consumption can also induce a hepatic steatosis in the animals [58, 59]. Thus, the increases of the metabolism hepatic of surplus proteins covered by the *Spirulina* in the diets were certainly at the origin of increases of liver weight in chickens. In addition, the low rises in heart weight of subjects on *Spirulina* diets were probably provoked by the high temperatures noted in building raising chickens at the end of experimental period which were higher than the normal of 18°C allowed at this age of animals. It is well established that these augmentations in ambiance temperatures can increase the breathing and cardiac rhythm as well as the heart weight in chickens which physiologically do not possess sweat glands [60].

Finally, the deposits of abdominal fat were more considerable in animals having consumed significant quantities of *Spirulina* during the experimental period. It seems that the principal nutritive elements contained in the *Spirulina* [12, 28, 31] were metabolized by the chickens for synthesize more lipids which were accumulated in abdomen in particular during the finish period of animals [46].

Organoleptic Tests: The colour of the Drumsticks and Breast Fillets of chickens nourished with the diet at 5% of *Spirulina* was appreciated by the panelists of a marked pink compared to those on control diet. It is well established that this microalgae contains various pigments such as (chlorophyl, carotenoids, phycocyanin and phycoérithrin) [2] which can accumulate in muscular tissues as well as in skin and induce great changes in the colour of carcasses of broiler chickens [46, 61, 62]. These various pigments contained in the *Spirulina* have not deteriorated the color of the meat after cooking. On the contrary, they have conferred to meat a characteristic colour, similar to that of farm poultry.

The panelists also found a better bitterness of Drumstick meat in animals nourished with diet at 5% of *Spirulina* than those which were alimented with the control diet. The many components constitutive of *Spirulina* such as lipids, glucides proteins, minerals and many other bioactive substances were contributed certainly to the improvement of this organoleptic criteria [3, 12, 28, 43]. Finally, it appears that the supplementation of the diets with *Spirulina* does not affect the others sensory criteria of the meats such as (tenderness, juiciness and flavour) which the panelists qualified as comparable with those of animals nourished with the control diet.

Biochemical Parameters: During the growth period of animals, the diets added with *Spirulina* have clearly improved the glycemia, as well as the plasmatic rates of HDL-c. These results were confirmed by Parikh *et al.* [63] who reported that ingestion of *Spirulina* reduces remarkably the plasmatic rate of glucose in type 2 diabetic patients. Other studies also revealed that the great richness of the *Spirulina* in poly unsaturated fatty acids omega 3 and omega 6 [64, 65] can contribute to the plasmatic increase in the good cholesterol and the stability of the cholesterolemy. It appears that the increase of HDL-c values in experimental chickens on diets added of *Spirulina* coincides with the stability of plasmatic Ldl-c rates and the reduction certainly of blood VLDL-c levels. Increasing hydrolysis of hepatic VLDL-c undoubtedly favored the synthesis of the HDL-c in chickens [66]. The previous data concerning the increases of HDL-c levels in chickens on diets added of *Spirulina* explain clearly the beneficial effect of this microalgae on serum lipids in these animals. Concerning the plasmatic cholesterol, the recorded values were not varied significantly in chickens alimented with the diets supplemented of *Spirulina* compared to those on control diet. In contrast, Yamamoto *et al.* [67] suggested that this microalgae can reduce considerably the plasmatic cholesterol level as well as its accumulation in the organism and improve remarkably the health among the patients with atherosclerosis. Also, it has been shown that the consumption of low fat diet induces a decrease in serum cholesterol concentration only when accompanied by body weight loss [68]. The findings in our study are in agreement with those announced by Sakr [69] who found in diabetic man a correlation between weight lost and decrease in total cholesterol levels.

In addition, the *Spirulina* was considered on the one hand as an energetic food because of its wealth in glucides as well as lipidic compounds and on the other hand this microalgae was qualified as an constructor food as a result of its remarkable protein contents (60 to 70% of dry matter) [2, 3, 12]. However, its addition (*Spirulina*) even to the important dose of 5% in the standard alimentary ration does not seem modified significantly the proteinic metabolism in chicken during his growth. Indeed, the tests diets with and without *Spirulina* did not change significantly the proteins values, uraemia and creatinin levels in animals. These stabilities of proteinous measurements were certainly due to the alimentary behavior of chicken which presents physiologically the faculty to increase or reduce the quantities of consumed food to assure its nutritional needs according to the levels proteinic and the energetic contents in the diets [59, 70].

Total Lipids and Muscular Fatty Acid: The intramuscular lipids of Breast Fillet of the meat (*Pectoralis major*) of chickens alimented without and with the *Spirulina* appear in comparable proportions. However, the recorded levels in the meat were slightly higher than 0, 9% value reported by Ratnayake et *al.* [71] and Mourot *et al.* [72].

The profile of the fatty acids has revealed a considerable richness of Breast fillet meat in poly unsaturated fatty acids (PIFA) in particular in animals which were alimented with the diet supplemented at 3% of *Spirulina*. The essential fatty acids of the series n-3 and n-6 accumulated in the Breast fillets meat with more interesting percentages in chickens alimented with *Spirulina* diets than those on control diet. In addition, the concentrations of n-6 were significantly higher than those of the n-3 series in the various chicken meats. According to Martin [73], the nutritional recommendations encourage the man to consume more fatty acids n-3 as well as few quantities of fatty acids n-6. Also, the linoleic acid (LA; C18:2) and the α linoleic acid (ALA; C18:3) must cover the alimentary diet with the ratios of C18:2/C18:3 = 5. The LA/ALA ratios which were found in our study on the quality of meats of chickens nourished with and without *Spirulina* are much higher than the normal value dictated by this author.

Recent data reported by Bourre [74] have shown that digestive physiology of birds preserves remarkably the polyinsaturés fatty acids contained in the consumed diets. In fact, the rate of arachidonic acid of the series n-6 of the Breast meat of *Pectoralis major* was directly proportional to the concentration of *Spirulina* consumed in diets by the broiler chickens. The values recorded in the meat of animals on *Spirulina* diet were higher from 01.37 to 01.50 times compared to those on control diets. However, similar variations in arachidonic acid rates in the meat of chickens alimented without *Spirulina* were reported by Mourot [75]. A dose effect was also observed in muscular tissues for α linoleic acid (ALA) multiplied by 1.18 times in animal on diets added of *Spirulina* than those on control diet. According to Bourre [74], the alimentation of birds with extracts of linseed or of colza can multiply the content of ALA in the animals carcasses by 10 in chickens, 6 in pigmeat, by 2 in ox and from 20 to

40 in eggs. However, the fatty acids with long chain, derived from the precursor C18:3 n-3 such as eicosapentanoic acid (EPA; C20:5 n-3) and docosapentanoic acid (DHA; C22:6 n-3), are not increased in animals. This can be the result of the under-expression and low activities of certain metabolic enzymes such as delta 5 and 6 desaturases which are required in majority of animals for the synthesis of highly fatty acids such as eicosapentanoic and docosapentanoic acids [76].

CONCLUSION

The results from this experiment suggest that the nutriment contained in Spirulina such as lipids and protein exercise an important role in the growth of chickens and remarkably improves the lipidic quality of the carcasses meat. However, its inclusion in the diet at 5% seems to induce bad performances in animals. Finally it appears clearly that the incorporation of this microalgae in the diet at 3% in growing chickens is possible.

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