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EFFECTS OF SPIRULINE (*Spirulina platensis*) SUPPLEMENTATION ON OXIDATIVE STRESS MARKERS, BIOCHEMICAL CHARACTERISTICS, AND HEMATOLOGICAL PARAMETERS IN RABBIT (*Oryctolagus cuniculus*) DOES

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ABSTRACT
The present study was carried out in order to evaluate the effects of feed supplementation with spiruline powder on the oxidative stress markers, biochemical characteristics, and hematological parameters in rabbit doe. Twenty-one nulliparous and sexually mature does (7–8 months old) were distributed into three groups of seven does each, comparable in terms of body weight. After a week of feeding with experimental feed, does of each group were mated. During the trial, does of group 1 (control group) were fed *ad libitum* with a feed free from spiruline (control feed), while those of groups 2 and 3 received the control feed supplemented with spiruline powder at 0.6 and 1.2%, respectively. Immediately after parturition, blood was collected for analyses of hematological, biochemical, and oxidative stress parameters. Studied parameters included serum concentrations of total proteins, cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine, and urea; malondialdehyde, reduced glutathione, catalase, and superoxide dismutase; and hematological parameters. The total protein concentration, number of white and red blood cells, platelets, hemoglobin, hematocrit, and mean corpuscular volume were significantly ($P<0.05$) higher in does treated compared to control. Oxidative stress parameters were comparable ($P>0.05$) among treatments. Hence, these results show that feed supplementation with spirulina powder can improve rabbit doe health, notably at 1.2%, since optimal results were obtained at this percentage.

Contribution/Originality: This study contributes to providing evidence on the effects of supplementing with spiruline in its raw powder form, in farm animal feedstuffs. This may contribute to other means of improving farm animal productivity by using medicinal plants to protect them from oxidative stress effects to which they are exposed.

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1. INTRODUCTION

Many endogenous factors (Valko, Morris, & Cronin, 2005) can be responsible for the perturbation of the animal system through the production of reactive oxygen species (ROS), which lead to induction of oxidative stress (OS). These include mitochondria, peroxisomes, the endoplasmic reticulum, prostaglandin synthesis, auto-oxidation of adrenalin, reduced riboflavin, inflammation, and infection (Poljsak and Milisav, 2013). Exogenous factors may also be involved, such as air and water pollution, heat, feed, aging, certain drugs, industrial solvents, cooking gas, radiation, and heavy metals.

Oxidative stress is a state that results from a disequilibrium within an individual, between the production of oxidants and the defense mechanism of antioxidants (Pastre, 2005) in favour of oxidants.

Many parameters, including enzymatic antioxidants (superoxide dismutase, catalase, and peroxidases), end products of lipid peroxidation like malondialdehyde, hepatotoxicity and nephrotoxicity markers of oxidative stress (ALAT, ASAT, creatinine, urea, total proteins, and cholesterol), and hematology can facilitate the evaluation and measurement of OS (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012).

Perturbation or dysfunction of the animal system by oxidative stress leads to negative consequences on productivity (Tamboura et al., 2006), resulting in important economic losses.

To deal with these problems, animal breeders turn inceasingly towards medicinal substances such as antibiotics and hormones, to fight against numerous infections, stimulate growth, and improve production (FAO/WHO, 2009). Awareness of the dangers of these medicines create distrust and worry in the minds of consumers, making it an obligation for governments to ban the use of antibiotics as growth activators in farm animal feedstuffs. The increasing demand for 'natural' products, which are seen as pure and ecologically sound, has stimulated the minds of researchers who are more and more interested in substitutes for synthetic products considered dangerous for animal and human health (AFSSA (French Food Safety Agency), 2007). Thus, studies have been carried out on diverse plants with antioxidant, anti-inflammatory, antiviral, anticancer, antibacterial, anti-protozoan, and hepatoprotective properties and that also stimulate growth and fertility (Chowdhury, Islam, & Khalequzzaman, 2009).

Equally, spiruline (*Spirulina platensis*) has been the object of many studies. It represents the most abundant photosynthetic micro-organism and is common in the lakes of Central Africa and Mexico (Ould, Bouchabchoub, Massoui, & Elyachioui, 2013). Spiruline is considered a food source with high nutritive value, due to its high digestibility and protein content (60–70%) (Ould et al., 2013), fat (15–25%) (Djaghoubi, 2013), and lipid (5.6–11%) (Sguera, 2008). It has antioxidant properties due to its phytocyanine content (Djaghoubi, 2013). These properties of spiruline make it a potential protector of animals from the effects of OS. Razafindrajaona, Rakotozandriny, Randria, and Ramampiherika (2010) observed that the administration of aqueous extract of spiruline at doses of 2 and 8 mg/kg bw led to increase in growth and better libido in male rats. Deutcheu (2016) reported an improvement in reproduction in rabbits treated respectively with 5, 15, or 25 mg methanolic extract of spiruline/kg bw and rabbit does having received 5, 10, or 20 mg aqueous extract of spirulina/kg bw.

All the above studies were performed on extracts of spiruline and, to the best of our knowledge, data are rare on the use of raw spiruline as a feed supplement and its anti-toxicity effects. Thus the current study was initiated to contribute to improvement in the health of farm animals using medicinal plants.

A search of the literature on spiruline and its effects showed that data are very scarce on its raw/powder form. Recent data on this product are equally hard to find, making comparison difficult for the results obtained from this work.

2. MATERIALS AND METHODS

2.1. Animal Material

Twenty-one healthy adult fertile rabbit does (New Zealand breed) 8 months of age, weighing 2.8–3.0 kg, produced at the Teaching and Research Farm of the University of Dschang, were used. They were treated against external (exo) and internal (hemo) parasites by subcutaneous injection of an ivermectin solution (0.1 ml/kg bw), repeated after two weeks.

2.2. Housing and Feeding

Animals were housed in a cement-block building with a sheet metal roof, plaster-rendered and an open 1/3 upper section. They were kept individually in wire cages (galvanized metal, 96 cm long, 40 cm wide, 15 cm high). Each cage was equipped with a feeder and a drinker (800 ml capacity). The building was previously disinfected with a solution of javel water and cresyl (1/2 l each mixed in 20 l water), which was sprayed inside the building and in all the cages. Animals were introduced to the cages two weeks after disinfection. Throughout the trial period, animals received

complete feed and water ad libitum. The composition and chemical characteristics of this feed are summarized in Table 1.

Table-1. Composition and chemical characteristics of the feed.

Ingredients	Proportion in feed (%)
Maize	27.00
Wheat bran	14.00
Kernel cake	18.00
Soy bean cake	5.00
Cotton cake	4.00
Premix10%*	5.00
Fish meal	3.00
Palm oil	2.00
Sea shells	1.50
Salt	0.50
Rice bran	20.00
Total (kg)	**100.00**
Chemical characteristics	
Metabolizable energy (kcal/kg)	2435.23
Crude protein (% DM)	16.47
Crude cellulose (% DM)	13.65
Calcium (% DM)	1.26
Phosphorus (% DM)	0.55
Sodium (% DM)	0.28
Lysine (% DM)	0.83
Methionine (% DM)	0.36

Note: *Premix10%: a mixture of vitamins A, B complex, D, K, and E plus Fe, Cu, Zn, Se, Mn, methionine and lysine principally and incorporated at 5% in diet.

2.3. Plant Material

Spiruline (*Spirulina platensis*) in the form of dried flat cake was collected from Lake Chad. The flat form was powdered to coarse particles using a grinding mill and incorporated in the feed.

2.4. Ethical Considerations

Experimental protocols used in this study were approved by the Ethical Committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES), and were in conformity with the internationally accepted standard ethical guide lines for laboratory animal use and care as described in the European Community guidelines; EEC Directive86/609/EEC, of the 24 November1986.

2.5. Experimental Design

Before starting the experiment, 21 does with the above-mentioned characteristics were weighed and randomly divided into thre groups of seven, comparable in terms of body weight (bw). Spiruline was incorporated in feed at the following rates:

- Group 1 (control): feed without spirulina,
- Group 2: control feed + 0.6 % spiruline,
- Group 3: control feed + 1.2% spiruline.

After one week of feeding the experimental feed rations, all does were put to reproduction. After parturition, they were followed up for 2 weeks.

2.6. Parameters and Characteristics Studied

2.6.1. Hematological and Biochemical Analysis

Blood samples were obtained by cardiac puncture and collected without anticoagulant for biochemical and OS indicator dosages, and with anticoagulant (EDTA) for complete blood count. The biochemical parameters analyzed from serum were performed with appropriate commercial Chronolab kits (Barcelona, Spain). The spectro-photometric method was used according to the manufacturer's instructions. The hematological parameters analyzed were performed using a veterinary hematology analyzer (Genius KT 6180, Shenzhen Genius Electronics Co., Ltd., Hong Kong, China).

2.6.2. Oxidative Stress Markers

The activities of superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) and the concentration of malondialdehyde (MDA) were measured.

2.6.2.1. Estimation of Catalase Activity

Catalase activity was estimated according to Aebi (1984), based on on the ability of H_2O_2 to decompose via the action of CAT to produce H_2O and O_2. Decrease in absorbance in the UV region over time corresponds to CAT activity. A volume of 2.0 ml of substrate (10 pmol/ml of H_2O_2 in 50 mmol/l sodium-potassium phosphate buffer, pH 7.0) was incubated with 100 ml of serum.

Decomposition of H_2O_2 was followed directly for 2 min by decrease in absorbance at 240 nm.

2.6.2.2. Estimation of GSH

The glutathione level was measured according to the method of Moron, Depierre, and Mannervik (1979), based on the reaction of GSH with 5,5-dithiobis-2-nitro-benzoic acid (DTNB) (Sigma Aldrich, Berlin, Germany) at pH 8.0, which was added to the tubes and the intense yellow color formed turned red at 412 nm in a spectrophotometer after 10 min. A standard curve of GSH was prepared using concentrations ranging from 2 to 10 nmol of GSH in 5% trichloroacetic acid (Labtech,Windsor, Australia). After centrifugation, absorbance of the yellow color was measured and the results were calculated from the glutathione standard curve.

2.6.2.3. Estimation of Lipid Peroxidation

Lipid peroxidation was estimated by the reaction of thiobarbituric acid (TBA) (Qulaikems, New Delhi, India) with malondialdehyde (MDA) according to Botsoglou et al. (1994). In the presence of an acid and heat (pH 2–3, 100 - mC), MDA condensed with two molecules of TBA to produce a pink color complex that absorbs at 532 nm. A total of 105 ml of orthophosphoric acid at 1% and 500 ml of the precipitation mixture (1% TBA in a 1% acetic acid solution) was added to 100 ml of homogenate.

The mixture in each tube was homogenized and placed in boiling water for 15 min. The tubes were then cooled in an ice bath and the mixture was centrifuged at 3500 rpm for 10 min. Absorbance was read at 532 nm against the control.

2.6.2.4. Estimation of SOD

Adrenaline is sufficiently stable at acidic pH but, when pH is increased, its rate of auto-oxidation increases. The dosage of SOD is thus based on the its capacity to inhibit or decelerate auto-oxidation of adrenaline to adreno-chromium in the milieu of a base. The method proposed by Misra and Fridovich (1972) was used in this study. Microtubes of serum were introduced into the spectrometer, as well as 1660 ml of carbonate buffer solution (pH 10) and 200 ml of adrenaline (0.3 mM). The absorbance of adreno-chromium formed was read at 480 nm 30 and 90 s after initiation of the reaction.

2.7. Statistical Analysis

Data were submitted to one-way analysis of variance (ANOVA) to test the effects of the rate of spiruline powder incorporation on the study parameters. The Duncan test was performed to separate means when there was a significant difference. The results are expressed as mean ± standard deviation, and the limit of significance was fixed at 5%. The software SPSS 20.0 was used for the analysis.

3. RESULTS

3.1. Effects of Spiruline Powder Feed Supplementation on OS Indicators in Rabbit Does

Table 2 presents the variation in rabbit doe serum concentration of Os indicators according to the level of spiruline powder in the feed. Serum levels of malondialdehyde and concentrations of catalase and superoxide dismutase were lower in animals administered feed containing spiruline powder as compared to the control group. Meanwhile, the reverse was observed with reduced glutathion. However, no significant difference (P>0.05) was found irrespective of the indicator considered.

Table-2. Variation of serum concentration of OS indicators according to dose of spiruline powder in rabbit doe.

OS indicator	Level of spiruline supplementation (%)			*P*
	0 (n = 5)	0.6 (n = 5)	1.2 (n = 5)	
Malondialdehyde (µM/l)	0.35±0.16	0.29±0.12	0.21±0.07	0.28
Reduced glutathion (µM/l)	47.48±12.63	69.88±23.75	60.46±14.28	0.25
Catalase (µM/min/ml)	0.24±0.03	0.21±0.07	0.21±0.05	0.73
Superoxide dismutase (µM/l)	0.37±0.07	0.32±0.11	0.32±0.02	0.80

Note: n: number of animals per group.

3.2. Effects of Spiruline Powder Supplementation in Feed on Biochemical Parameters in Rabbit Doe

Variation of serum concentration of biochemical characteristics with respect to dose of spiruline powder in rabbit doe is summarized on Table 3. It appears that, serum levels of creatinine, urea, ASAT and total cholesterol were

comparable (P>0.05) among the different treatments. On the contrary, concentrations of total proteins and ALAT were significantly (P<0.05) higher in rabbits given feed with highest rate of spiruline with respect to the other two treatments.

Table-3. Variation in rabbit doe serum concentrations of selected biochemical parameters with respect to level of spiruline powder administered.

Biochemical parameter	Level of spiruline supplementation (%)			*P*
	0 (*n* = 5)	0.6 (*n* = 5)	1.2 (*n* = 5)	
Creatinine (mg/dl)	1.21±0.38	1.36±0.49	1.47±0.56	0.75
Urea (mg/dl)	342.71±42.27	350.00±89.24	259.25±38.94	0.12
ALAT (U/l)	16.19±5.41^{b}	14.44±3.14^{b}	23.63±2.93^{a}	0.02
ASAT (U/l)	16.56±4.84	24.44±6.50	18.13±5.36	0.17
Total protein (g/dl)	4.10±0.85^{b}	3.86±0.19^{b}	5.48±0.53^{a}	0.01
Total cholesterol (mg/dl)	21.01±5.56	14.81±5.87	19.92±7.61	0.39

Note: a,b: values with the same subscript letter in the same line are not significantly different (*P*> 0.05). *n*: number of animals per group. ALAT: alanine aminotransferase. ASAT: aspartate aminotransferase.

3. 3. Effects of Spiruline Powder Supplementation in Feed on Hematological Parameters in Rabbit Doe

Table 4 summarizes the variation in rabbit doe serum concentrations of hematological characteristics according to the level of spiruline powder administered.

The number of white blood cells was significantly (*P*<0.05) higher in animals fed with a ration containing spiruline than in those those without spiruline. Red blood cells, hematocrit, mean corpuscular volume and level of hemoglobin were higher in T2 females (1.2% spiruline), though comparable (*P*>0.05) to the control, except for hemoglobin level. Contrary to this, the numbers of platelets were significantly (*P*<0.05) higher in T1 rabbits (0.6% spiruline) in comparison to the other groups. Nevertheless, the other hematological characteristics were comparable (*P*>0.05) among the different treatments.

Table-4. Variation in rabbit doe serum concentration of hematological parameters according to level of spiruline powder administered.

Hematological parameter	Level of spiruline supplementation (%)			*P*
	0 (*n* = 5)	0.6 (*n* = 5)	1.2 (*n* = 5)	
WBC (10^3/µl)	4.43±1.87^{b}	7.60±1.20^{a}	7.13±0.47^{a}	0.03
Lymphocytes (10^3/µl)	3.27±1.86^{b}	6.10±1.17^{a}	4.67±0.91^{ab}	0.04
Monocytes (10^3/µl)	0.23±0.06^{b}	0.40±0.14^{ab}	0.60±0.10^{a}	0.01
Granulocytes (10^3/µl)	0.93±0.57	1.10±0.24	1.87±0.50	0.17
RBC (10^6/µl)	5.86±0.99^{ab}	4.87±0.78^{b}	6.63±0.70^{a}	0.04
Hgb (g/dl)	9.37±1.55^{b}	10.70±1.73^{b}	14.54±1.67^{a}	0.01
HCT (%)	38.30±6.00^{ab}	30.13±4.95^{b}	45.73±9.15^{a}	0.04
MCV (%)	66.63±3.91^{ab}	62.05±0.97^{b}	68.53±2.25^{a}	0.03
MCH (PG)	17.96±7.68	21.98±1.61	22.43±0.51	0.40
MCHC (g/dl)	27.00±10.59	35.53±2.21	32.87±1.80	0.23
PLT (10^3/µl)	78.67±5.69^{b}	129.25±24.78^{a}	84.67±19.55^{b}	0.02
MPV (fl)	8.57±1.56	6.58±0.57	7.17±1.27	0.14
RDW (fl)	9.77±4.22	5.85±0.67	8.30±1.73	0.18
PCT (%)	0.06±0.01	0.08±0.03	0.05±0.02	0.26

Note: a,b: values with the same subscript letter in the same line are not significantly different (*P* > 0.05). *n*: number of animals per group. RBC: red blood cell count, WBC: white blood cell count; Hg: hemoglobin ; HCT: hematocrit ; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; RDW: red cell distribution width; PCT: plaquetocrit.; MPV: mean plaquetocrit volume.

4. DISCUSSION

Many parameters, including hematology, enzymatic antioxidants (superoxide dismutase, catalase, and peroxidases), end products of lipid peroxidation like MDA, hepatotoxicity and nephrotoxicity markers of OS such as aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), creatinine, urea, total protein, and cholesterol can facilitate the evaluation and measurement of OS. The detection of oxidation derivatives of these different substances will therefore serve as markers of the presence of OS (Birben et al., 2012). Variation in the values of these characteristics is used as test to show the action of extracts of substances that act as antioxidants against OS to which animals are exposed, from both endogenous and exogenous sources.

Enzymes commonly used in the evaluation of liver function are aspartate ASAT and ALAT; increase in aminitransferase activity reflects cytolysis.

The biochemical analyses in this study showed higher values of ASAT and ALAT in treated groups compared to controls, although this difference was only significant for ALAT. These results are in disagreement with Kader and Kalapuram (2017), who recorded a marked decrease in the activities of ALAT and ASAT in rats treated with *E. officinalis* at a dose of 250 mg/kg bw; and with Jotham, Hilou, Ouedraogo, Sombie, and Traore (2018), who

administered extracts of *A. hispidum* and recorded a significant decrease in ALAT. This contradiction may be due to the form and dose of spiruline used.

Meanwhile, a non-significant decrease in total cholesterol and urea (at 1.2%) and a rise in creatinine levels were observed in spiruline-treated animals compared to those given the control feed. These findings are in agreement with Cimanga et al. (2015), who administered oral doses of 0.5 and 1 g/kg bw aqueous extract of *Triclisia gille*tii to Wistar rats.

Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body, depending on muscle mass (Zuo, Wang, Zhou, Sachdeva, & Ruelos, 2008). The increase in creatinine level found in this study could be due to dysfunction of glomerules, which are the structures responsible for renal filtration, while that in urea could be explained by the increase in protein catabolism due to the high synthesis of the enzyme arginase, which intervenes in the urea production.

Equally, an increased rate of total serum proteins was noticed in animals that received the highest dose of spiruline powder (1.2%). These results are in agreement with those of Kameni (2011), who observed an increase in the rate of total serum protein in rats treated with an aqueous extract of *Nymphaea lotus* at a dose of 75 mg/kg bw. Such a rise in serum protein concentration may be due to enhanced digestibility of proteins by spiruline. These results suggest that plant extracts could improve OS via their free-radical-scavenging mechanism, which could be linked to the effects of bioactive constituents including phenols, flavonoids, and alkaloids present in plant extracts.

Assessment of hematological characteristics can be used to explain the hematological functions of a chemical compound or plant extracts in an organism. Blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents (Olafedehan et al., 2010). Spiruline has immunological properties (Shih, Cheng, Wong, Kuo, & Chou, 2009) and phycocyanine, which it contains, has the capacity to stimulate the production of cells within bone marrow, with a direct consequence which is the stimulation of synthesis of red blood cells, white blood cells, and platelets (Le Bail, 2010) necessary for the survival of rabbit kids. Also, certain polysaccharides possess immunostimulatory and immunoregulatory properties (Evets, 1994), as in the case of the spirulan contained in spiruline (Rechter et al., 2006). This could explain the increase found in the numbers of red blood cells, white blood cells, and platelets and levels of haemoglobin and hematocrit recorded in this study in rabbits given feed supplemented with spirulina, with respect to the control. The increment in hematocrit and haemoglobin can only have arisen as a consequence of the increase in red blood cells; and that of lymphocytes and monocytes a result of the rise in white blood cells. These results are similar to those obtained by Ngoula et al. (2014) in male guinea pigs treated with essential oil of guava leaves; by Ezejiofor and Orisakwe (2017), who administered aqueous leaf extract of *Costus afer* at doses of 750, 1150, and 2250 mg/kg bw to rats; and by Cimanga et al. (2015) who used an aqueous extract of *Triclisia gille*tii in Wistar rats at an oral dose of 5 g/kg bw.

The results registered in the present work indicate a decline in SOD and CAT activities and MDA levels, with an increase in reduced glutathione in rabbits given spiruline powder with respect to the control, though these were not significant. This finding iss similar to a report by Vemo et al. (2017), who treated guinea pigs with ethanolic extract of *B. engleriana* leaves and obtained a significant decrease compared to those not receiving the extract.

Similar observations were made by Ngoumtsop et al. (2017) in Japanese quail receiving 200 mg/kg bw of aqueous leaf extract of *Persea americana*, by Babatunji et al. (2016) after treatment of rats with an aqueous extract of *Aframomum melegueta* (400 mg/kg bw), and by Jotham et al. (2018) in rats treated with *Acanthospermum hispidum* extract (250 mg/kg bw). These mitigate the antioxidant capacity of plant extracts, which can be attributed to their bioactive components such as phenols, flavonoids, and alkaloids.

The major enzymatic antioxidants of the lungs are SOD, CAT, and glutathione peroxidase. SOD ensures the elimination of the superoxide anion, the first toxic species formed from oxygen. It thus provides the first line of defense against OS. Low values of SOD can be explained by low levels of these trace elements. In the case of OS, SOD will behave in two different ways. Initially the body will respond to moderate OS by overexpressing SOD. If the stress persists and produces massive reactive oxygen species (ROS), SOD will be destroyed and its concentration will drop. Like SOD, seleno-dependent glutathione-peroxidase or reduced glutathione will behave in two different ways faced with OS. Paradoxically, excess concentration of SOD can be dangerous because, in this case, it is at the root of overproduction of hydrogen peroxide (Birben et al., 2012).

Knowing that SOD behaves in two ways when faced with OS, the slight decrease found in this study may be attributed to the fact that excessive free radicals may have been produced, thereby destroying this antioxidant enzyme and reducing its concentration.

Polyunsaturated fatty acids in cell membranes are the primary target of ROS (Birben et al., 2012), resulting in the formation of lipid peroxides (Meagher & FitzGerald, 2000). Lipid peroxidation yields MDA, which is generally a product of the oxidative decomposition of unsaturated lipids (Haj-Mouhamed et al., 2003).

At birth, the system of rabbit kids is very rich in lipids and is thus potentially susceptible to thre influence of free radicals. Some components of spirulina, including phenolics, phycocyanine, and tocopherol, possess antioxidant activities (Guan et al., 2009; Patel, Mishra, & Ghosh, 2006) and may played a role in protecting kids from free radicals. This can be demonstrated by the decrease in MDA level and increased concentration of reduced glutathione observed in spirulina-treated groups in the current study. MDA is a product of the oxidative decomposition of unsaturated lipids (Haj-Mouhamed et al., 2003), while glutathion is mainly used as a substrate of glutathion-peroxidase that ensures the elimination of lipid peroxides. Thus, the values recorded for these two OS markers show low lipid peroxidation in females fed with rations containing spiruline powder.

5. CONCLUSION

The present study shows that supplementation of feed with spiruline powder improved hematological parameters, as seen in white blood cells, and biochemical parameters, as indicated by an increase in total protein but no significant change in most parameters as well or in OS markers. However, there was a general tendency of improvement in the parameters considered in animals that received feed containing spiruline powder, in a dose-dependent manner. Thus, supplementing feed with a higher dose of spiruline powder may protect rabbits from OS to which they are exposed from various sources.

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REFERENCES

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121-126.
AFSSA (French Food Safety Agency). (2007). Proposals for an evaluation process for "new" substances or products intended for animal feed special case of plant-based substances and products.
Babatunji, E. O., Basiru, O. A., Oluwafemi, A. O., Habiba, M. M., Sunday, A. O., & Abiodun, A. O. (2016). Ameliorative potential of Aframomum melegueta extract in cadmium-induced hepatic damage and oxidative stress in male Wistar rats. *Journal of Applied Pharmaceutical Science*, *6*(7), 094-099. Available at: https://doi.org/10.7324/japs.2016.60714.
Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, *5*(1), 9-19.
Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry*, *42*(9), 1931-1937. Available at: https://doi.org/10.1021/jf00045a019.
Chowdhury, N., Islam, W., & Khalequzzaman, M. (2009). Insecticidal activities of stem bark extracts from Vitex negundo L. against Tribolium castaneum (Herbst). *Journal of Bio-Science*, *17*, 63-70. Available at: https://doi.org/10.3329/jbs.v17i0.7103.
Cimanga, K. R., Kikweta, M. C., Mbamu, M., Kambu, K. B., Apers, O., Vlietinck, A. J. S., & Pieters, L. (2015). Assessment of acute and subacute toxicological profiles of the aqueous extract (decoction) from the leaves of Triclisia gilletii (De Wild.) Staner (Menispermaceae) in Wistar rats. *Scholars Academic Journal of Pharmacy*, *4*(7), 351-357.
Deutcheu, N. S. (2016). *Effects of aqueous spirulina extract (Spirulina platensis) on reproductive performance in rabbits (Oryctolagus cuniculus)*. Master of Science Thesis. Faculty of Agronomy and Agricultural Sciences. University of Dschang, Cameroon.
Djaghoubi, A. (2013). *Impact of the salinity of the waters of the Ouargla region on the behavior of Spirulina "Spirulina platensis" (Tamanrasset strain)*. Academic / Professional Master thesis, Kasdi Merbah Ouargla University.
Evets, L. (1994). Means to normalize the levels of immunoglobulin E, using the food supplement spirulina. Patent (19) RU (11) 2005486. Russia.
Ezejiofor, A., & Orisakwe, O. (2017). Evaluation of protective effect of aqueous leave extract of Costus afer on female albino Wistar rats exposed to lead acetate. *EC Pharmacology and Toxicology*, *4*(2), 75-92.
FAO/WHO. (2009). Seventieth rapport of joint FAO/WHO expert committee on food additives. WHO Technical Report Series; No. 954.
Guan, X., Zhang, W., Zhang, X., Li, Y., Wang, J., Lin, H., & Qin, S. (2009). A potent anti-oxidant property: Fluorescent recombinant α-phycocyanin of Spirulina. *Journal of Applied Microbiology*, *106*(4), 1093-1100. Available at: https://doi.org/10.1111/j.1365-2672.2008.04051.x.
Haj-Mouhamed, D., Ezzaher, A., Neffati, F., Douki, W., Gaha, L., Dalle-Donne, I., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*, *329*(1-2), 23-38. Available at: https://doi.org/10.1016/s0009-8981(03)00003-2.
Jotham, N. D. Y.-p., Hilou, A., Ouedraogo, N., Sombie, E. N., & Traore, T. K. (2018). Phytochemistry, antioxidant, and hepatoprotective potential of Acanthospermum hispidum DC extracts against diethylnitrosamine-induced hepatotoxicity in rats. *Medicines*, *5*(2), 42. Available at: https://doi.org/10.3390/medicines5020042.
Kader, A., & Kalapuram, S. (2017). Ameliorative effect of Emblica officinalis in potassium dichromate induced toxicity in rats. *European Journal of Pharmaceutical and Medical Research*, *4*(3), 267-274.
Kameni, P. M. (2011). *Evaluation of the effects of the aqueous extract of the flowers of Nymphaea lotus L. (Water Lilies) on the reproductive function of normoglycemic and type 1 diabetic rats*. Masters Thesis, University of Yaoundé. (1)
Le Bail, D. (2010). The blue jewel of spirulina: Phycocyanin, Nutritherapy (pp. 83). BS No. 122.
Meagher, E. A., & FitzGerald, G. A. (2000). Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radical Biology and Medicine*, *28*(12), 1745-1750. Available at: https://doi.org/10.1016/s0891-5849(00)00232-x.
Misra, H. P., & Fridovich, I. (1972). The generation of superoxide radical during the autoxidation of hemoglobin. *Journal of Biological Chemistry*, *247*(21), 6960-6962. Available at: https://doi.org/10.1016/s0021-9258(19)44679-6.
Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochemistry and Biophysics Acta (BBA) -General Subjects*, *582*(1), 67-78. Available at: https://doi.org/10.1016/0304-4165(79)90289-7.
Ngoula, F., Nouboudem, S. C., Kenfack, A., Tadondjou, C. D., Kana, J. R., Kouam, J.-M., . . . Tchoumboue, J. (2014). Effect of guava (Psidium guajava) leaves essential oil on some reproductive parameters in male guinea pig (Cavia porcellus). *Biological Systems*, *3*(1), 1-4. Available at: https://doi.org/10.4172/2329-6577.1000125.

Ngoumtsop, V., Ngoula, F., Kenfack, A., Mutwedu, B., Nguemmogne, T., Tchoffo, H., & Manjeli, Y. (2017). Effects of oxidative stress induced by Antouka Super (Insecticide)® on Some Reproductive Parameters of Male Japanese Quail (Coturnixcoturnix japonica) and Mitigation Strategies Using Aqueous Leaves Extract of Persea americana. *Global Veterinary*, *18*(4), 242-249.

Olafedehan, C. O., Obun, A. M., Yusuf, M. K., Adewumi, O. O., Oladefedehan, A. O., Awofolaji, A. O., & Adeniji, A. A. (2010). *Effects of residual cyanide in processed cassava peal meals on hematological and biochemical indices of growing rabbits.* Paper presented at the Proceedings of 35th Annual Conference of Nigerian Society for Animal Production.

Ould, B. T., Bouchabchoub, A., Massoui, M., & Elyachioui, M. (2013). Cultivation and production of Spirulina platensis in domestic wastewater. *Larhyss Journal*, *14*, 107-122.

Pastre, J. O. (2005). *Interest of antioxidant supplementation in the diet of domestic carnivores.* Veterinary Doctorate Thesis. National Veterinary School of Toulouse.

Patel, A., Mishra, S., & Ghosh, P. (2006). Antioxidant potential of C-phycocyanin isolated from cyanobacterial species Lyngbya, Phormidium and Spirulina spp. *Indian Journal of Biochemistry & Biophysics*, *43*(1), 25-31.

Poljsak, B., & Milisav, I. (2013). Aging, oxidative stress and antioxidants. *InTech*, *14*, 331-356.

Razafindrajaona, J. M., Rakotozandriny, J. N., Randria, J. N., & Ramampiherika, K. D. (2010). Study of the nutritional performance of Madagascar spirulina (Spirulina platensis, variety Toliarensis) on mice, ERAILS Madagascar.

Rechter, S., Konig, T., Auerochs, S., Thulke, S., Walter, H., Dornenburg, H., & Marschall, M. (2006). Antiviral activity of Arthrospira-derived spirulan-like substances. *Antiviral Research*, *72*(3), 197-206.

Sguera, S. (2008). *Spirulina platensis and its constituents; nutritional interests and therapeutic activities.* Doctoral Thesis, Henri Poincare University - Nancy 1.

Shih, C.-M., Cheng, S.-N., Wong, C.-S., Kuo, Y.-L., & Chou, T.-C. (2009). Antiinflammatory and antihyperalgesic activity of C-phycocyanin. *Anesthesia & Analgesia*, *108*(4), 1303-1310. Available at: https://doi.org/10.1213/ane.0b013e318193e919.

Tamboura, H. H., Bayala, B., Pellicer, M. T., Zongo, D., Traoré, A., Ouédraogo, L., & Sawadogo, L. (2006). Estrogenic effects of the aqueous macerate of the leaves of Holarrhena floribunda (G. Don) in the ovariectomized spleen. *Base*, *10*(3), 173-180.

Valko, M., Morris, H., & Cronin, M. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, *12*(10), 1161-1208. Available at: https://doi.org/10.2174/0929867053764635.

Vemo, B. N., Kenfack, A., Ngoula, F., Nantia, E. A., Kodjio, N., Guiekep, A. J. N., & Teguia, A. (2017). Effects of ethanol extract of Bersama engleriana leaves on oxidative stress and reproductive parameters in male Guinea pig (Cavia porcellus) exposed to cypermethrin. *International Journal of Biological and Chemical Sciences*, *11*(5), 2243-2253. Available at: https://doi.org/10.4314/ijbcs.v11i5.23.

Zuo, Y., Wang, C., Zhou, J., Sachdeva, A., & Ruelos, V. C. (2008). Simultaneous determination of creatinine and uric acid in human urine by high-performance liquid chromatography. *Analytical Sciences*, *24*(12), 1589-1592. Available at: https://doi.org/10.2116/analsci.24.1589.