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EVALUATION OF CHEMICAL-NUTRITIONAL CHARACTERISTICS OF RAINBOW TROUT SAMPLES AFFECTED BY THE "RED-MOUTH DISEASE" COMPARED TO HEALTHY TROUT SAMPLES

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ABSTRACT

Enteric Redmouth (ERM) disease is a serious systemic infection due to a gram-negative bacterium (Yersinia Ruckeri) which causes significant economic losses in salmonid aquaculture all over the world. This disease is called "Red-mouth" for the reddening of the mouth. Other clinical manifestations of this disease are: exophthalmia, ascites and haemorrhage with ulceration of palate, gill and operculum resulting in anorexia. Although this disease has been reported in other fish species, rainbow trout (Oncorhynchus mykiss) are particularly susceptible to ERM. Rainbow trout is one of the most popular fish species in nature and in many countries it is also recognized as cultivated/farmed fish species, due to its fast growth and excellent nutritional quality. The target of this research being undertaken is to analyze the chemical-nutritional characteristics and evaluation of the oxidative processes in samples of rainbow trout fish affected by ERM compared to the healthy group. The results of analysis show significant differences concerning the contents of some qualitative and chemical-nutritional parameters in fish-meat samples belonging to animals that have recovered from the "red-mouth" disease and healthy ones. Despite this, the unhealthy rainbow trouts are good source of nutrition, similar than healthy trouts.

Keywords: Enteric redmouth disease, Rainbow trout, Fatty acids, Lipid oxidation, Malondialdehyde, Histamine.

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Contribution/ Originality

This study is one of the very few studies evaluating nutritional characteristics of rainbow trout samples affected by the "Red-Mouth Disease" compared to healthy trout samples

1. INTRODUCTION

Enteric Redmouth disease (ERM) is one of the most important diseases of salmonids [1]. The illness is caused by *Yersinia Ruckeri*, a Gram negative rod-shaped enterobacterium [2] which was first isolated from rainbow trout (*Oncorhynchus mykiss*) in the Hagerman Valley of Idaho, USA [3, 4] and it is currently found throughout North and South America, Europe, Australia, South Africa, the Middle East and China [5, 6]. Rainbow trout is a member of Salmonidae family, one of the most popular fish species in nature and it is also recognized as farmed intensively fish species for consumption, because of its fast growth and exceptional meat nutritive quality [7] rich in polyunsaturated acids.

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From a nutritional point of view, the trout's lipids contain an amount of DHA acid and polyunsaturated acids which is larger than in other farmed fishes, such as the sea bass and bream [8] and their composition is affected by the environment where they live, by their diet, by the fishing practice and preservation, as well as their health status during the fish farming operations. The polyunsaturated fatty acids, even if considered essential in the human diet, are susceptible to oxidation processes that lead to formation of aldehydes, ketones and alcohols, which are compounds associated with fish flavor.

Lipid oxidation in food is the greater non microbiological factor that can adversely affect the quality of the fish flesh . In addition, it depends both on the content of polyunsatured acids and on the camunt of lipid-soluble antioxidants (such as Vitamin E, coenzyme Q10, carotenoids etc.) and on water-soluble antioxidants (such as anserine, carnosine, vitamin C, etc.) which can be found in the fish itself. Some of these compounds derive from the fishes' diet, while others are directly contained in their muscle fibers, such as Coenzyme Q10, anserine and carnosine [9-12]. The amount of antioxidants which can be found in the fish flesh depends on their diet and on their intramuscular fat accumulation. In addition to lipid oxidation, histamine is another component responsible of qualitative decay of fish flesh.

Histamine concentration is considered an indicator of the freshness of fish. It is produced by the bacterial enzymatic decarboxylation catalyzed by histidine decarboxylase enzyme and L-histidine amino acid and is considered the most important biogenic amine in fish and the most toxic of the amines detected in food [13, 14].

The present study aims to assess meat quality of rainbow trout fishes affected by "red-mouth" disease compared to healthy fishes evaluating lipids, fatty acids, malondialdehyde (MDA) and histamine values.

2. MATERIAL AND METHODS

2.1. Experimental Design and Sampling

Forty-eight rainbow trouts (*Oncorhynchus mykiss*) (i.e., 24 healthy and 24 unhealthy) were sampled from an Italian trout farm, located in Abruzzo, in Province of Pescara.

The trouts came from the same farm, they had been fed with the same diet and raised under the same conditions. These fishes received the same commercial feed, with a known Fatty Acids profile (Tab.1).

According to the manufacturer, fishes received an amount of feed equal to 1.5-1.8% of their live weight, taking into account the environmental conditions (such as water temperature and dissolved).)

The fishes were randomly removed from the water when they reached a body weight of 300-350 g.

Among them, 24 *exhibited* signs of the *disease*, *like the* reddening of the mouth (unhealthy group) and the other 24 were picked from a healthy group (control group). The trouts, after the electrical stunning, as required by the law concerning animal protection during the slaughter, were cool to refrigeration temperature (under ice) and immediately dissected. The flesh sections of the farmed rainbow trouts were stored at -20°C until the analysis. Before the analysis, these samples were homogenized separately in order to obtain homogeneous specimens. The homogenate fishes were used for the analysis of intramuscular fat and fatty acids.

In order to evaluate the amount of histamine and oxidation processes of lipids using the TBARS test, the animals were kept for 5 days at 0-4 ° C and subsequently fish fillet samples, taken from the muscle (fillet) after having removed the skin, were analyzed.

Table-1. Chemical and fatty acid composition of the commercial diets fed to white trout

Chimical composition		
Dry matter	%	90,50
Crude protein	% DM	46,41
Crude fats	"	24,31
Crude fiber	"	3,10
Ash	"	6,30
Calcium		0,88
Posphorus		0,83
Sodium		0,33
Fatty acid		**(%)**
C14:0		3,44
C16:0		16,24
C18:0		3,87
C20:0		1,01
SFA		**24,56**
C14:1		0,20
C16:1 n7		3,45
C18:1 n9		19,89
C18:1 n7		1,95
C20:1		1,01
C22:1		0,20
MUFA		**26,70**
C18:2 n6		31,30
C18:3 n3		4,27
C22:6 n3		2,86
PUFA		**38,43**

Source: results obtained in the laboratory by the authors

2.2. Reagents

All the chemicals used were reagent grade commercial products and were used without any further purification. 2-thiobarbituric acid (TBA) (Sigma-Aldrich, Italy) in acetic acid 90% (Carlo Erba, Italy); trichloroacetic acid (TCA) (Carlo Erba, Italy) in distilled water; perchloric acid ($HClO_4$) (Carlo Erba, Italy) in distilled water; butylated hydroxytoluene (BHT) (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); sodium hydroxide (NaOH) (Carlo Erba Italy); Fatty Acid Methyl Ester (FAME) (Sigma–Aldrich, Italy).

2.3. Lipid extraction and Fatty Acid Analysis

Total lipids were extracted with a mixture of chloroform/methanol (2/1, v/v) from fishes using the method of Folch, et al. [15]. In order to go on with the analysis of fatty acids,, the total lipids extracted through the method of Folch were transmethylated into methyl esters (FAMEs) at room temperature by using potassium hydroxide (KOH) 2 M in methanol. FAME composition was determined by gas chromatography using gas chromatograph Perkin Elmer Auto System XL with flame ionisation detection (FID) equipped with a Varian column CP-SIL 88 of 100m (Chrompack Capillary Column). The carrier gas was hydrogen. Oven temperature programming was as follows: 160°C held for 3 min; 175°C at 3°C/min, held for 25 min; 220°C at 3°C/min, held for 40 min, 160°C at 10°C/min. Fatty acid identification was carried out with standard mixture and fatty acid values were expressed in percentage.

2.4. Histamine Analysis

Histamine analysis was carried out in RP-HPLC. 5g of fish were weighted and homogenized in perchloric acid ($HClO_4$) 0,4 M with Ultra-Turrax T25 at 10000 rpm for 1 min and centrifuged at 3000rpm for 10 min. 10 ml of supernatant were filtered with filters of cellulose acetate 0,45 µm. Histamine was derivatized with dansil-cloride in basic ambient: 0,5 ml of perchloric extracted were added with 0,5 ml HClO4, 200 µl of sodium hydroxide (NaOH)

2M and 300 µl of saturated solution of sodium bicarbonate. After homogenization, 1ml of dansil-cloride 1% in acetone was added. The mixture was heated at 40°C for 50 min. After having added 150 µl of NaOH 30%, the sample was left in a dark place for 1 h. Chromatographic separation was performed with a RP-18 column at 40°C. Mobile phase was constituted by methanol-water (80:20 v/v) and the flux was 1 ml/min. UV detection was performed at 254 nm. Limit of detection was 4mg/Kg (ratio S/N ≥3). Histamine concentration was calculated from external standard curves.

2.5. Determination of Muscle Fatty Acid Oxidation (Tbars Test)

Oxidation of samples was carried out with the 2-thiobarbituric acid (TBA) distillation method. The TBARs test was performed as described by Tarladgis, et al. [16] except for he butylated hydroxytoluene (BHT) that was added before homogenization. Fish (6-6,5 g) was added with 500 µl of BHT (0,01%) dissolved in methanol and homogenized with 50 ml of aqueous trichloroacetic acid (TCA) 5%, with UltraTurrax T25 at 4000 rpm for 5 min. Homogenate was distilled and two ml of distilled were supplemented with two ml of 0,02 M TBA in acetic acid (90%). This mixture was heated in a water bath at 80°C for 1 hour and then cooled for 10 min with cold tap water. The absorbance was determined by JENWAY 6305 UV/vis Spectrophotometer at 534 nm against a blank containing 2 ml of distilled TCA (with 500 µl of BHT) and 2 ml of 0,02 M TBA solution. The TBA number was calculated from standard curves.

3. STATISTICAL ANALYSIS

The mean value and standard deviation were established using one way ANOVA test.

Differences were significant for P≤0,05 and highly significant for P≤0,01. All statistics were performed using SPSS for Windows.

4. RESULTS AND DISCUSSION

4.1. Total Fat and Fatty Acid Profile

Total fat data were reported in Tab.2. The obtained results demonstrated a quantity of lipids in meat statistically significantly lower (p≤0,05) in the group of animals affected by disease.

This can probably be justified by a lower food consumption caused by the lesions present in the mouth's mucosa in the phases of the disease. The fatty acid composition of the rainbow trout is presented in Table 2.

The fatty acids analyzed were grouped in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).

Palmitic acid (C16:0) was the main fatty acid in both groups (healthy and unhealthy) of the rainbow trouts. Furthermore, among saturated fatty acids (SFA), except for the palmitic acid, the most abundant fatty acids were myristic acid (C14:0) and stearic acid (C18:0), according to Tkaczewska, et al. [17]. *No differences have been observed concerning saturated fatty acids between the two groups.*

Among monounsaturated fatty acids (MUFA), oleic acid (C18:1*n-9*), palmitoleic acids (C16:1*n-7*) and erucic acid (C22:1) were the predominant fatty acids. These fatty acids showed significant differences within the two examined groups (p≤0,01).

Linoleic acid (C18:2 *n-6*) and docosahexaenoic acid (DHA) (C22:6 *n-3*) were the most abundant polyunsaturated fatty acids (PUFA). Similar results have been reported by Ehsani, et al. [18] and by Zakipourm, et al. [19]. Linoleic acid is more present in the healthy group, while the docosahexaenoic acid (DHA) (C22:6 *n-3*) in the unhealthy one, showing statistically significant differences (p≤0,01).

Table-2. *Total fat percentage and* intramuscular fatty acid composition in healthy and unhealthy groups *(% of total fatty acid methyl esters).*

	Trouts	
	Healthy	***Unhealthy***
Total fat %	[a]2.65	[b]2.18
Fatty acids %		
C14:0	3.82	4.57
C16:0	22.54	22.07
C18:0	4.54	4.46
C20:0	0.69	0.68
SFA	31.59	31.78
C14:1	0.31	0.35
C16:1 n7	[A]3.71	[B]4.46
C18:1 n9	[A]20.95	[B]19.21
C18:1 n7	[a]2.24	[b]2.65
C20:1	[a]0.58	[b]0.71
C22:1	[A]3.30	[B]3.89
MUFA	31.09	30.92
C18:2 n6	[A]20.01	[B]17.40
C18:3 n3	2.41	2.26
C22:5 n3	1.02	1.43
C22:6 n3	[A]13.88	[B]16.21
PUFA	37.32	37.30

A,B = (p≤0,01); a,b = (p≤0,05).

The ratio between polyunsaturated (PUFA) and saturated (SFA) fatty acids as well as the P/S index are among the most reliable indicators of nutritional values. In particular, normal nutritional value for P/S index should be above 0,5 [20]. *A P/S index below 0.45 is considered inadequate because it can lead to hypercholesterolemia* [21]. *In our study P/S index resulted to be 1,18 and 1,17 in healthy and unhealthy trouts, respectively.*

Our results, according with Vranić and Đinović-Stojanović [22] *show that the* ratio between unsaturated (UFA) and saturated fatty acids (SFA) in unhealthy rainbow trout is 2,15, while it is 2,17 in the healthy group (Tab. 3).

Tab-3. Contents of SFA, MUFA, PUFA (% of total fatty acids), n–3, n–6, n–3/ n–6, P/S, UFA/ SFA ratios

	SFA	**MUFA**	**PUFA**	**n-3**	**n-6**	**n-3/n-6**	**P/S**	**UFA/SFA**
Healthy trouts	31,59	31,09	37,32	2,41	20,01	0,12	1,18	2,17
Unhealthy trouts	31,76	30,94	37,30	2,26	17,4	0,13	1,17	2,14

Source: results obtained in the laboratory by the authors

4.2. Histamine Analysis

In this study, histamine level is one of the quality parameters examined because it is an important index of fish freshness and an indicator of its edibility and this amine as well as other present in the muscle tissue of chickens are also produced due to tissue enzymes. Meat is very susceptible to chemical and physical changes and to biological agents; among them, microorganisms and endogenous or microbial enzymes can make the meat unsuitable for consumption. *In fact, fishes containing high levels of histamine cause an acute illness called Scombroid fish poisoning in human beings* [23]. *In relation to the content of histamine (Tab.4), the results suggest a significant difference (p≤0,01) between the two groups with higher values in the unhealthy group compared to healthy one.*

This is probably due to deterioration of the meat, which is faster in the unhealthy fishes infected by the pathogenic bacterium Yersinia Ruckeri, that is able to synthesize h*istamine from the amino acid histidine. Nevertheless, both groups present histamine levels below the recommended limit, required to prevent toxic effects* [24, 25].

Table-4. Content of histamine (mg/Kg) in the two groups

Trouts		
	Healthy	Unhealthy
Histamine (ppm)	$^{A}5.35 \pm 0,55$	$^{B}6.60 \pm 0,63$

A,B = (p≤0,01); a,b = (≤0,05).

4.3. Lipid Oxidation Determination

The determination of the oxidants was performed on muscle samples collected after 3 days of storage at 0-4 ° C.

The oxidation of fats depends on a number of factors, apart from the level of polyunsaturated fatty acids, among which the pro-oxidant and anti-oxidant concentrations. Oxidation of polyunsaturated fatty acids (PUFA) leads to the formation of hydro- and endo-peroxides, which undergo fragmentation in order to yield a wide range of reactive intermediates, including alkanals, alkenals, hydroxyalkenals and MDA. The obtained results of this do not show significant differences between the two groups although in the "healthy" group the mean value average value is slightly lower (Tab.5). Higher MDA values in muscle of affected trouts may depend on the minor amount of lipid-soluble antioxidants accumulated in the tissues and on the ones introduced by the diet.

Tab-5. Content of MDA (mg MDA/ kg fillet) in healthy and unhealthy experimental groups

Trouts		
	Healthy	Unhealthy
MDA (ppm)	$0.51 \pm 0,048$	$0.60 \pm 0,053$

A,B = (p≤0,01); a,b = (p≤0,05).

5. CONCLUSION

The results show that trouts affected by the "red mouth" disease have a lower amount of fat than the healthy ones and a greater production of histamine during the storage, showing tendency to an easy deterioration and a difficult preservation. Among unsaturated fatty acids, linoleic acid (C18:2 *n-6*) and oleic acid (C18:1 n-9) are more present in the healthy group compared to the unhealthy one, *but it is important to underline that the ratio between polyunsaturated (PUFA) and saturated (SFA) is adequate in both groups..*

Finally, results regarding lipid oxidation show no significant variation between the two groups.

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REFERENCES

[1] M. T. Horne and A. C. Barnes, *Enteric redmouth disease (Yersinia Ruckeri). In: Woo PTK, Bruno DW, (Eds). Fish diseases and disorders. Viral, bacterial and fungal infections*. Wallingford: CABI Publishing, 1999.

[2] E. Tobback, A. Decostere, K. Hermans, F. Haesebrouck, and K. Chiers, "Yersinia ruckeri infections in salmonid fish," *Journal of Fish Diseases*, vol. 30, pp. 257-68, 2007.

[3] M. D. Furones, C. J. Rodgers, and C. B. Munn, "Yersinia ruckeri, the causal agent of enteric redmouth disease (ERM) in fish," *Annual Review of Fish Diseases*, vol. 3, pp. 105-125, 1993.

[4] A. J. Ross, R. R. Rucker, and W. H. Ewing, "Description of a bacterium associated with redmouth disease of rainbow trout (Salmo Gairdneri)," *Canadian Journal of Microbiology*, vol. 12, pp. 763–770, 1966.

[5] S. Karatas, A. Candan, and D. Demircan, "Enteric red mouth disease in cultured rainbow trout (Oncorhynchus Mykiss) on the black sea coast of Turkey," *Israeli Journal of Aquaculture- Bamidgeh*, vol. 56, pp. 226-231, 2004.

[6] W. D. Shaowu, L. Hongbai, and L. Tongyan, "Isolation of Yersinia ruckeri strain H01 from farm-raised amur sturgeon acipenser schrencki in China," *Journal of Aquatic Animal Health*, vol. 25, pp. 9–14, 2013.

[7] Simonović, *Ribe Srbije. NNK International, Zavod za zaštitu prirode Srbije*. Beograd: Biološki Fakultet, 2001.

[8] S. Testi, A. Bonaldo, P. P. Gatta, and A. Badiani, "Nutritional traits of dorsal and ventral fillets from three farmed fish species," *Food Chemistry*, vol. 98, pp. 104–111, 2006.

[9] G. Martino, L. Grotta, and V. Ponzielli, "Influence of dehydrated medicago sativa on quality characteristics of marchigiana beef," *Animal Review*, vol. 1, pp. 37-44, 2014.

[10] G. Martino, C. Mugnai, D. Compagnone, L. Grotta, M. Del Carlo, and F. Sarti, "Comparison of performance, meat lipids and oxidative status of pigs from commercial breed and organic crossbreed," *Animals*, vol. 4, pp. 348-360, 2014.

[11] G. Martino, M. N. Haouet, C. Reali, O. Olivieri, and F. Valfre, "Coenzyme Q10 and its relationship to other nutritional components of animal products," *Italian Journal of Food Science*, vol. 7, pp. 19-26, 1995.

[12] G. Martino, H. M. Naucer, S. Marchetti, L. Grotta, and V. Ponzielli, "Effect of Vitamine E supplementation on egg yolk quality and oxidative stability," *Asian Journal of Agriculture and Food Science*, vol. 02, pp. 248-255, 2014.

[13] P. Izquierdo, M. Allara, G. Torres, M. Sánchez, G. Peña, and M. Sangronis, "Aminas biógenas y crecimiento bacteriano en carne de Hamburguesas," *Revista Científica*, vol. 14, pp. 7-12, 2004.

[14] G. Martino, S. Marchetti, L. Grotta, and V. Ponzielli, "Biogenic amines content f poultry breast stored under different conditions at 4°C from two different industrial genotype chickens slaughtered at 3,5-4,0 kg of live weight," *Agriculture & Food*, vol. 3, pp. 12-20, 2015.

[15] J. Folch, M. Lees, and S. G. H. Sloane, "A simple method for the isolation and purification of lipids from animal tissues," *Journal of Biological Chemistry*, vol. 226, pp. 497-509, 1957.

[16] B. G. Tarladgis, B. M. Watts, M. T. Younathan, and L. Dugan, "A distillation method for the quantitative determination of malonaldehyde in foods," *Journal of the American Oil Chemists Society*, vol. 37, pp. 44-50, 1960.

[17] J. Tkaczewska, P. Kulawik, and W. Migdał, "The quality of rainbow trout (Oncorhynchus Mykiss) cultured in various Polish regions," *Annals of Animal Science*, vol. 15, pp. 527–539, 2015.

[18] A. Ehsani, M. S. Jasour, and M. Khodayari, "Differentiation of common marketable-size rainbow trouts (Oncorhynchus Mykiss) based on nutritional and dietetic traits: A comparative study," *Journal of Applied Animal Research* vol. 41, pp. 1–5, 2013.

[19] R. I. E. Zakipourm, M. Jasour, A. Ehsani, M. A. Rahnama, and A. Arshadi, "Dietary supplementation versus direct postmortem addition of α-tocopherol acetate on fatty acid composition of rainbow trout (Oncorhynchus Mykiss) fillets during refrigerated storage," *International Food Research Journal*, vol. 19, pp. 1145–1151, 2012.

[20] B. Žlender and L. Gašperlin, "Značaj i uloga lipida mesa u bezbednoj i balansiranoj ishrani," *Tehnologija Mesa*, vol. 46, pp. 11–21, 2005.

[21] J. Santos-Silva, R. J. B. Bessa, and F. Santos-Silva, "Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat," *Livest Production Science*, vol. 77, pp. 187–194, 2002.

[22] D. Vranić and J. Đinović-Stojanović, *Rainbow trout (Oncorhynchus Mykiss) from aquaculture– meat quality and importance in the diet, Spirić Aurelija1 tehnologija mesa*. Osnivač i Izdavač: Institut za Higijenu i Tehnologiju Mesa, 2011.

[23] L. Auerswald, C. Morrom, and A. Lopata, "Histamine levels in seventeen species of fresh and processed South African seafood," *Food Chemistry*, vol. 98, pp. 231-239, 2006.

[24] A. Önal, "A review: Current analytical methods for the determination of biogenic amines in foods," *Food Chemistry*, vol. 103, pp. 1475-1486, 2007.

[25] S. Pons-Sànchez-Cascado, M. T. Veciana-Nogués, S. Bover-Cid, A. Mariné-Font, and M. C. Vidal-Carou, "Use of volatile and non-volatile amines to evaluate the freshness of anchovies stored in ice," *Journal of the Science of Food and Agriculture*, vol. 86, pp. 699-705, 2006.