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Fish feeding

EFFECT OF UREA INTRODUCED INTO FEED ON STRUCTURAL CHANGES AND MUCOPOLISA CHARIDES CONTENT IN BRANCHIAL EPITHELIUM OF RAINBOW TROUT (SALMO GAIRDNERI RICHARDSON)

WPŁYW MOCZNIKA WPROWADZONEGO DO PASZY NA ZAWARTOŚĆ MUKOPOLISACHARYDÓW W NABŁONKU SKRZEL PSTRĄGA TĘCZOWEGO (SALMO GAIRDNERI RICHARDSON)

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> Histochemical and histological surveys on gills of rainbow trout (Salmo gairdneri Rich.) fed control feed and feed with 2 and 4% of urea added were carried out. Irrespective of an urea amount added to feed, microscopic structure schedule of gills was identical. However, in case of some structural elements essential differences were noted, particularly for fish fed feed with 2% of urea added. Thickening of branchial epithelium on blood vessels side and increase in number and size of mucous cells were observed. Within the branchial epithelium mucous cells, the neutral mucopolisaccharides were most numerous for all experimental variants, while acidic ones dominated in fish fed feed with 2% of urea added.

INTRODUCTION

For many years now, science has dealed with the problem of replacement and supplement of protein in feedstuffs with nonprotein nitro-compunds (urea and ammonium compounds). The problem has been succesfully solved in case of ruminants. In some countries (mostly in USA and USSR) ruminants are being fed houndreds thousands tonns of urea with good results (Chomyszyn, 1961). It gave hint to research workers for further surveys on urea as a relatively cheap nitrogen source in feeding other animals, including fish. De Long et al. (1959) were first to apply nonprotein nitrogrn compounds in fish feeding. Their surveys indicated the salmon to be unable to use area as nitrogen source for growth. Nehring (1963) tested an influence of urea introduced to water in conjuction with an urease on freshwater fish and carried out a toxicity test of urea after its per os introduction to the alimentary canal of fish. No toxic effect of ammonium produced during an enzymatic urea decomposition was noted for carp and trout after joined introduction of urea and urease to the alimentary canal of fish. Besides, confirmation to other research workers results was given (Schäperelaus, 1952; Allan, 1955). The conclussion to that was, that toxic activity of ammonium was mainly through gill and skin epithelium causing there several histopatological changes (Flis, 1968 I, II; Waluga and Flis, 1971). The water solutions of urea, however, were being practically untoxic for fish. According to Krauss (1936) carp and rainbow trout were resistant to the urea concentration equal to 20000 mg/l for many hours. Scheuring and Leopoldseder (1934) stated, that the 4% urea concentration caused death, within 4-6 hours, in some fish species only, while others resist to that concentration much longer.

Surveys on the urea application in fish feeding were carried out by Privolniev et al. (1965) and Kniazieva (1969, 1970). The highest weight increase of the rainbow trout was obtained for the feed with 10% addition of urea.

Poles have been involved in the above problem also. Dabrowski and Wojno (1978 a,b,c,d) carried out surveys on usage of non – protein nitrogen compounds (urea and ammonium citrate) in carp feeding. They indicated the urea had improved the nitrogen balance of that fish and influenced benefficially its growth. Efforts as to use animal waste materials (including urea) as the ingredients of standard value in feedstuff granules production for rainbow trout (Wojno in print) have been undertaken. According to an opinion of the majority of authors, influence of urea present in feed on some fish growth seems to be undoubtfull. However, up to the date, works includes no information on possible changes the urea can cause within the tissues of fish osmoregulatory organs, particularly in gill epithelium.

Many research workers dealed with gill epithelium of fish and its slime cells secretion. Porcelli and Novell (1970) tested development, distribution and histochemical data concerning cells of the *Salmo fario* gill epithelium excreting slime. A detailed analysis of the ultrastructure of the rainbow trout gill epithelium was done by Morgan and Tovell (1973) and Morgan (1974). Five types of cells were distinguished: light and dark unspecialized epithelium cells, mucous cells, chloride cells and granulocytes. Histochemical analysis of the gill epithelium mucous cells indicated the mucopolisaccharides to be the main slime ingedient (Capuro, 1967; Porcelli and Novell, 1970). Neutral and acidic mucopolisaccharides were found in gill epithelium mucous cells of *Mugil cephalus* and *Anoptichthys jordani* as well as in gristle of gill arches of *Torpedo ocellata* and

Effect of urea ...

Seylliorhinus canicula (Zaccone, 1972, 1975). While testing acidic mucopolisaccharides in gills of eel (Anguilla anguilla (L)) Zbanyszek found out its presence in the mucous cells of outer layer of gill epithelium, in the gristle part of gill leaflet and also in the basic membrane. In gills of *Tilapia mossambica* the most numerous were, however, neutral mucopolisaccharides (Narasimham and Parvatheswarao, 1974). Colombo (1960) testing the mucous cells of eel gills noted a positive PAS reaction there. Histochemical surveys of the mucous cells of gill epithelium of *Tilapia shirana chilwae* were done also by Cockson (1970). He noted there the presence of acidic and neutral mucopolisaccharides. Mucous cells of *Carassius auratus* gills stained deeply by the PAS method (Jozuka, 1967).

Many authors tested also slime exerction by the gill epithelium of *Teleostei*. Smith (1929, 1930, 1931) pointed out to its role in the osmotic pressure regulation in *Teleostei* and Selachii, although he admitted the extranephritic salt (NaCl, KCl) excretion through the gill aparratus to be highly energetic. Philpott (1966) suggested the acid mucopolisaccharides might take part in ion exchange in fish and Whitelaw (1975) proved its share in the regulation of ammonium excretion. As for physiological function of slime, except the above mentioned regulatory function in osmotic exchangees, its presence in gills might have protect the epithelium against penetration of external substances through the gill aperture and function as lubricate (Zaccone, 1972). However osmotic pressure regulation can be mainly due to presence of chloride cells within the gill's area (Keys and Willmer , 1932; Copeland, 1950; Colombo, 1960), which other authors, namely Bevelander (1935), Parry, Halliday and Baxter (1959) take for the slime elements.

Because urea plays a significant role in an osmoregulators process in some fish (*Elasmobranchii*), it seems purposefull to test the effect of this compound enclosed into feed on the basic osmoregulatory fish organ – gills, and gill's epithelium in particular. It seems to be justified by the fact, that urea and product of its enzymatic decomposition – ammonia-acting as external agents towards fish gill's epithelium were already carefully tested by many authors, while only few have checked the effect of ammonia towards gill's epithelium when acting from inside. It has been known from many surveys, the epithelium, in young individuals in particular, not to be insensible to any changes in an external (Flis, 1968 a,b; Cockson, 1970; Waluga and Flis, 1971; Waluga, 1975; Skidmore and Tovell, 1972; Zbanyszek, 1975; Whitelaw, 1975; Dąbrowska, 1976; Narasimham and Parvatheswarao, 1974; Cykowska, 1978) or internal environment (Whitelaw, 1975). That was why it seemed purposefull:

- 1. To trace possible changes in gill's epithelium microstructure due to the urea administration per os and.
- 2. To test mucopolisaccharides activity expressed by its affinity degree to the Schiff's agent and alcian blue and how are being localized in gills of rainbow trout.

MATERIAL AND METHOD

The material used was the fry of rainbow trout (*Salmo gairdneri* Rich.) of the spring breed, cultured at the Fish Wronki (PGRyb Oleśnica). The fry was fed feed of a "starter"

type (feed's ingredients are given in Table 1), prepared by the feed's granules factory at the State Fish Farm "Oleśnica". To the feed 2 or 4% of fertilizer urea, produced by the Nitric Factory in Pulawy (including 46% of pure urea according to standards - 48% in fact) was added. Feeds analysis gave no urea in the control feed's batch "S" and 1.55% and 2.82% of urea for feeds batches signed "2" and "4", respectively.

Table 1

Feed	S	2	4 4
cod roe wheaten bran	30 25	30 25	30
fish meal	25	25	25 25
feed grade yeasts powdered milk	10 8	10	10 8
polfamix	2	2 	2
fertilizer urea	100° -	100 2	100 4

Ingredients of feeds used in the experiment

In ten rotary basins, about 1,500 l in volume each) $2 \times 2 \times 0.5 \text{ m}$) 512 fry units (about 1.5 kg) were placed in each one. Water flow was settled at about 201/min with average oxygen content in an inflow being 7 mg/l (which responded to 7.5 mg/l of water in the basin, with water level at about 30 cm and rotary speed around 10 cm/sec). For two weeks fry was given control feed "S". Next two basins kept as control ones, while others were used to test feed with 2 and 4% addition of the lime urea. Feed administered stated for 3% of an actual fish weight. Control measures were taken every two weeks and corrections in the amount of feed administered every week. Fish were fed twice a day, except for Sunday, when measures were taken. Water temperature being 19°C at the beginning lowered to 11°C during ten weeks of the experiment (Jakubowski in press).

Histological and histochemical surveys were carried out since autumn 1981 at Zoology Department, at the Institute of Biology WSP in Słupsk.

Gill segments were collected from the middle part of first gill arch of 20 fish from each of the three experimental groups. Then fixed parallely in either Bouin fluid or 10% neutralized formaldehyde. Fragments 10 μ m thick were stained with hematoxyline and eosine. As to differentiate mucopilisaccharides and glycogen digestion with malt diastasis was applied. As to identify neutral mucopolisaccharides PAS method of staining according to Mc Manus with Schiff's reagent was used, while acid mucopolisaccharides were identified with alcian blue according to Steedeman.

RESULTS

Observation of the rainbow trout gill's leaflets proved its general microscopic structure schedule to be identical for all the applied lime urea dose in feed. As for some structural elements, however, visible differences, both in number and quality were noted. It concerned mainly, both the respiratory epithelium and the mucous cells (Tab. 2). For fish fed feed with 2% lime urea the gill's epithelium thickness increase on both the afferent and efferent artery sides were respectively $12.9 \,\mu\text{m}$ and $2.4 \,\mu\text{m}$, when compared to control one. For rainbow trout fed feed "4" an average increase in epithelium thickness was $1.5 \,\mu\text{m}$, being observed on the afferent artery side only. Differences in numbers and sizes of mucousells were also observed. For fish fed feed "2", an average increase in number was 8.1 and $1.1 \,\mu\text{m}$ in size compared to the control ones, while for fish receiving feed with 4% lime urea added increase both in number and size was respectively 5.9 and $0.3 \,\mu\text{m}$.

Cytochemical studies indicated all three fish groups, namaly "S", "2", "4", to have very active gill epithelium cells when stained by PAS method according to Mc Manus, which differed visibly from other types of the epithelium cells (Tab. 3,). The differences became clear after staining with alcian blue according to Steedeman. Mucous cells of the individuals fed feed "2" gave strong reaction for acid mucopolisaccharides, while those fed feed "S" and "4" only a mild one (Tab. 3). Table 3 presents a complete data including histochemical data of other cells of gill epithelium, base membrane, gristle and connective tissue capsule.

Table 2

Tested object		Experiment					
		Control		- 2%		4%	
		x	±	x	±	x	± Dec 11 - 55
E pi thelium	1 . aa	31.5	4.5	44.4	2.4	33.0	3.8
thickness μm	2.ae	• 22.2	2.5	24.6	3.1	22.2	2.5
Number of mucous c	Number of mucous cells		4.9	26.6	3.4	24.4	3.7
Size of mucous cells µm	2. 	11.5	1.5	12.6	2.3	11.8	2.4

Changes within the gill epithelium of rainbow trout due to the urea present in feed

1. Afferent artery. 2. Efferent artery. n = 50

Type of mucopoli- saccharides	Histoche- mical method	Fixative	Experiment	Object of histochemical studies				
				Mucous cells c.m.	Epithelium cell c.ep.	Base membra. m.b.	gristle ch.	Connective tissue capsule. t.c.
Neutral Diastase mucopoli- PAS saccha- according rides to Mc Manus	10% for-	Standard	++++	-	++	+++	• + + +	
	according	maldohy- de Bouin	2%	++++	_	+++	++++	+++
		4%	++++	_	+++	++++	+++	
mucopoli- blue saccha- accord	Alcian		Standard	+ + (v.few)	_	+ .	· + +	-
	according to de	maldehy- de Bouin	y- 2%	+++	-	++	+++	+
			4%	++	_	+	+ +	+

Histochemical reactions demonstrating mucopolisaccharides activity in various cells and connective tissue of the rainbow trout gill leaflets

Activity levels: ++++ very strong; +++ strong; + weak; - negative.

Table 3

DISCUSSION AND CONCLUSSIONS

Gill function was numerously tested by Smith (1930), Keys and Willmer (1932), Zaccone (1972) and the authors agreed on gills being, due to their structure and fragile epithelium, deeply involved in ion exchange. Intensive slime excretion is characteristic gill's reaction to any change around. Mucopolisaocharides playing an important role in numerous and patological processes are main slime ingredient (Brimacombe, and Webber, 1964; Capuro, 1967; Porcelli and Novell, 1970). They function as boundary agents, influence regeneration processes as well as electrolite and water regulation within the extracellular fluids.

For some animals urea is being physiologically important compoud. Sometimes present in great ammounts, e.g.: Elasmobranchii fish, for which the urea concentration in blood keeps steady and on high level. According to the up to date publications, the authors opinion on the urea role in ion exchange is unequivocal. Schlieper (1963) states no increase in the chlorine ion excretion due to an urea, while Parry (1966) adds the urea enhances ions excretion. This problem hasn't been solved so far.

In the present work two histochemical tests were applied, which proved slime, often a mixture of various matters, to be produced mainly by the one cell mucous glands. A positive PAS reaction obtained may points out to the presence of glicogen or neutral mucopolisaccharides within the mucous cells or other structural elements. However, because the gill's scraps were subjected to malt diastas activity resulted in glicogen digestion it might have been the neutral mucopolisaccharides only. Some microscopic slides were stained with an alcian blue as to find out acidic mucopolisaccharides.

Domination of neutral mucopolisaccharides in branchial epithelium was stated, both in control and experimental individuals, similarly as did Cockson (1970), Narasimhama and Parvatheswarae (1974). Possibly urea in feed doesn't influence the number and activity of neutral mucopolisaccharides. Essential differences showed, however, while testing the acid mucopolisaccharides activity. The acidic mucopolisaccharides were most numerous within the mucous cells of branchial epithelium of rainbow trut fed feed with 2% of urea added. For individuals fed feed with 4% of urea their number resembled that for control ones. Presence of acid mucopolisaccharides in gills was noted also by Zbanyszek (1975) and Zaccone (1975). According to that and including results of surveys of others on various substances, e.g. salts (Jozuka, 1967), one can say, that with average concentrations of substances, gills- osmo- and ion-regulatory organ, increase its excretion activity resulted in higher acidic mucopolisaccharides production. Besides those substances take part in an active transport of ions and ammonium through cell membranes (Whitelaw, 1975). For higher concentrations, ions excess is being removed by diffusion (Maetz, 1964; Kniazieva, 1970). Putting excretion mechanisms in motion is attended by branchial epithelium microstructure changes. When histochemical structure is concerned differences, both, in quality and quantity were noted. The differences reffered to mucous cells (neir size and number) and respiratory epithelium (its thickness). Obtained results correspond partly with the data presented in Cykowska's work (1978), who studied gills microstructure of the rainbow trout adapted to marine water conditions.

It was stated, the mucous cells to be most numerous in the branchial epithelium of rainbow trout fed feed with 2% addition of urea. Their number, when compared to control was higher by 43.8% and size by 9.6%. As for the respiratory epithelium thickness, it grew bigger on both sides of the gill leaflet with 40.9% and 10.8% increase, respectively, on an afferent and efferent artery side. For individuals fed feed with 4% of urea added, number of mucous cells was higher by 31.9% and their diameter by 2.6%. Branchial epithelium thickness on the afferent artery side being higher by, only, 4.8% and for efferent one the same as for control individuals.

Obtained results of this work correspond with opinion of other research workers (Nehring, 1963; Kniazieva, 1970) on considerable adaptative possibilities of the rainbow trout to feedstuffs with syntetic nitrogen compounds added. Better growth of fish fed feed with urea addition attest to it. The adaptative abilities result from high functioning efficiency and platicity of the osmoregulatory organ – gills, and of its respiratory epithelium, in particular. (Smith, 1930; Cykowska, 1978). Urea within the fish body is being included into aminoacids cycles (Kniazieva, 1970), with ammonium as a by-product of it. Ammonium is being actively expelled by the acid mucopolisaccharides by way of counter-exchange with sodium, or, in case of an excess, by way of diffusion (Maetz, 1964; Whitelaw, 1975). Putting in motion excretion mechanisms causes changes within the branchial epithelium microstructure.

Our own results analysed and compared with available literature data let to draw following conclussions:

- 1. Urea introduced into feed cause no histopatological changes in the branchial epithelium of rainbow trout, while its solutions including arease do, when acting externally.
- 2. Urea used in the experiment caused reconstruction of the branchial epithelium microscopic structure, with notable and bigest changes stated for individuals fed feed with 2% of urea added.
- 3. Neutral and acidic mucopolisaccharides were identified in the mucous cells of branchial epithelium, in base membrane, gristle and connective tissue capsule.
- 4. Urea had no effect on the number of neutral mucopolisaccharides which dominated in all three exporimental variants.
- 5. Acidic mucopolisaccharides content increase within the mucous cells of experimental fish "2" was stated when compared to the control ones.
- 6. Increased content of acidic mucopolisaccharides within mucous cells of the respiratory epithelium of experimental fish "2" gives evidence to their increased exerction activity.

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WPŁYW MOCZNIKA WPROWADZONEGO DO PASZY NA ZAWARTOŚĆ MUKOPOLISACHARYDÓW W NABŁONKU SKRZEL PSTRĄGA TĘCZOWEGO (SALMO GAIRDNERI RICHARDSON)

STRESZCZENIE

Przeprowadzono badania histologiczne skrzeli narybku pstrąga tęczowego Salmo gairdneri Rich. karmionego paszą z dodatkiem mocznika 2% i 4% oraz kontrolną (bezmocznikową). Stwierdzono, że niezależnie od stosowanej ilości mocznika w paszy, ogólny plan budowy mikroskopowej jest jednakowy, jednakże w odniesieniu do niektórych elementów strukturalnych wystąpiły znaczne różnice. Zmiany te były szczególnie zauważalne u ryb karmionych paszą z dodatkiem 2% mocznika i dotyczyły one nabłonka skrzelowego i komórek śluzowych. Zaobserwowano zwiększenie ilości komórek śluzowych, ich rozmiarów oraz grubości nabłonka oddechowego.

Badania histochemiczne wykazały występowanie mukopolisacharydów obojętnych i kwaśnych w komórkach śluzowych nabłonka wielowarstwowego. Najliczniej występowały mukopolisacharydy obojętne, natomiast kwaśne najwyższy stopień aktywności wykazywały w komórkach śluzowych osobników karmionych paszą z dodatkiem 2% mocznika.

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