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Arginine effects on biochemical composition of sperm in rainbow trout, *Oncorhynchus mykiss*

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Summary

The present study investigated the effect of arginine on seminal plasma composition in rainbow trout. Male rainbow trout broodstocks (2500 ± 200 g) were fed five practical diets (each consisting of three triplicates) supplemented with Arginine at 0.50%, 1.50% and 2.00%. The control group were fed without arginine. Broodstock feeding lasted for 90 days, and then fish semen was sampled. Results indicated no significant differences in LDH, ALP, Fe^{2+} and phosphorous content among the different treatments. The lowest levels of AST and ALT and the highest levels of Ca^{2+} and Mg^{2+} ions were observed in the treatment fed with 1.50% arginine, which showed significant differences from other treatments ($P < 0.05$). Moreover, the amount of Cl^{-}, Na^{+} and K^{+} ions was significantly increased in the seminal plasma in fish fed diets containing arginine in comparison with the control. As the amount of arginine was increased, the levels of uric acid became significantly greater in contrast to urea and glucose levels. The highest amounts of cholesterol, fructose and total protein were observed in treatments fed on 2.00%, 0.50% and 1.00% arginine, respectively, showing significant differences from other treatments ($P < 0.05$). The highest pH value was assayed in the 1.50% arginine treatment. Results indicated that arginine had a potential efficacy on semen quality in rainbow trout broodstocks.

Keywords: seminal plasma, rainbow trout, biochemical composition, amino acids, reproduction

Introduction

Semen consists of seminal plasma and spermatozoa and its quality most surely affects the production of healthy larvae (Bromage & Roberts 1995). Moreover, seminal plasma has a special composition that support sperm cells for better external fertilisation and is affected by nutritional, physiological and environmental factors (Rurangwa, Kime, Ollevier & Nash 2004; Gardeur, Mathis, Kobilinsky & Brun-Bellut 2007; Canyurt & Akhan 2008; Lahnsteiner 2009). Among the factors, the nutrition has a key role in semen quality and some indices of sperm that relate directly to its fertilisation ability are affected by dietary intake. Inorganic constituents (K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+}) are in charge of sperm motility while, organic compounds (triglycerides, glycerol, fatty acids, glucose, and lactate) provide the energy for metabolism, and several enzymes (acid phosphatase, alkaline phosphatase, malate dehydrogenase, lactate dehydrogenase, adenosine triphosphatase and aspartate aminotransferase) take part in the spermatozoal metabolic process (Rurangwa *et al.* 2004). Several micro-nutrient such as Arginine have been recognised as highly valuable for male fish brooders and able to make changes in biochemical composition of semen in adult male animals (Wu 2009).

Arginine is an essential amino acid that must be provided orally and has an important role in adult male of reproductive age (Lall, Kaushik, Le Bail, Keith, Anderson & Plisetskaya 1994; Küçükbay, Yazlak, Sahin, Akdemir, Orhan, Juturu & Sahin 2008). It is involved in many metabolic processes, such as protein synthesis, urea production, glutamic acid and proline metabolism, and the synthesis of ceratine, nitric oxide and polyamine (Nikolic, Stojanovic, Pavlovic, Sokolovic, Bjelakovic & Beninati 2007). Arginine takes part in the process of sperm formation and has been found to be a basic component of the nucleoprotein of the

spermatozoa of different species (Miroueh 1970). Arginine boosts sperm motility by developing the rate of glycolysis, which elevates the rate of Adenosine-5′-triphosphate (ATP) and lactate generation in spermatozoa (Patel, Srivastava, Phadke & Govil 1998). Moreover, it prevents bilayer phospholipid membrane peroxidation in various peroxidation situations by producing a nitric oxide (NO) mechanism which protects the structural and functional integrity of spermatozoa (Govil, Phadke & Srivastava 1992; Srivastava, Desai, Coutinho & Govil 1999).

This preliminary study on male rainbow trout investigated the effects of supplemented diet with pure arginine on seminal plasma biochemical parameters, such as inorganic compounds (sodium, potassium, iron, magnesium, phosphorous, chloride and calcium ions), organic compounds (total protein, glucose, fructose, urea, uric acid and cholesterol) and semen enzymes [Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH)] in matured rainbow trout.

Materials and methods

Experimental diets

Five isocaloric and isonitrogenous experimental diets containing basic pure Arg content (control) or 0.50%, 1.00%, 1.50% and 2.00% Arg supplemented. The basic Arg content in control diet was provided by ingredients and set as the minimum Arg requirement of growing rainbow trout (NRC 2011). Other test diets were supplemented with different levels of pure synthetic pure L-Arginine. The pure L-Arginine (98%) purchased from SIGMA-ALDRICH Company with molecular formula and weight of $C_6H_{14}N_4O_2$ and 174.2, respectively. Ingredients were purchased from an extruder fish feed company and were analysed for proximate composition (Table 1). All ingredients were mixed for 20 min and mechanically extruded to obtain 5 mm pellets. The pellets were dried in a convection oven at 80°C and stored in airtight plastic bags until use. Amino acid composition of test diets was determined and presented in Table 2.

Table 1 Formulation and proximate composition of the experimental diets (% dry matter)

	0.00 (Control)	0.5%	1.0%	1.5%	2.0%
Ingredients					
Fishmeal	45.0	45.0	45.0	45.0	45.0
Soybean meal	25.0	25.0	25.0	25.0	25.0
Wheat gluten	10.0	10.0	10.0	10.0	10.0
rice	5.0	4.5	4.5	4.5	4.0
corn flour	5.0	5.0	4.5	4.0	4.0
Yeast	2.0	2.0	2.0	2.0	2.0
Gelatin	0.5	0.5	0.5	0.5	0.5
Casein	0.5	0.5	0.5	0.5	0.5
Fish oil	3.5	3.5	3.5	3.5	3.5
Methionine	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
CMC	0.5	0.5	0.5	0.5	0.5
Vitamin mix	2.0	2.0	2.0	2.0	2.0
Arginine	–	0.5	1.0	1.5	2.0
Proximate composition					
Protein	52.1	52.2	52.5	52.6	52.7
Lipid	12.4	12.07	12.6	12.1	12.09
Ash	8.5	9.4	8.6	9.3	9.8
Dry matter	95.0	95.0	94.0	94.0	94.0

Table 2 Analysed amino acid composition of the practical diets (% dry diet)

	Control	0.5%	0.1%	1.5%	2.0%
Aspartic acid	9.34	9.14	8.25	9.2	8.28
Glutamic acid	24	19.25	18.31	19.3	21.13
Serine	6.18	7.21	6.99	6.98	7.48
Histidine	1.05	1.02	1.1	1.22	0.96
Glycine	1.6	14.49	14.61	14.41	14.37
Threonine	9.36				
Arginine	**5**	**5.29**	**5.73**	**6.36**	**7.16**
Taurine	1.16	1.22	1.22	1.33	nd
Alanine	6.52	7.45	6.9	7.84	6.37
Tyrosine	2.67	2.72	2.5	2.53	2.56
Methionine	2.57	9.39	10.55	9.76	9.65
Valine	7.74				
Phenylalanine	4.02	3.9	3.9	3.63	3.68
Isoleusine	5.74	4.95	5.6	5.31	5.24
Leucine	8.63	8.58	8.5	8.04	8.03
Lysine	2.73	3.09	2.96	2.63	2.48
∑ Amino acid	98.31	97.7	97.12	98.54	97.39
∑ Essential amino acid	51.47	48.59	45.77	48.78	47.42
∑ Non-essential amino acid	46.84	49.11	51.55	49.72	49.97

Proximate analysis

Feed ingredients and experimental diets were analysed for dry matter (DM) proximate composition of crude protein, crude lipid, fibre and ash content following (AOAC, 1997). Crude protein was determined according to KJeldahl procedure (Crude protein = nitrogen × 6.26). Samples were extracted with chloroform methanol (2:1, v/v) to determine crude lipid, crude ash was measured by ashing in

a muffle furnace for 5 h at 550°C and crude fibre by loss on ignition of dried residue after successive digestion with 5% H2SO4. Nitrogen free extract (NFE) was calculated by subtracting the sum of crude protein, crude fat, ash and crude fibre from the total dry matter content. The gross energy content of diets was determined based on 17.2, 39.5 and 23 KJ g^{-1} for carbohydrate, lipid and protein, respectively. All determinations were carried out in triplicate (n = 3) and results are presented as mean ± SD.

Amino acid analysis

The amino acid profile of the experimental diets was determined by hydrolysing 0.1 g (dry weight) of the sample with 6N HCl at 110°C for 24 h and then derivatised with AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) before undertaking chromatographic separation using an AccQ TagTM reversed phase (3.9 × 150 mm) analytical column (Waters). Amino acid analysis was performed on a HPLC (High Performance Liquid Chromatography, USA) system comprising a Waters 1525 Binary HPLC Pump, 717 Plus auto-sampler (Waters®) and Waters 2475 Multi λ Fluorescence detector (wavelength excitation 250 nm, emission 395 nm). Chromatographic peaks were integrated, identified and quantified with BreezeTM software, version 3.20 by comparing to known standards (Amino acid standard H, Pierce, Rockford, Illinois, USA).

Fish and experimental design

Prior to breeding season, number of 120 three-year-old matured male rainbow trout (2500 ± 200 g) were distributed in 15 raceways (30 × 3 × 1 m^3) in the research centre of genetic and breeding of cold-water fishes, Yasouj, Iran. During 2 weeks acclimation, the fish were fed with a commercial diet (40% CP, 12% CL). Later, the fish were fed test diets at the rate of 1% of BW twice a day (9:00 and 16:00) for 3 months. During trails dissolved oxygen remained between 5.9 and 6.8 ppm, pH: 6.9–7.1 and the temperature was 11 ± 1 °C. The tanks equipped with aeration and normal sunlight were applied during the experiment.

Semen collection and seminal plasma preparation

After 90 days of the feeding trial, fish were fed a restricted diet for 5 days to prevent the contamination of semen with faeces during stripping. Male fish from each concrete tank were anaesthetised in a clove oil bath (50 μL^{-1}). After cleaning the genital area with fresh water and drying, semen was collected by abdominal massage. All semen samples were pooled in sterile micro tubes. Care was taken to avoid contaminating semen with water, urine, blood or faecal matter. Finally, semen samples were frozen at −80°C and transferred to the laboratory for further examination.

Inside the laboratory, the semen sample from each fish was centrifuged at 14 000×g for 10 min, and the supernatant seminal fluid was frozen and stored at −20°C until used in seminal plasma parameter examinations.

Seminal plasma compositions

Seminal plasma biochemical components were measured by methods used in the articles named below:

glucose (Lott & Turner 1975), total protein (Lowry, Rosebrough, Farr & Randall 1951), AST (Reitman & Frankel 1957) ALP (Kind & King 1954), ALT (Reitman & Frankel 1957), LDH (Babson & Babson 1973), Na^+, K^+ and Ca^{2+} (by Pars Azmoon kit, Karaj, Iran) based on the ion-exchanger colorimetric method (Yoshimura, Waki & Ohashi 1976), urea and uric acid (Liao, Zhao, Zhao, Tao, Zhu & Liu 2006), cholesterol (Sullivan, Kruijswijk, West, Kohlmeier & Katan 1985), Cl^- (Ng, Altaffer, Ito & Statland 1985), pH meter (pH meter, Iran 762), Fe^{2+} concentration (Hoppe, Hulthén & Hallberg 2003), fructose (Mann 1964), magnesium (Ehrhardt, Appel & Paschen 1992) and phosphorus (Burtis, Ashwood & Bruns 2012).

Statistical analysis

Results are presented as means ± SE. Differences between parameters were analysed by one-way analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) at a level of α = 0.05, using the SPSS statistical software package. The data for semen parameters were analysed for each characteristic using triplicate samples taken from five treatment groups.

Results

Results of different percentages of oral arginine on enzymes, ion components, organic components,

and pH of seminal plasma are presented in Tables 3–6, respectively. According to the results, the predominate ions in *Oncorhynchus mykiss* seminal plasma are Na, Cl and K. There were no statistically significant differences between LDH, ALP, Fe and P content in different treatments. Conversely, significant differences were observed in AST and ALT. The lowest amounts of these factors were in the 1.5% group. Mg^{2+} and Ca^{2+} were highly significant in 1.5% arginine group. Moreover, K^{+} content was significantly higher in 0.5% arginine treatment and there are no significant differences between other groups. The highest and lowest Na^{2+} was in the 1% and control group, respectively.

Discussion

Because the components of seminal plasma and dietary arginine have a great impact on the biological quality of the milt, and finally associated with fertilisation success, the effect of pure arginine on the biochemical composition of seminal plasma in rainbow trout, *O. mykiss* tested in current study. The lowest amounts of ALT observed in the 1.5% Arginine treatment, however, no significant difference with control and 2% arginine treatments. Significant decreased in the level of AST was also observed in 1%, 1.5% and 2% Arginine treatments compared to control group. Enzyme activity in seminal plasma can also be introduced as a relevant stress indicator. In order to deal with the energy crisis during stress, an increase in transamination occurs due to amino acid input to the TCA cycle (Velisek, Wlasow, Gomulka, Svobodova, Dobsikova, Novotny & Dudzik 2006). Inevitably, fish face a more stress during the period of culture, so a convenient antioxidant can alleviate oxidative stress, protect the spermatozoa membrane from lipid peroxidation, and even reduce the release of enzymes such as AST, ALT and LDH into seminal plasma. This study showed that arginine possesses the capacity to protect sperm membrane from AST and LDH release. Both AST and LDH are considered to be enzymes that are released by damaged sperm cells; therefore, their increasing levels in seminal plasma may reduce semen quality. The extra-cellular activity of transaminases is caused by their leakage into seminal plasma which is caused by damage inflicted upon spermatozoa (Kapila 1992). Therefore, seminal plasma transaminases are evaluated as an index of measurement of injury to spermatozoa incurred during different conditions (Sirat, Sinha, Singh & Prasad 1996). Enzyme release from spermatozoa has generally been viewed as a cellular injury indices (Ingale, Suthar & Sharma 2000), whereby membranes become inactive or are destroyed, resulting in the loss of material therein (Sidhu, Pangawkar & Chaudhary 1996). Sperm damage through oxidative stress increases membrane permeability for enzymes and other substances, and therefore, reduces sperm metabolic activity (Storey 1997). Changes in the activity of enzymes such as AST or ALT in semen plasma are associated with defects in sperm membranes. Alkaline phosphatase is a main secretion of the epididymis, an organ that plays a crucial role in the maturation of sperm cells; the activity of this enzyme was also determined in the cytoplasmic droplets of the sperm cells, which suggests its association with glycogen metabolism in the epididymal epithelium, thus supplying the maturing spermatozoa with energy (Arangasamy, Singh, Ahmed, Ansari & Ram 2005). Alkaline phosphatase also participates in producing free fructosis in semen, which, after fructolysis, provides the energy needed for sperm cell motility (Borkowski & Strzezek 1994). Variable levels of alkaline phosphatase activity have been evaluated in the semen plasma of men, dogs, toms, bulls, rabbits, rams, goats, buffalo, cocks, turkeys, boars and camels where it is believed to be involved in sperm glycolytic reactions and fructose formation.

Table 3 Effects of different levels of arginine on enzymes in fish seminal plasma

Enzymes	0.0 (control)	0.5%	1.0%	1.5%	2.0%
LDH (u l^{-1})	2821.66 ± 36.66	3166.66 ± 33.33	3433.33 ± 120.18	2733.33 ± 192.20	2936.66 ± 417.94
ALT (u l^{-1})	100 ± 3.6^{ab}	114 ± 16.86^{a}	129 ± 10.44^{a}	75.33 ± 6.35^{b}	80.66 ± 5.54^{b}
AST (u l^{-1})	2370 ± 295.35^{a}	2400 ± 267.64^{a}	1816.66 ± 44.09^{ab}	1410 ± 26.45^{b}	1526.66 ± 119.76^{b}
ALP (u l^{-1})	12.33 ± 0.88	14.66 ± 1.45	14 ± 1.15	14.66 ± 0.88	13 ± 0.57

Different letters in the same row show significant differences between treatments.

Table 4 Effects of different levels of arginine on inorganic composition (ions) in seminal plasma

inorganic composition	0.0 (control)	0.5%	1.0%	1.5%	2.0%
Fe^{2+} (μg dl^{-1})	162 ± 11.53	156 ± 19.85	135 ± 5.77	121 ± 13.57	142 ± 16.16
Mg^{2+} (mg dl^{-1})	3.1 ± 0.25^{b}	3.4 ± 0.25^{ab}	3.4 ± 0.10^{ab}	4.0 ± 0.11^{a}	3.1 ± 0.15^{b}
P (mg dl^{-1})	12.06 ± 0.46	12.10 ± 1.46	11.8 ± 0.30	12.66 ± 1.20	14 ± 1.15
Cl^{-} (mmol l^{-1})	85.33 ± 6.93^{b}	107.66 ± 5.04^{a}	107.66 ± 1.45^{a}	99.66 ± 4.91^{ab}	90 ± 2.88^{b}
Ca^{2+} (mg dl^{-1})	4.56 ± 0.32^{b}	4.20 ± 0.05^{b}	4.66 ± 0.14^{b}	5.80 ± 0.30^{a}	4.53 ± 0.24^{b}
Na^{+} (mmol l^{-1})	58.66 ± 4.63^{c}	89.33 ± 4.25^{a}	91 ± 0.57^{a}	85.33 ± 7.75^{ab}	72 ± 2.51^{bc}
K^{+} (mmol l^{-1})	24 ± 1.52^{b}	33 ± 1.52^{a}	28.33 ± 0.33^{b}	27 ± 2.08^{b}	26 ± 0.57^{b}

Different letters in the same row show significant differences between treatments.

Table 5 Effects of different levels of arginine on organic composition in seminal plasma

Organic composition	0.0 (control)	0.5%	1.0%	1.5%	2.0%
Cholesterol (mg dl^{-1})	28.66 ± 1.45^{a}	16.33 ± 1.20^{c}	17.66 ± 0.88^{bc}	22.66 ± 1.85^{b}	31.33 ± 2.33^{a}
Uric acid (mg dl^{-1})	0.93 ± 0.03^{c}	1.50 ± 0.11^{ab}	1.10 ± 0.05^{bc}	1.16 ± 0.24^{bc}	1.83 ± 0.20^{a}
Urea (mg dl^{-1})	10 ± 1.50^{a}	7.33 ± 0.88^{ab}	7.33 ± 0.33^{ab}	8 ± 0.57^{ab}	6.33 ± 0.88^{b}
Fructose (mg dl^{-1})	32.33 ± 1.20^{b}	36.33 ± 2.02^{a}	27 ± 0.57^{c}	29.66 ± 0.88^{bc}	27.33 ± 0.88^{c}
Glucose (mg dl^{-1})	13.33 ± 1.45^{a}	7.66 ± 0.88^{b}	5.66 ± 0.33^{bc}	5.66 ± 0.88^{bc}	3.66 ± 0.33^{c}
Total protein (mg dl^{-1})	336 ± 12.42^{ab}	314.33 ± 12.99^{b}	355.33 ± 11.89^{a}	268 ± 10.59^{c}	304.33 ± 3.48^{b}

Different letters in the same row show significant differences between treatments.

Table 6 Effects of different levels of arginine on pH seminal plasma

	0.0 (control)	0.5%	1.0%	1.5%	2.0%
pH	8.50 ± 0.05^{a}	8.46 ± 0.11^{ab}	8.53 ± 0.03^{a}	8.53 ± 0.06^{a}	8.25 ± 0.07^{b}

Different letters in the same row show significant differences between treatments.

Most of the energy needed by spermatozoa for motility is provided by fructose oxidation in the process of anaerobic glycolysis, the product of which is lactic acid, and in its passage through the cell membrane, lactate dehydrogenase plays a role.

The formation of seminal fluid (inorganic as well as organic compounds) is an active secretion process of the spermatic duct epithelium (Marshall 1986; Marshall, Bryson & Idler 1989). The semen quality, particularly in aquaculture species, is dependent on various external factors such as feeding regime, feed quality, rearing temperature and spawning season of males (Bromage & Roberts 1995; Rurangwa *et al.* 2004). The seminal plasma of fish, compared with higher vertebrates, is characterised by a low total protein concentration, substantial mineral compounds (Na^{+}, K^{+}, Cl^{-}, Ca^{+2}, Mg^{+2}), and low concentrations of organic substances (Billard, Cosson, Crim & Suquet 1995). Several studies have proven that the presence of organic and inorganic components (especially Na^{+} and Cl^{-}) supports the viability of spermatozoa (Morisawa, Suzuki, Shimizu, Morisawa & Yasuda 1983; Piironen & Hyvärinen 1983; Lahnsteiner, Patzner & Weismann 1994; Ciereszko & Dabrowski 2000). In this regard, interactions of ions present in the seminal plasma with the sperm membrane influence the membrane potential greatly and represent a mechanism of spermatozoa inhibition in the seminal plasma or sperm duct, allowing the maintenance of the potential of motility before release to the surrounding medium (Ciereszko & Dabrowski 2000). Inorganic ions are crucial to maintaining optimal osmotic pressure for sperm survival (Hajirezaee, Mojazi Amiri & Mirvaghefi 2009). High levels of Na^{+} in semen can be associated with a high percentage of sperm motility. In this study, Na^{+} and K^{+} contents were increased in the broodstocks fed arginine compared with the control treatment, and a significant increase in the Ca^{2+} level in seminal plasma was observed in arginine treatments compared with the control group. In rainbow trout semen, extra

sperm calcium stimulates adenylate cyclase activity and subsequently increases intra-sperml cAMP, which regulates the initiation of flagellar movement (Morisawa & Ishida 1987), intra-sperml calcium levels also influence motility behaviour via axonemal binding. The relationship between the high concentration of potassium and low concentration of calcium seems to be important for the inhibition of sperm motility. Baynes, Scott and Dawson (1981), in *Salmo gairdneri* showed that high calcium concentrations antagonise the potassium effect on sperm motility.

The ionic components of seminal plasma have a significant influence on sperm motility in fish. In salmonids, the motility of spermatozoa is mainly controlled by the K^+ level. It is generally known that a higher K^+ level inhibits sperm motility in salmonids (Morisawa & Suzuki 1980) but it increases sperm motility in carp (Billard & Cosson 1992). Divalent cations (mainly Ca^{2+} and Mg^{2+}) are more effective in antagonising the inhibitory effect of K^+ on sperm motility than the monovalent Na^+ ion (Baynes *et al.* 1981; Billard & Cosson 1992). The inhibition of sperm motility by K^+ can be overcome by an increased external Ca^{2+} concentration.

White and MacLeod (1963), noted that protein in fish semen is protective, however, its specific role is unknown. Urea is considered correlated with protein metabolism and total protein, because it occurs as a result of the digestion of protein, which contains N_2. The role of glucose in fish semen is unclear. The presence of glucose in seminal plasma has been connected to the high energy demand of the testis during spermatogenesis or to the lipid synthesis of spermatozoa (Soengas, Sanmartin, Barciela, Aldegunde & Rozas 1993). According to Piironen (1994), seminal plasma lipids are related to metabolism in spermatozoa. While cholesterol was found in the seminal plasma of freshwater fish (Billard *et al.* 1995), there is a little information about its role. Lipids and cholesterol may have been protective against environmental changes (especially temperature) when semen is released. As arginine was increased in practical diets, the amount of glucose in seminal plasma specimens was significantly reduced, which can reduce the energy storage of the milt. This reduction is caused by the role of arginine in improving the use of glucose in the Krebs cycle and glycolysis, which increases the rate of adenosine triphosphate and lactate production in arginine sperm, thereby providing access to energy for sperm motility. When dietary arginine was increased, a significant linear decrease in the glucose of seminal plasma was observed. Generally, the amount of fructose in diets containing arginine decreased in comparison with the control.

According to previous studies, during the passage of spermatozoa from the testis to the spermatic duct, an increase in external pH may be responsible for the acquisition of motility in some salmonid fish (Morisawa & Morisawa 1986, 1988; Billard *et al.* 1995).Therefore, the seminal plasma pH might also affect the final maturation of spermatozoa.

It can be concluded that the findings of this research can be used in selecting high-quality mature males for egg fertilisation in a commercial aquaculture operation, and, as a result of reducing the number of male broodstocks, the economic efficiency of the farm can be increased. The information on quantities characteristics and chemical compositions obtained in the present study could lead to more efficient gamete management, increase yields and enhance the suitability of semen for short-term storage.

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References

AOAC (1997) *Official Method of Analysis*. Association of Official Analytical Chemist, Arlington, TX, USA.

Arangasamy A., Singh L., Ahmed N., Ansari M. & Ram G. (2005) Isolation and characterization of heparin and gelatin binding buffalo seminal plasma proteins and their effect on cauda epididymal spermatozoa. *Animal Reproduction Science* **90**, 243–254.

Babson A.L. & Babson S.R. (1973) Kinetic colorimetric measurement of serum lactate dehydrogenase activity. *Clinical Chemistry* **19**, 766–769.

Baynes S., Scott A. & Dawson A. (1981) Rainbow trout, *Salmo gairdnerii* Richardson, spermatozoa: effects of cations and pH on motility. *Journal of Fish Biology* **19**, 259–267.

Billard R. & Cosson M.P. (1992) Some problems related to the assessment of sperm motility in freshwater fish. *Journal of Experimental Zoology* **261**, 122–131.

Billard R., Cosson J., Crim L.W. & Suquet M. (1995) Sperm physiology and quality. In: *Brood Stock Management and Egg and Larval Quality* (ed. by N.R. Bromage & R.J. Roberts), pp. 25–52. Blackwell Science, Oxford, UK.

Borkowski E. & Strzezek J. (1994) The use of biochemical indicators to evaluate semen quality-a review. *Medycyna Weterynaryjna* **50**, 200–200.

Bromage N.R. & Roberts R.J. (1995) *Broodstock Management and Egg and Larval Quality*. Blackwell Science Ltd, Oxford, UK.

Burtis C.A., Ashwood E.R. & Bruns D.E. (2012) *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Elsevier Health Sciences, Washington, DC, USA.

Canyurt M.A. & Akhan S. (2008) Effect of ascorbic acid supplementation on sperm quality of rainbow trout (*Onchorynchus mykiss*). *Turkish Journal of Fisheries and Aquatic Sciences* **8**, 171–175.

Ciereszko A. & Dabrowski K. (2000) In vitro effect of gossypol acetate on yellow perch (*Perca flavescens*) spermatozoa. *Aquatic Toxicology* **49**, 181–187.

Ehrhardt V., Appel W. & Paschen K. (1992) Evaluierung eines Xylidyl-Blau-Reagenz zur Bestimmung von Magnesium. *Wiener klinische Wochenschrift* **104**, 5–11.

Gardeur J.-N., Mathis N., Kobilinsky A. & Brun-Bellut J. (2007) Simultaneous effects of nutritional and environmental factors on growth and flesh quality of *Perca fluviatilis* using a fractional factorial design study. *Aquaculture* **273**, 50–63.

Govil G., Phadke R.S. & Srivastava S. (1992) Physical/Chemical studies of vitamin E in membranes. In: *Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications* (ed. by A.S.H. Ong & L. Packer), pp. 29–34. Birkhäuser, Basel, Switzerland.

Hajirezaee S., Mojazi Amiri B. & Mirvaghefi A.R. (2009) Effects of stripping frequency on semen quality of endangered Caspian brown trout, *Salmo trutta caspius*. *American Journal of Animal and Veterinary Sciences* **4**, 65–71.

Hoppe M., Hulthén L. & Hallberg L. (2003) Serum iron concentration as a tool to measure relative iron absorption from elemental iron powders in man. *Scandinavian Journal of Clinical and Laboratory Investigation* **63**, 489–496.

Ingale N., Suthar B. & Sharma V. (2000) Pellet freezing of ram semen and associated alterations in enzyme activity. *Indian Journal of Animal Sciences* **70**, 839–840.

Kapila R. (1992) *Leakage of Enzymes During Freezing of Goat Semen*. MSc dissertation, N.D.R.I. (Deemed University), Karnal, India.

Kind P. & King E. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *Journal of Clinical Pathology* **7**, 322.

Küçükbay F., Yazlak H., Sahin N., Akdemir F., Orhan C., Juturu V. & Sahin K. (2008) Effects of dietary arginine silicate inositol complex on mineral status in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* **14**, 257–262.

Lahnsteiner F. (2009) The role of free amino acids in semen of rainbow trout *Oncorhynchus mykiss* and carp *Cyprinus carpio*. *Journal of Fish Biology* **75**, 816–833.

Lahnsteiner F., Patzner R. & Weismann T. (1994) Testicular main ducts and spermatic ducts in some cyprinid fishes I. Morphology, fine structure and histochemistry. *Journal of Fish Biology* **44**, 937–951.

Lall S., Kaushik S., Le Bail P., Keith R., Anderson J. & Plisetskaya E. (1994) Quantitative arginine requirement of Atlantic salmon (*Salmo salar*) reared in sea water. *Aquaculture* **124**, 13–25.

Liao F., Zhao Y.-S., Zhao L.-N., Tao J., Zhu X.-Y. & Liu L. (2006) Evaluation of a kinetic uricase method for serum uric acid assay by predicting background absorbance of uricase reaction solution with an integrated method. *Journal of Zhejiang University Science B* **7**, 497–502.

Lott J.A. & Turner K. (1975) Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. *Clinical Chemistry* **21**, 1754–1760.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Mann T. (1964) *The Biochemistry of Semen and of the Male Reproductive Tract*. Methuen, London, UK.

Marshall W.S. (1986) Sperm duct epithelium of brook trout: Na^+ transport and seminal plasma composition. *Canadian Journal of Zoology* **64**, 1827–1830.

Marshall W.S., Bryson S.E. & Idler D.R. (1989) Gonadotropin stimulation of K^+ secretion and Na^+ absorption by brook trout (*Salvelinus fontinalis*) sperm duct epithelium. *General and Comparative Endocrinology* **75**, 118–128.

Miroueh A. (1970) Effect of arginine on oligospermia. *Fertility and Sterility* **21**, 217–219.

Morisawa M. & Ishida K. (1987) Short-term changes in levels of cyclic AMP, adenylate cyclase, and phosphodiesterase during the initiation of sperm motility in rainbow trout. *Journal of Experimental Zoology* **242**, 199–204.

Morisawa S. & Morisawa M. (1986) Acquisition of potential for sperm motility in rainbow trout and chum salmon. *Journal of Experimental Biology* **126**, 89–96.

Morisawa S. & Morisawa M. (1988) Induction of potential for sperm motility by bicarbonate and pH in rainbow trout and chum salmon. *Journal of Experimental Biology* **136**, 13–22.

Morisawa M. & Suzuki K. (1980) Osmolality and potassium ion: their roles in initiation of sperm motility in teleosts. *Science* **210**, 1145–1147.

Morisawa M., Suzuki K., Shimizu H., Morisawa S. & Yasuda K. (1983) Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. *Journal of Experimental Biology* **107**, 95–103.

Ng R., Altaffer M., Ito R. & Statland B. (1985) The Technicon RA-1000 evaluated for measuring sodium, potassium, chloride, and carbon dioxide. *Clinical Chemistry* **31**, 435–438.

Nikolic J., Stojanovic I., Pavlovic R., Sokolovic D., Bjelakovic G. & Beninati S. (2007) The role of L-arginine in toxic liver failure: interrelation of arginase, polyamine catabolic enzymes and nitric oxide synthase. *Amino Acids* **32**, 127–131.

NRC 2011. *Nutrient Requirement of Fish and Shrimp*, National Research Council, Washington, DC, USA, 77.

Patel A.B., Srivastava S., Phadke R.S. & Govil G. (1998) Arginine activates glycolysis of goat epididymal spermatozoa: an NMR study. *Biophysical Journal* **75**, 1522–1528.

Piironen J. (1994) Composition and cryopreservation of sperm from some Finnish freshwater teleost fish. *Fisheries Research* **15**, 27–48.

Piironen J. & Hyvärinen H. (1983) Composition of the milt of some teleost fishes. *Journal of Fish Biology* **22**, 351–361.

Reitman S. & Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transminases. *American Journal of Clinical Pathology* **28**, 56–63.

Rurangwa E., Kime D., Ollevier F. & Nash J. (2004) The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture* **234**, 1–28.

Sidhu S., Pangawkar G. & Chaudhary R. (1996) Effect of some additives on the release at enzymes from buffalo spermatozoa during cryopreservation. *Indian Veterinary Journal* **73**, 154–158.

Sirat M., Sinha A., Singh B. & Prasad R. (1996) Effect of cryoprotectants on release of various enzymes from buck spermatozoa during freezing. *Theriogenology* **45**, 405–416.

Soengas J., Sanmartin B., Barciela P., Aldegunde M. & Rozas G. (1993) Changes in carbohydrate metabolism in domesticated rainbow trout (*Oncorhynchus mykiss*) related to spermatogenesis. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **105**, 665–671.

Srivastava S., Desai P., Coutinho E. & Govil G. (1999) Protective effect of L-arginine against lipid peroxidation in goat epididymal spermatozoa. *Physiological Chemistry and Physics and Medical NMR* **32**, 127–135.

Storey B.T. (1997) Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. *Molecular Human Reproduction* **3**, 203–213.

Sullivan D.R., Kruijswijk Z., West C.E., Kohlmeier M. & Katan M.B. (1985) Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol. *Clinical Chemistry* **31**, 1227–1228.

Velisek J., Wlasow T., Gomulka P., Svobodova Z., Dobsikova R., Novotny L. & Dudzik M. (2006) Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Veterinarni Medicina-praha-* **51**, 469–000.

White I. & MacLeod J. (1963) Composition and physiology of semen. In: *Mechanism Concerned with Conception* (ed. by C.G. Hartman), pp. 135–172. Pergamon Press, London, UK.

Wu G. (2009) Amino acids: metabolism, functions, and nutrition. *Amino Acids* **37**, 1–17.

Yoshimura K., Waki H. & Ohashi S. (1976) Ion-exchanger colorimetry—I: Micro determination of chromium, iron, copper and cobalt in water. *Talanta* **23**, 449–454.

