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Physiologic laboratory parameters of adult pond-kept koi (*Cyprinus carpio*) in Southern Germany – Influence of season and sex

Physiologische Laborparameter von adulten Koi (Cyprinus carpio) aus süddeutschen Teichhaltungen – jahreszeitliche und geschlechtsspezifische Einflüsse

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Summary

Husbandry of exotic pets like ornamental fish demands for the same holistic veterinary care, which is provided in companion animal medicine. The aim of this study was to provide basic values for laboratory parameters of adult koi kept in outdoor ponds as a basis for evaluation of fish general health and detection of organic disturbances. In addition, seasonal and sexual differences were examined. Six natural and conventional ponds with long-lasting veterinary health care and good pond water quality for koi husbandry were selected. Out of these, 42 koi with equal sex distribution and different age and size range underwent parasitological and thorough clinical examination to confirm general health status. Plasma biochemistry, haematological parameters and enzyme activities were determined. Differences between sexes include significantly increased alanine transaminase (ALT) activities and total protein, albumin and triglyceride concentrations in females. Seasonal differences obtained in 32 (76.2%) koi between late spring and late summer mainly included significantly increased activities of aspartate transaminase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) during summer. Further seasonal differences in plasma biochemistry values and neutrophil fraction were minor. This study provides basic values for koi kept in outdoor ponds as a basis for good veterinary practice, which includes routine blood parameter evaluation in a plethora of species. Furthermore, potential influence of sex and season/water temperature are highlighted to prevent misinterpretation of different physiological states like reproductive activity as pathological conditions.

Keywords: biomarker, fish, haematology, plasma biochemistry, reference value

Zusammenfassung

Auch die Haltung von exotischen Haustieren, insbesondere von Zierfischen, erfordert die gleichen holistischen Standards in der tiermedizinischen Gesundheitsversorgung wie bei klassischen Haustieren. Diese Studie hatte zum Ziel, Basislaborwerte zu erheben, um eine Grundlage für die Interpretation des Gesundheitszustandes von adulten Koi in Teichhälterung zu schaffen. Zusätzlich wurden jahreszeitliche und zwischengeschlechtliche Unterschiede der Laborparameter untersucht. Sechs Natur- und Kunstteiche mit jahrelanger Bestandsbetreuung und optimalen Wasserwerten wurden ausgewählt. Zur Bestätigung des Gesundheitsstatus wurden 42 Koi unterschiedlichen Alters bzw. Größe, aber gleicher Geschlechtsverteilung klinisch und parasitologisch untersucht. Plasma Biochemie, hämatologische Parameter und Enzymaktivitäten wurden bestimmt. Bei weiblichen Koi waren die Konzentrationen von Albumin, Gesamtprotein und Triglyzeriden sowie die Aktivität der Alanin-Aminotransferase (ALT) signifikant erhöht. Bei 32 (76,2%) Koi wurden jahreszeitliche Unterschiede der Laborwerte verglichen. Diese beinhalteten signifikant erhöhte Aktivitäten von Aspartat-Transaminase (AST), Creatin-Kinase (CK) und Laktat-Dehydrogenase (LDH) im Spätsommer (verglichen mit spätem Frühling). Weitere signifikante jahreszeitliche

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Unterschiede der Plasma Biochemie Werte und neutrophilen Granulozytenfraktion waren gering. Das Ergebnis dieser Studie liefert Basiswerte für in Teichen gehälterte adulte Koi, um eine Grundlage für eine fundierte tierärztliche Versorgung zu liefern. Darüber hinaus, zeigt diese Studie jahreszeitliche beziehungsweise Temperatur-assoziierte sowie geschlechtsspezifische Unterschiede auf. Diese können herangezogen
werden, um eine Fehlinterpretation von physiologischen Zuständen (beispielsweise Reproduktionszyklen) als Organerkrankungen zu verhindern.

Schlüsselwörter: Biomarker, Fisch, Hämatologie, Plasma Biochemie, Referenzwert

Introduction

Ornamental fish husbandry around the world is constantly increasing. As of 2007, global ornamental fish export value totalled over USD 314 million, according to the Food and Agricultural Organization of the United Nations (FAO), with most ornamental carp coming from Japan (Monticini 2010). In Japan, ornamental carp, called Nishikigoi or goi (which means carp), have been selectively bred at least since the 19th century (Balon 1995). Internationally they are referred as koi. After a steady increase starting in the 1950s, the value of ornamental carp production exceeded the production of carp as human food already in 1995 (Balon 1995). In Germany, garden ponds with ornamental fish are represented in 1.4 million households, which is approximately 3 % of German pet animal husbandry. With an estimated average of 19 ornamental fish per pond (Lange 2009), estimated number of ornamental fish on the single animal level is about the same when compared with the number of dogs and cats kept in Germany (IVH 2021). Koi ponds increase in popularity and with a life expectancy of over 40 years (Carey et al. 2001), single animals represent pets with a high financial and emotional value for their owners for a long period of time.

The increasing number of pond-kept koi and their considerable value for their keepers demands for high standards of husbandry and medical care including pond health management. In companion animal medicine, i.e. companion mammal medicine, wellness and pre-anaesthetic laboratory screenings are often routinely recommended in geriatric patients (Webb et al. 2012). In dogs, reference values for laboratory parameters have been established for years and more and more interbreed variations are detected (Gomez-Fernandez-Blanco et al. 2018, Hoglund et al. 2018).

In wild carp and carp kept for consumption, several studies established serum and haematological parameter to detect the effects of certain stressful conditions on laboratory parameters (Hoseini et al. 2012, Karan et al. 1998, Kopp et al. 2011, Kotonska-Feiga et al. 2015). However, koi diverged from wild carp and carp kept for human consumption centuries ago, thus the representativeness of these laboratory values is limited for adult pond-kept koi. Few studies have addressed this problem, and established haematological and plasma chemistry parameters including serum enzyme activities for young koi. The three main studies addressing laboratory parameters of koi used fish weighing between 200-839 g and 15-39 cm of body length. None of these studies determined or reported the sex of the fish. The usefulness and representativeness of these parameters for adult koi kept in garden ponds is questionable, because pond-kept koi are often several years older and are much bigger (Lynch et al. 2007, Ott Knüsel et al. 2015). Until now, the utilization of haematological and plasma parameters plus enzyme activities is limited due to missing representative reference parameters for pond-kept koi. However, these are urgently needed as a diagnostic guidance in diseased individuals and for the early detection of pathological processes or organic disturbances in koi without clinical symptoms.

In this study, basic values of selected laboratory parameters from healthy pond-kept koi are provided as a first basis for assessment of koi general health. In addition, these parameters are compared with already published data to evaluate their overall applicability for routine laboratory diagnostic practice. Furthermore, differences in physiologic parameters between two different seasons and males and females are highlighted.

Material and Methods

This study was approved by the Government of Upper Bavaria (Az. 55.21-1-54-2532.2-23-11). Animal handling was carried out according to institutional guidelines and the German Animal Welfare Act. The koi owners declared their written consent to participate in this study.

The database of the koi-specialized veterinary practice of Dr. Hoedt in Rosenheim (Germany) was used to select natural and conventional koi ponds with outdoor koi husbandry during all four seasons of the year. Natural ponds refer to ponds providing shallow water areas, planted borders, other aquatic plants, and natural substrate on the bottom for the fish. Conventional ponds were outdoor tanks with a biological filter system but without environmental enrichment. Only ponds with regular health care for at least 7 years, at least biannual pond health monitoring and excellent pond management conditions were selected. After pond selection, the following inclusion criteria were applied for individual koi: age ≥1 year, latest veterinary treatment ≥1 year ago and a thorough clinical examination performed by one of the authors (M.S.), an experienced fish veterinarian, in which the health status was assessed. Examinations and sampling were performed in late spring. To detect seasonal differences in the levels of laboratory parameters, an additional examination and sampling was performed in late summer. Both examinations were eight months apart.

For clinical examination, gonadal sonography and blood sampling, koi were anaesthetized by immersion in a water bath using tricaine methanesulfonate (MS-222, Thomson & Joseph Limited, Norwich, UK, 70 mg/l). The following parameters/organs were assessed during clinical examination: appetite, eyes, native faecal examination of whole pond samples, fins, general condition, gill colour and integrity, oral cavity, palpation of body cavity, respiration, skin/mucosal integrity, native skin/gill smears to exclude ectoparasites, and swimming behaviour.

Heparinised blood was collected by ventral puncture of the caudal vein. Directly after sampling, a blood smear was performed for differentiation count of cells. Plasma samples for biochemical and enzymatical analyses were cooled directly after sampling to 4-6 °C using a refrigerator and transferred to the laboratory on the same evening. Albumin, calcium, cholesterol, glucose, phosphor, total protein (TP), triglycerides, urea, and uric acid concentrations, alanine transaminase (ALT), α-amylase, aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) and lipase activities were analysed from the plasma with a Hitachi Chemistry Analyzer in the Synlab laboratory in Augsburg, Germany. Globulin and albumin/globulin (A/G) ratio were determined arithmetically. Total leukocyte and erythrocyte concentrations were manually counted using heparin-anticoagulated blood and a Neubauer-improved haemacytometer chamber within maximal 5 minutes after blood sampling. Blood was diluted 1:100 in Natt and Herrick's solution with gentle shaking for two minutes. The haemacytometer was loaded and kept still for 30 seconds. Erythrocyte number was counted in 5 group squares of the centre primary square (each 0.04 mm²) and raw number multiplied by 5,000 to calculate total red blood cells (RBCs) per microliter. White blood cell (WBC) and thrombocyte numbers were calculated likewise by multiplication of the raw number counted in four corner primary squares (each 1 mm²) times 250.

Calculations and statistical tests were performed using commercial software: Microsoft Excel (Microsoft Office 2016) and GraphPad Prism version 5.04 for Windows (GraphPad Software, La Jolla, California, USA). A P-value <0.05 was regarded significant.

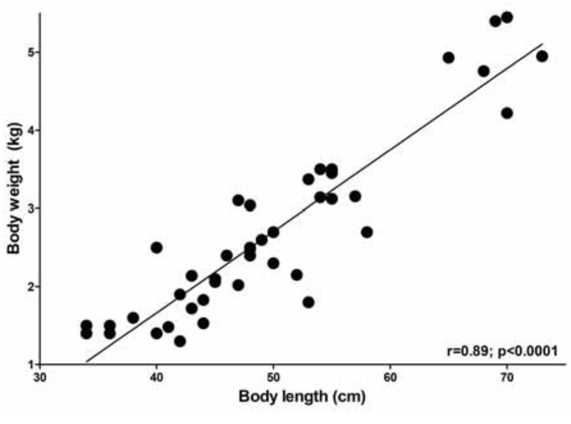

FIGURE 1: *Body weight and body length correlation with regression line and Spearman's r (Graphic: Maite Schneider)*

TABLE 1: *Pond water parameters*

Pond/Parameter	1	2	3	4	5	6	Threshold tap water[1]
Ammonia (mg/l)	0.01	0	0	0	0	0.01	0.5
Hardness carbonate (°dH)	10	10	10	10	10	10	14.7[2]
Hardness general (°dH)	13	15	13	12	12	15	15.8[2]
Nitrate (mg/l)	30	75	30	100	45	30	50
Nitrite (mg/l)	0.01	0.01	0	0	0	0.01	0.5
O_2 (mg/l)	7.9	7.5	8.3	9	8.5	8.4	9.8[2]
pH	8	8.3	8.6	8.2	8	7.9	7.54
Temperature spring (°C)	15.4	14.9	15.9	15.3	16	15.8	–
Temperature summer (°C)	19.8	20.2	19.8	21.1	18.9	[3]	–

1 According to German Drinking Water Ordinance (Trinkwasserverordnung)

2 For these parameters there is no threshold reported, values for comparison are means from Munich tap water analysis from 01/2019

3 No summer sampling performed

The ranges for koi haematology, serum chemistry and enzymes activities were determined from 42 clinically healthy koi. Values outside of three times interquartile range from the 25th and 75th percentile were defined as outliers and eliminated. Thereafter, the ranges were determined non-parametrically as interval between the 2.5th and 97.5th percentile.

The following pond water parameters were determined: ammonia, hardness (carbonate and general), nitrate, nitrite, oxygen, pH value, and temperature.

Results

Of >200 possible ponds in total, six met the inclusion criteria and were eventually selected. Three of these were natural ponds and three were conventional ponds. The water quality parameters were good and comparable with tap water requirements in Germany (Table 1).

From a total amount of >100 fish, 42 koi were selected. All of them were examined and sampled in late spring. In late summer, 32/42 koi (76.2%) were examined and sampled a second time. The precise age of the koi was known in 25/42 animals (59.5%). The age range was 1-15 years with a mean of 6 years (standard deviation [SD]: 3.3), the remaining 17 koi were adult. The body weight ranged from 1.3-5.5 kg. The body length was between 34 cm and 73 cm (Fig. 1). The following different colour variations were represented: variations of grey (5), variations of orange (5), Platinum Ogon (4), Showa (4), variations of red, white, and silver (3 each), Sanke (3), Bekko (2), Chagoi (2), Kohaku (2), Shiro-Utsuri/Utsuri (2). Yamabuki, Doitsu red, black and white and Tancho were each represented once.

In 40/42 koi (95.2%) the gender was unequivocally determined, 21 were females and 19 were males. Every koi displayed normal behaviour with upright physiologic swimming position and unremarkable respiratory activity. Faecal samples of whole ponds were negative or displayed only few parasites. Feed intake was normal, gill colour was physiologic and oral mucosal lesions were not detected. Table 2 gives an overview over the clinical data. Laboratory parameters are summarized in Table 3.

To detect differences of laboratory parameters between females and males, values of the same 32 koi were statistically compared. To detect seasonal/temper-

TABLE 2: *Clinical health parameters of 42 adult koi*

Koi	Pond	Sex	BW (kg)	L (cm)	Integument	Skin smear	Gill smear	Faecal smear
1	1	♀	2.5	48	intact	NAD	NAD	NAD
2	1	♂	2.1	45	intact	NAD	NAD	NAD
3	1	n/a	2.1	43	intact	NAD	NAD	NAD
4	2	♂	2.2	52	intact	NAD	NAD	NAD
5	2	♂	1.8	53	intact	NAD	NAD	NAD
6	2	♀	1.3	42	intact	NAD	Positive[1]	NAD
7	3	♀	5.4	69	intact, lost scales	Positive[2]	NAD	NAD
8	3	♂	2.3	50	intact	NAD	NAD	NAD
9	3	♀	3.4	53	intact	NAD	NAD	NAD
10	3	♀	2.5	40	intact	NAD	NAD	NAD
11	3	♂	4.8	68	intact	NAD	NAD	NAD
12	3	♀	4.9	65	intact	NAD	NAD	NAD
13	3	♀	1.4	36	intact	NAD	NAD	NAD
14	3	♀	1.4	34	intact	NAD	NAD	few *Capillaria* spp.
15	4	♀	1.5	34	intact	NAD	NAD	NAD
16	4	♂	3.1	54	intact	NAD	NAD	few *Capillaria* spp.
17	4	♂	1.5	36	intact	NAD	NAD	NAD
18	4	♀	2.4	46	intact	NAD	NAD	NAD
19	4	♂	2.1	45	intact, focal pock	NAD	few Costia	NAD
20	5	♀	2.7	58	intact	NAD	NAD	NAD
21	5	n/a	1.7	43	intact	NAD	NAD	NAD
22	5	♀	1.8	44	intact	NAD	NAD	NAD
23	5	♀	1.5	44	intact	NAD	NAD	NAD
24	5	♀	3.2	57	intact	NAD	NAD	NAD
25	5	♀	3.5	55	intact	Positive[3]	NAD	NAD
26	5	♂	5.0	73	intact	NAD	NAD	NAD
27	5	♀	1.5	41	intact	NAD	NAD	NAD
28	5	♀	2.0	47	intact	NAD	NAD	NAD
29	5	♂	3.1	55	intact	NAD	NAD	NAD
30	5	♀	3.0	48	intact	Positive[3]	NAD	NAD
31	5	♂	2.6	49	intact	NAD	NAD	NAD
32	5	♂	1.4	40	intact	NAD	NAD	NAD
33	5	♂	2.7	50	intact	NAD	NAD	NAD
34	5	♂	3.5	55	intact	NAD	NAD	NAD
35	5	♂	4.2	70	intact	NAD	NAD	NAD
36	6	♂	3.5	54	intact	NAD	NAD	NAD
37	6	♂	2.4	48	intact	NAD	NAD	NAD
38	6	♂	1.6	38	intact	NAD	NAD	NAD
39	6	♀	2.5	48	intact	NAD	NAD	NAD
40	6	♂	5.5	70	intact	NAD	NAD	NAD
41	6	♀	1.9	42	intact	NAD	NAD	NAD
42	6	♀	3.1	47	intact	NAD	NAD	NAD

n/a = not available; NAD = nothing abnormal detected

[1] A single ***Gyrodactylus*** spp.; [2] A single ***Dactylogyrus*** spp.; [3] Few ***Trichodina*** spp.

TABLE 3: *Physiological laboratory parameters of 42 clinically healthy adult koi and comparison with literature*

Parameter	n	Mean	SD	Percentiles		Palmeiro et al. 2007, n = 71		Tripathi et al. 2003/2004, n = 20/30	
				2.5%	97.5%	Median	Range	Mean	SD
Enzymes									
AST (U/l)	37	384.6	254.3	118.2	939.3	98	41–340	85	28
ALT (U/l)	39	90.1	23.6	52.3	144.8	31	9–98	31	8
LDH (U/l)	35	937.4	718	202.9	2579.3	nd	nd	207	136
α-Amylase (U/l)	41	17.5	8.5	7.0	34.0	nd	nd	nd	nd
Lipase (U/l)	38	7.1	1.6	4.9	10.0	nd	nd	nd	nd
CK (U/l)	38	8014	6872	1545	23686	4008	1170–9716	5855	2992
Plasma biochemistry									
Cholesterol (mmol/l)	38	4.6	1.6	2.1	8.5	3.9	2.4–7.3	4.3	0.6
Triglycerides (mmol/l)	40	4.7	2.5	1.4	8.4	nd	nd	nd	nd
Urea (mmol/l)	39	1.9	0.9	0.6	3.9	0.7	0.1–1.8	nd	nd
Uric acid (μmol/l)	39	12.9	4.7	5.9	23.1	6	0–29.8	0.9	2.1
Calcium (mmol/l)	36	2.5	0.4	1.9	3.2	2.8	2–5	2.6	0.2
Phosphorus (mmol/l)	38	3.8	1.1	2.4	6.5	2	1.2–4.1	1.6	0.5
Glucose (mmol/l)	38	2.1	1.2	0.7	4.6	2.7	1.1–7	2.7	0.6
Albumin (g/l)	39	10.4	3.3	5.0	17.1	15	6–25	14	1
Albumin:Globulin ratio	38	0.5	0.2	0.3	0.9	1	0.4–1.3	0.8	0.1
Total protein (g/l)	39	30.7	5.4	19.0	39.3	30	20–45	30	3
Globulin (g/l)	38	20.4	4.4	13.8	29.1	15	14–20	16	2
Haematology									
RBCs $\times 10^6$/μl	42	0.4	0.1	0.3	0.6	nd	nd	1.8	0.2
HCT	41	42.6	3.8	35.0	49.0	nd	nd	31.8	5.5
Thrombocytes $\times 10^3$/μl	42	11.2	5.9	0.8	20.5	nd	nd	nd	nd
WBCs $\times 10^3$/μl	42	5.3	1.4	3.0	8.0	nd	nd	24	5.6
Lymphocytes %	42	75.5	0.6	75.0	77.0	nd	nd	79.1	12.4
Monocytes %	42	3.3	0.5	3.0	4.0	nd	nd	2.9	1.3
Granulocytes									
Neutrophils %	42	16.5	0.9	15.0	18.0	nd	nd	10.9	7.7
Basophils %	42	3.8	0.4	3.0	4.0	nd	nd	4.6	2.6
Eosinophils %	42	0.9	0.3	0.0	1.0	nd	nd	nd	nd

AST = aspartate transaminase; ALT = alanine transaminase; LDH = lactate dehydrogenase; CK = creatine kinase; WBCs = white blood cells; RBCs = red blood cells; HCT = haematocrit; nd = not determined

ature effects, both comparisons were performed with parameters obtained in spring and summer. Triglycerides, albumin and total protein concentrations were significantly increased in female koi in both summer and spring (Mann-Whitney test, $p<0.05$). ALT activities were significantly increased in female koi only in the summer (Mann-Whitney test, $p<0.05$; Table 4). The body weight and body length were not significantly different between male and female koi (Mann-Whitney test, $p>0.05$).

To detect seasonal differences between laboratory parameters in spring and summer, different values of the same 32 koi were statistically compared. Five parameters were significantly increased, and seven parameters significantly reduced in the spring when compared to summer (Mann-Whitney test, $p<0.05$; Table 5).

Discussion

There is a demand among animal owners to seek animal health care not only for small mammal companions, but also for exotic animal species (Awosanya et al. 2015, Klaphake et al. 2002). Such exotic pet owners expect the same good health care for their beloved pets, just as they expect it for themselves in human health care, an experience that can be made in everyday practice. Veterinarians are sought out to provide animal health care, information on disease prevention and control, and treatment and diagnostic opportunities (Awosanya, et al. 2015). In companion animal medicine, i.e. mostly small mammal medicine, blood and serum parameters are routinely used to detect organic disturbances for decades. In addition, most laboratories also provide organ profiles to

TABLE 4: *Significant differences of laboratory parameters between male and female koi*

	Summer			Spring		
Parameter	**n**	♂	♀	**n**	♂	♀
Enzymes						
ALT (U/l)	13/15	87	110	18/19	ns	ns
Plasma biochemistry						
Triglycerides (mmol/l)	13/16	3.1	5.2	18/21	2.1	6.5
Albumin (g/l)	12/13	8	12	18/20	8	12
Total protein (g/l)	13/15	29	35	17/20	29	33

Depicted are medians (Mann-Whitney test, p<0.05); ALT = alanine transaminase;

ns = not significant

detect single organ diseases or geriatric profiles to cover age-associated maladies.

In carp kept in laboratory environment or ponds, reference values and laboratory parameters have been established and reported for several conditions, including physiologic ones. Values have been investigated in carp kept for human consumption, wild carp and there are many articles in the field of environmental toxicity (Böttcher 1998, Dobsikova et al. 2006, Groff et al. 1999, Hoseini, et al. 2012, Karan, et al. 1998, Kopp, et al. 2011, Kotonska-Feiga, et al. 2015, Sepici-Dincel et al. 2009, Velisek et al. 2009). Only few articles established laboratory parameters for ornamental carp using young animals in a laboratory environment (Palmeiro et al. 2007, Tripathi et al. 2004, Tripathi et al. 2003). In our opinion, these values, collected in a controlled laboratory environment, are of limited value for adult koi kept in outdoor ponds. Outdoor husbandry exposes piscine individuals to increased infection/infestation pressure, potential predators, fluctuating temperatures and deposition of exogenic substances (e.g. dust or leaves). Furthermore, applicable organ or geriatric profile examinations are not available for adult koi. The availability of laboratory parameters is imperative to provide state-of-the-art veterinary health care for koi. In the present study, laboratory parameters of adult koi kept in outdoor ponds were assessed to establish first basic values for blood parameters and to provide an initial foundation for basic veterinary care in evaluating organic diseases using organ-associated values. Furthermore, seasonal and sexual differences between the different parameters were investigated to highlight physiologic metabolic states, which may change laboratory parameters without underlying pathological conditions.

TABLE 5: *Statistically significant differences of laboratory parameters between two seasons*

Parameter	n	Spring	Summer
Enzymes			
AST (U/l)	30/28	371.5	380
LDH (U/l)	27/29	756	816
CK (U/l)	30/30	7834	17636.5
Plasma biochemistry			
Cholesterol (mmol/l)	30/30	4.5	4.9
Urea (mmol/l)	31/29	1.8	1.5
Calcium (mmol/l)	28/22	2.4	2.6
Glucose (mmol/l)	30/29	2	2.3
Albumin (g/l)	31/26	10	9.5
Albumin:Globulin ratio	30/25	0.51	0.46
Total protein (g/l)	30/29	31.5	31
Haematology			
Neutrophils %	32/32	16	15

Depicted are medians (Mann-Whitney test, p<0.05); AST = aspartate transaminase;

LDH = lactate dehydrogenase; CK = creatine kinase

As briefly mentioned above, there are several important differences between the present study and those already published. Two studies from the same workgroup used koi, which measured 15-18 cm in length and had an average body weight of 200 g (Tripathi, et al. 2004, Tripathi, et al. 2003). Another study used fish with a body weight of 75-839 g and a body length of 17-39 cm (Palmeiro, et al. 2007). These fish were housed in closed animal facilities or aquariums. With the awareness, that laboratory parameters of fish are influenced among others by age, breeding status, water parameters and season (De Pedro et al. 2005, Hrubec et al. 1997, Svetina et al. 2002), these parameters determined in a laboratory environment very likely are not representative for garden pond-kept adult koi. The koi in the present study were older, heavier, and longer and opposing to these studies the sex of the fish was additionally determined.

Still, the concentrations of albumin, calcium, cholesterol, glucose, and total proteins as well as blood leukocyte fractions were within a comparable range. Globulin concentration was slightly higher in the present study. This is probably due to the outdoor pond-keeping of the koi in this study when compared to the tank husbandry in the previous studies. Koi kept in outdoor ponds are much more exposed to environmental microorganisms and temperature and lighting fluctuations than laboratory-kept ones. Further differences were observed in the enzyme activities of ALT, AST, LDH, and CK, which were increased 2-3.5 times in the koi of the present study. In a study on the effects of analgesics on koi clinical and behavioural parameters after exploratory coeliotomy, the enzyme activities of AST, LDH, and CK were increased 48 hours post-surgery (Harms et al. 2005). This implicates that at least CK is an enzyme specific for muscular damage in koi, just as it is in other species. However, because the coelomic cavity was explored in that study, the increased AST and LDH activities could be caused by either muscle or liver damage during exploration. In ruminants, all three enzymes increase after muscular damage (Russell et al. 2007). Anaesthesia alone influences several haematological and plasma biochemistry parameters in fish (Carter et al. 2011, Wagner et al. 2003). Thus, in the present study the effects of capturing and anaesthesia likely influenced those values. Furthermore, the puncture of the caudal vein can lead to muscular damage and subsequent increase of muscle-associated enzyme activities. As ALT activity seems to be a liver specific biomarker in carp in vitro and in vivo (Jia et al. 2012, Liu et al. 2017), the increased parameter could hint towards an increased liver cell turnover, maybe due to increased metabolic stress in the examined koi when compared to the values of the literature. In addition, other factors like husbandry, feeding, environment as well as season could explain the differences in these values.

Phosphorous, urea and uric acid concentrations were about twice as high as the values obtained by Palmeiro, et al. (2007). These parameters are products of protein degradation. An explanation for the increase might be different feeding modalities: A diet rich in protein more likely produces more products of protein metabolites. As fish can effectively excrete excess nitrogen and electrolytes not only via the kidneys but also via the gills, the effects of these increased concentrations are probably minor.

WBC and RBC concentrations in the present study and a previous one (Tripathi, et al. 2004) differ quite considerably (means of 5.3 vs 24 $\times 10^3$/µl and 0.4 vs 1.8 $\times 10^6$/µl, respectively), although counting of RBCs and WBCs was done similarly, using a Neubauer-improved haematocytometer. The magnitude of difference, however, is the same with both parameters (~4.5-fold). Negenborn (2009) provided a mean WBC concentration of 6.86 $\times 10^3$/µl determined in young common carp. Other authors present much higher values, like 37.8 $\times 10^3$/µl (Groff, et al. 1999). The low reproducibility of manual blood cell counting cannot explain such a high variance. Reasons for the apparently decreased RBC and WBC counts can only be speculated about.

Regarding the WBC concentration: An infection of the chosen koi, which reduces WBC concentration seems unlikely, because koi implemented in this study were clinically healthy as determined by clinical examination and were housed in ponds with good management and very low parasite loads. Furthermore, differential blood counts and other laboratory parameters do not support an acute infection. Early or chronic infections, which could provide an explanation for the decrease, seem unlikely, because monitoring of sampled animals after/before this study did not reveal any clues regarding an infection. A convincing explanation for the lower WBC concentration in this study could be the different age of koi used when compared to the literature, where much younger animals were examined (Tripathi, et al. 2004). High WBC concentration in young animals and lower ones in adult individuals are common in different species including humans (Bourges-Abella et al. 2015, Monke et al. 1998, Zierk et al. 2015), thus the observed 'reduced' WBC concentration in our study probably reflects the physiological state of older koi.

Regarding the reduced RBC concentration, clinical examination did not indicate anaemia, because gill colouration was unremarkable and respiratory rate was not increased. Furthermore, the determined haematocrit values also point out against anaemia because they were above published values for koi and common carp (Kopp, et al. 2011, Negenborn 2009, Tripathi, et al. 2004). Comparing the mean RBC concentration and haematocrit of one study on koi and two additional studies on common carp, mean values were 1.2/1,9/1.8 $\times 10^6$/µl and 0.23/0.36/0.32, respectively (Kopp, et al. 2011, Negenborn 2009, Tripathi, et al. 2004) and thus within a closer range than in the study presented here. One of the main differences between these studies and our study is the weight and length and thus the age of the studied koi population. Older animals are difficult to obtain for experimental studies in a laboratory setup; most of the published experiments were conducted using young animals. It is tempting to speculate that older koi downregulate their absolute RBC concentration, but upregulate erythrocyte volume to maintain oxygen exchange. This is reflected by calculation of mean corpuscular volume (MCV), which was five times higher in our study than in the above-mentioned ones. Further studies are needed to test this hypothesis using young, adult and very old carp or koi in the future.

One aim of the present study was to figure out if laboratory parameters differ between sexes and seasons. The significantly different parameters between male and female koi were independent from the two examined seasons. Female koi revealed higher ALT activity, higher triglyceride and albumin/total protein concentrations. All of these parameters are connected to hepatic (lipoprotein) metabolism and are likely associated with reproductive activity. For reproduction, female fish produce a magnitude of follicles during spawning season. Oocytes mature by incorporation of plasma vitellogenin, which is a yolk precursor lipoprotein produced in the liver. This process needs high metabolic activity and increased protein and lipid concentrations (Hiramatsu et al. 2015).

Although there were several statistically significant differences in plasma biochemistry parameters and relative neutrophil concentration between seasons, the biological significance of these is very likely minor. The medians of some parameters differ only by very small changes of the respective values. In contrast, statistically significant increases in AST, LDH and CK activities were observed in summer. The differences between AST activities are minor and probably do not represent biological difference but rather biological variation. The higher CK activities suggest increased muscular metabolism during summer months. Warmer temperature induces generally increased activity of poikilothermic individuals and increased activity or exercise results in increased CK activities in several species (Chulayo et al. 2013, Jahr et al. 2019). The increased LDH activity as an expression of anaerobic capacity during summer (where water oxygen-binding capacity is reduced) could also reflect the increased fish activity during this season (Lind 1992). Although pond water temperature was not that high, the increased enzyme activities were certainly additionally influenced by the increased water temperature in late summer.

Enzyme specificity for certain organs can be different between species, e.g. biochemical indicators for liver damage can be ALT or alkaline phosphatase (AP) in cats and dogs, but sorbitol dehydrogenase (SDH) and gamma glutamyltransferase in horses and AST and LDH in ruminants (Camus et al. 2010, Russell, et al. 2007). In carp, experimental studies show increase of AST, ALT and LDH, after hepatic challenge with hepatotoxins in vitro and in vivo (Jia, et al. 2012, Liu, et al. 2017). As AST and LDH activities are also increased after explorative coeliotomy (Harms, et al. 2005), the combination of increased AST, LDH and CK activities suggests muscular damage. Increased AST and LDH activities without concurrent increased CK activities are supportive of liver disease (Russell, et al. 2007). Independent of that and after combining the available data, at least ALT seems to be a single hepatospecific biomarker in carp and koi.

Overall, the findings of the present study provide a first basis for plasma biochemical and haematological data interpretation in every day veterinary care of pond-kept adult koi. Plasma enzyme activities can indicate muscular damage or hepatic disease. This study emphasizes the importance of seasonal and sexual variations, which have to be taken into account when evaluating these parameters. One advantage of this study is the implementation of adult individuals highlighting the importance of age on the interpretation of laboratory parameters. Future studies could include biomarker differences between older and younger individuals for the establishment of geriatric profiles and comparison of obese and cachectic animals and the influences of these conditions on laboratory parameters.

Ethical approval

The authors hereby declare that they have followed the universally accepted guidelines of good scientific practice while preparing the present paper. All relevant national and institutional ethical guidelines for the handling and care of the animals used in the study were observed. The information regarding the application for approval of animal experimentation and its authorisation is given in the publication.

Conflict of interest

The authors hereby declare that they have no proprietary, professional or other personal interests in any product, service and/or company that could have influenced the contents or opinions expressed in this publication.

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Authors Contribution

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Data collection: MS.
Data analysis and interpretation: MS, WH, MCL, PK.
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