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Seasonal values of selected blood parameters of farm-raised channel catfish (*Ictalurus punctatus*) in the Mississippi Delta

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Abstract. Hematocrit, sodium, chloride, potassium, calcium, glucose, and pH were measured in whole blood of 1,522 channel catfish collected from 3 commercial food-fish ponds in the Mississippi Delta. Samples were collected from March 1995 to March 1996 to monitor seasonal fluctuations. A total of 10–20 fish were arbitrarily collected with snag lines from each pond on each sample day. The mean monthly hematocrits fluctuated seasonally from a low of 14.5% in midwinter to a high of 25.7% in midsummer (annual $\bar{x}$ = 21%, SE = 0.15). Sodium levels were consistent throughout the year with a mean (SE) of 134 (0.13) mM/liter. Mean chloride values for the year were 120 (0.14) mM/liter but increased to 132 mM/liter in midwinter. By March 1996, the chloride levels had returned to levels observed during spring 1995. Potassium and glucose levels varied throughout the year with means of 4.43 (0.06) mM/liter and 26.9 (0.46) mg/dl, respectively, and coefficients of variation of 51.8% and 63.3%, respectively. Calcium and pH values were fairly stable with means of 1.31 (0.004) mM/liter and 7.13 (0.004), respectively. All parameters except glucose and potassium may be adequately evaluated with a sample size of 25 or less. These data were collected to provide baseline information for ongoing pond health studies.

The commercial catfish industry has undergone rapid expansion in recent years. With the increasing demand for catfish, farmers have intensified production resulting in higher stocking and feeding rates. Concurrent with this increase in production, the industry has experienced an increase in fish health problems. These changes reflect those experienced by other animal agriculture industries with the development of intensive production systems. An understanding of the normal values for healthy animals and identification of predictors of the onset of health problems may enable the fish health specialist to intervene before major losses occur.

This study was designed to collect data on normal blood parameters in channel catfish (*Ictalurus punctatus*) reared under commercial conditions in the delta region of Mississippi, where 58% of the domestic catfish pond acreage is located.[27] Published data on channel catfish blood parameters are limited and do not include values throughout the year from fish in levee-type ponds in the Mississippi Delta. Clinical pathology values have been reported for the channel catfish[3–5,13,28,29] and have been previously summarized.[12]

Defining expected blood values is critical to interpreting effects associated with factors that influence fish health. For parameters that do not fluctuate much throughout the year, a single baseline value with some range to incorporate variation is appropriate. If a parameter fluctuates seasonally, then it becomes important to develop seasonal or monthly baseline values. Under normal culture situations, if a parameter exhibits excessive variability, variability associated with different ponds, or variability over time in different ponds, then delineation of baseline values may not be practicable and these parameters must be evaluated with caution.

Catfish are commonly reared in multibatch pond systems to which fish are intermittently stocked and harvested, thus a pond will contain fish of various sizes and ages at any time.[26] The practicality of examining fish blood parameters would be enhanced if it could be applied to an arbitrary sample of all fish in the pond instead of attempting to evaluate various classes or sizes of fish. In this study, parameters were measured on a group of fish arbitrarily collected from each pond regardless of fish size; however, correlations between blood values and fish size are reported.

The objectives of this study were to obtain values for 7 channel catfish blood parameters, including hematocrit (Hct), sodium (Na), chloride (Cl), potassium (K), calcium (Ca), glucose (Glu), and pH, in typical commercial pond production systems and to investigate seasonal patterns under the same conditions. Consideration was given to the number of fish that must be sampled to obtain an accurate estimate of the parameter.

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Table 1. Observed values ($\bar{x}$, SE) of selected clinical pathology data over a 1-yr period for channel catfish in Mississippi Delta ponds.

	Hct (%)		Na (mM/liter)		Cl (mM/liter)		K (mM/liter)		Ca (mM/liter)		Glu (mg/dl)		pH	
Month	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Jan	14.5	0.67	131	0.75	125	0.62	3.60	0.26	1.17	0.02	5.1	0.83	7.40	0.01
Feb	18.6	1.21	134	0.71	123	0.46	5.17	0.32	1.25	0.02	ND*	ND	7.20	0.02
Mar	17.5	0.30	133	0.34	120	0.26	4.77	0.11	1.25	0.01	19.9	0.87	7.23	0.01
Apr	19.4	0.30	133	0.42	120	0.25	5.88	0.15	1.27	0.01	17.7	1.12	7.10	0.01
May	21.4	0.29	133	0.37	118	0.44	4.00	0.11	1.33	0.01	22.0	0.76	7.02	0.01
Jun	22.8	0.31	135	0.28	119	0.39	3.40	0.17	1.39	0.01	26.3	1.17	7.00	0.01
Jul	25.7	0.41	135	0.30	117	0.23	3.95	0.23	1.39	0.01	32.1	1.26	7.12	0.01
Aug	25.7	0.59	135	0.30	118	0.24	4.00	0.23	1.38	0.01	44.6	1.30	7.15	0.01
Sep	23.3	0.38	135	0.26	117	0.21	3.99	0.21	1.37	0.01	44.9	1.31	7.20	0.01
Oct	19.2	0.52	130	0.60	119	0.43	6.04	0.30	1.26	0.01	33.1	1.69	7.22	0.01
Nov	16.7	0.81	127	0.63	126	0.57	5.30	0.33	1.13	0.02	24.9	1.74	7.33	0.01
Dec	16.2	0.51	134	0.73	132	0.98	4.08	0.37	1.24	0.03	11.0	1.27	7.32	0.01
Year	21.0	0.15	134	0.13	120	0.14	4.43	0.06	1.31	0.004	26.9	0.46	7.13	0.004
n	1,503		1,514		1,511		1,478		1,514		1,386		1,509	

* ND = not determined.

Materials and methods

Four food fish production ponds on a commercial catfish farm in the Mississippi Delta were included in the study. From March 1995 to March 1996, 10–20 fish were arbitrarily collected with snag lines from each pond between 8:00 and 11:00 AM of each sample day. Blood was collected within 5 min of landing the fish to reduce changes associated with the sampling procedure. The blood sample was drawn from the caudal vein into lithium heparin tubes using a vacutainer system and was placed on ice pending analysis, which was initiated within 4 hr of sampling. The fish were weighed and measured (total body length), and 4 fish were retained for diagnostic evaluation, which included wet mount examination of the gills, visual examination of internal organs, and bacterial cultures of the brain and posterior kidney. All blood samples were analyzed on an automated blood analysis machine[a] for Hct, Na, Cl, K, Ca, Glu, and pH. The machine was calibrated with standard solutions provided by the manufacturer. A second Hct reading was obtained on 176 samples measured with a microcentrifuge operated at 13,700 relative centrifugal force (RCF) for 3 min; the results were compared with those obtained by the analysis machine for the same samples.

Annual and monthly means and standard errors were computed for each blood parameter. Multivariate linear regression models were constructed for each parameter to investigate seasonal patterns using the general linear models procedure in a commercial computer software package.[b] Models included the sine and cosine transformations of the number of days elapsed as well as dummy variables for the pond and interactions between the time components and pond variables. Partial correlation coefficients were calculated to examine the linear relationship among blood parameters after controlling for the effect of fish length. Sample sizes needed to estimate a monthly mean for a specified parameter were calculated using a formula for estimating sample sizes for means.[21] For sample size estimation, the population being sampled was assumed to be at least 10,000 fish and the estimate of the population variance was the maximum observed monthly variance for each parameter. The precision of the estimate was standardized by calculating the sample size needed to achieve a boundary on the error (±2SE) of at most 10% of the annual mean.

Results

A disease outbreak was identified in 1 pond during the sampling period, and data from this pond were excluded. Disease outbreaks did not occur in the remaining 3 ponds based on clinical examinations and bacterial cultures, and the data collected were assumed to be typical of healthy pond-raised channel catfish. Monthly and annual descriptive statistics for the blood parameters are presented in Table 1. Mean (SE) yearly Hct was 21% (0.15%) and fluctuated from a low of 14.5% in midwinter to a high of 25.7% in midsummer. Mean yearly K was 4.43 (0.06) mM/liter with a coefficient of variation of 51.8%. Mean yearly Glu was 26.9 (0.46) mg/dl with a coefficient of variation of 63.3%. Potassium and Glu fluctuated throughout the year and did not follow a statistically identifiable seasonal trend, although a seasonal pattern in mean monthly Glu values was observed. Sodium levels were consistent throughout the sampling period with a mean of 134 (0.13) mM/liter. Mean Cl values for the year were 120 (0.14) mM/liter but rose to 132 mM/liter in midwinter. Calcium and pH values were stable with a mean of 1.31 (0.004) mM/liter and 7.13 (0.004), respectively. Fish size varied widely, with a mean weight of 547 (12.09) g and a mean length of 35.3 (0.25) cm. Fish length and weight were positively correlated with Ca, Na, and Hct and negatively correlated with K and pH (Table 2). Partial correlations were calculated to control for the effect of fish size. Significant partial correlations exist among

Table 2. Blood parameter correlations with channel catfish size variables (length and weight). (*P* values are in parentheses.)

Blood parameter	Fish size	
	Weight	Length
Hematocrit	0.2040	0.2106
	(0.0001)	(0.0001)
Sodium	0.2829	0.03369
	(0.0001)	(0.0001)
Chloride	−0.0358	−0.0145
	(0.1647)	(0.5727)
Potassium	−0.2309	−0.2656
	(0.0001)	(0.0001)
Calcium	0.3446	0.4252
	(0.0001)	(0.0001)
Glucose	0.0542	0.0334
	(0.0437)	(0.2138)
pH	−0.1310	−0.1670
	(0.0001)	(0.0001)

most blood parameters (Table 3), both positive and negative. Correlations were not observed between Na and K, and between Cl and K or Ca. Sodium and Ca had the strongest positive partial correlation ($r = 0.697$, $P < 0.001$). Partial correlation coefficients, which are adjusted for length, closely followed the simple linear correlation coefficients. Sample sizes required to obtain precise estimates of the parameters ranged from 8 for Ca to 119 for Glu (Table 4).

Seasonal models were investigated for all blood parameters. Only the multiple regression models for K, Na, Ca, and Hct did not include significant interactions between pond and time in the models. The seasonal models for Ca and Hct explained 21.4% and 32.6% of the variation (r^2) in their respective parameters. The K and Na models, although significant, demonstrated a weak relationship between the parameters and time ($r^2 = 0.046$ for both parameters). A paired comparison of Hct values revealed that Hct values measured by centrifuge were significantly higher than those measured by the machine (mean difference = 2.6, $P < 0.001$). An adjustment for machine values was calculated by regressing machine-derived Hct (Mv) values against centrifuge-derived Hct (Cf) values, which produced the following equation: $Cf = 0.6 + (Mv * 1.11)$ ($r^2 = 0.73$). Analysis of the residual plot indicated a consistent deviation pattern with increasing Hct levels.

Table 4. Number of fish per sample needed to estimate the mean of selected blood parameters in channel catfish based on observations from Mississippi Delta ponds.

Blood parameter	Annual values		
	mean $\bar{x}$	2SE	*n*
Hct (%)	21	2	25
Na (mM/liter)	134	3	13
K (mM/liter)	4.43	0.5	116
Ca (mM/liter)	1.32	0.1	8
Glu (mg/dl)	26.9	3	119
pH	7.13	0.1	12
Cl (mM/liter)	120	3	13

Discussion

Of the 7 blood chemistry parameters evaluated, Glu and K were the most variable, and obtaining an accurate estimate of these 2 parameters would require large sample sizes from a pond. A pattern of decline

Table 3. Partial correlations between blood parameters for channel catfish from Mississippi Delta ponds after removing the effect of fish size. Values shown are Pearson correlation coefficients, Prob > IRI under H_o: Rho = 0, and number of observations.

	Na	K	Ca	Glu	pH	Cl
Hct	0.4202	−0.2982	0.4621	0.2781	−0.4168	−0.2792
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	1,503	1,468	1,503	1,377	1,498	1,500
Na	…	−0.5512	0.7422	0.0324	−0.4059	0.2566
	…	0.0001	0.0001	0.2275	0.0001	0.0001
	…	1,478	1,514	1,386	1,509	1,511
K	…	…	−0.5702	−0.0529	0.2340	−0.0215
	…	…	0.0001	0.0519	0.0001	0.4084
	…	…	1,478	1,353	1,474	1,475
Ca	…	…	…	0.1541	−0.5323	0.0057
	…	…	…	0.0001	0.0001	0.8238
	…	…	…	1,386	1,509	1,511
Glu	…	…	…	…	0.1468	−0.1769
	…	…	…	…	0.0001	0.0001
	…	…	…	…	1,383	1,383
pH	…	…	…	…	…	0.1449
	…	…	…	…	…	0.0001
	…	…	…	…	…	1,506

in Glu and K in fall and winter was observed in this study and has been previously reported.[3] Glucose levels would be expected to vary with feeding activity, stress, and perhaps unknown factors.[7,23] Fish in this study were sampled in the morning hours prior to feeding. Although Glu was erratic, there was a pattern of higher values in warm weather, which would likely be related to increased feeding activity. This finding is contrary to reports of higher Glu at colder temperatures,[23] which would be expected with higher cortisol levels in colder months.

The remaining parameters were less variable and may therefore be more useful than Glu and K in fish health evaluations. When sampling pond fish, it is imperative to obtain a representative sample to reflect the status of the general population. If blood parameters varied substantially by fish size, then fish from different size classes would need to be collected to precisely estimate blood parameters for the population. However, based on the data gathered throughout a full year on a sample of catfish arbitrarily snagged from ponds, the sample sizes needed to estimate the mean of the remaining blood parameters with sufficient precision is 25 or less. Thus, it appears feasible to monitor this set of blood parameters in commercial operations without collecting fish of a specific size. The fish health specialist must balance the logistics of sampling against the accuracy desired in a clinical setting. The sample sizes will provide guidelines on which to base this judgment. Based on a comparison of the partial correlation coefficients with the simple linear correlation coefficients, blood parameter relationships did not appear to change with fish length. Because fish length is strongly correlated with fish weight ($r = 0.925$, $P < 0.001$), the same is true for the relationship between weight and the blood parameters.

The potential for seasonal fluctuations of blood parameters in commercial ponds should be taken into consideration when evaluating farm-reared channel catfish. Although the K and Na models demonstrated a significant relationship with time, the relationship was weak. During the growing season, Na appears to be relatively stable; Thus in practical applications, a single Na level, with an accompanying measure of variability, may provide an appropriate reference. Simple models for seasonal fluctuations in parameter levels were constructed for Hct and Ca. Observed mean yearly Hct values closely approximated those in a previous study.[5] There was a positive correlation between Hct and fish size in the present study, which is contrary to the results of a previous study, in which a negative correlation was reported between Hct and size in 4 species of catfish.[10]

A number of factors have been reported to effect channel catfish blood parameters, particularly Hct. Dietary folic acid and vitamin C can have significant effects on Hct,[8] and low Hct values were associated with low dietary folic acid or high dietary pteroic acid.[19] Dietary lipid from menhaden oil was associated with a significant reduction in Hct compared with that observed with other sources of dietary lipid.[17] Suppression of Hct has also been associated with diets deficient in iron,[11,18] exposure to aflatoxin B sub(1),[14] and chronic exposure to sublethal nitrite concentrations.[25] Very low Hct values have been reported with a channel catfish anemia syndrome,[6,16] and in some instances this anemia has been linked to the presence of *Bacillus* in catfish feeds.[15] Experimental infection with *Edwardsiella ictaluri* resulted in decrease in Hct and Glu.[2] Exposure to lethal concentrations of un-ionized ammonia resulted in increased Hct[22] and decreased Na.[24]

Blood values are typically measured with automated systems, although the microcentrifuge is commonly used for Hct determination. This laboratory used an automated system that measured each parameter, including Hct. The accuracy of the automated Hct readings could be affected by the larger nucleated red blood cells of fish. These data provided insight into the correlation between the 2 methods and the usefulness of the automated Hct reader in future studies. The automated Hct results correlated well with the centrifuge results. The regression coefficient of 1.11 for the automated Hct results indicated that centrifuge values will become increasingly larger than automated Hct readings as the Hct values increase. These differences must be taken into consideration when comparing automated and microcentrifuge Hct data, and comparison of the Hct data from this study to microcentrifuge data should only be performed after conversion of the Hct values using the regression formula provided here. This formula would only be applicable to measuring channel catfish Hct on the Nova analyzer.

Chloride and pH could not easily be modeled because of variability between ponds. It may be necessary to develop values for individual ponds, although reference ranges could be established from these and previously reported data. The cause of the spike in blood Cl levels in midwinter was undetermined. Farmers routinely add NaCl to ponds in the fall of the year as a deterrent to nitrite toxicity, but the addition of NaCl to these ponds occurred in midsummer and did not coincide with the elevation of blood Cl. It is not known if the Cl spike is a seasonal occurrence or an incidental event or if it reflects increased susceptibility to stress of handling in cold weather. The Cl spike occurred in fish from all 3 ponds and appeared to coincide with similarly timed changes in Na levels. A pattern was also observed with pH; it was higher in winter and lower in summer. The cause of this pattern is unknown, but it may be related to environmental or physiological factors that are subject to seasonal influence.

Consideration must be given to the effects of sampling on blood parameters. There is stress associated with the snagging procedure and rapid increases in blood cortisol are reported following handling in channel catfish.[1] Increases in serum cortisol of channel catfish were also reported in fall and winter months and associated with increased stocking densities;[1] however, stocking density has not been shown to effect blood parameters.[3] No change in Hct was observed in channel catfish subjected to a 15-minute transport stress.[9] In rainbow trout (*Oncorhynchus mykiss*), elevation of K and Cl and decreases in Na and pH were demonstrated within 1 minute of handling.[20] In channel catfish, electrolytes were very stable in the face of harvest stress but Glu increased during harvest.[7] An effort was made to reduce the effects of stress by collecting the blood sample soon after the fish in this study were landed.

This study provided clinical pathology data from a large number of channel catfish observed over an entire year, thus providing fish health specialists with a reference on which to base observations obtained from commercial catfish ponds. As further understanding develops with regard to the interaction of blood parameters and fish health, this information may prove useful as part of an integrated management system with the goal of understanding changes that predict the onset of disease, allowing appropriate intervention and prevention of fish losses.

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