Effects of acute and chronic ammonia exposure on survival, growth and intestinal microbiota composition of hybrid carp

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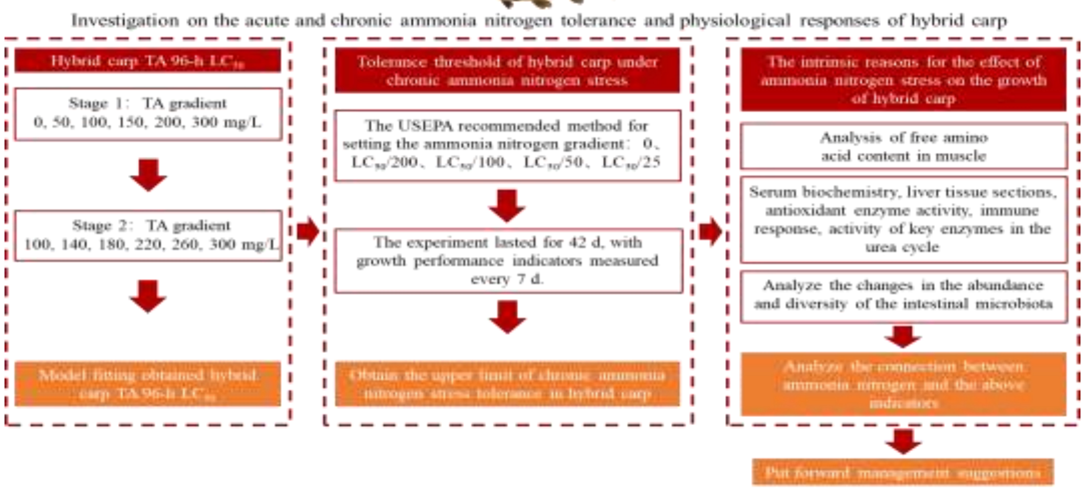
Background

- Ammonia is an environmental stress factor harmful to aquatic organisms, which is produced by the decomposition of residual feed and animal feces.
- Different fish species exhibit variations in their tolerance to ammonia stress.
- This study used the sequential method to assess the acute/chronic ammonia tolerance of hybrid carp.

Materials and methods

- hybrid carp *(Cyprinus carpiovar Jian ♂ × C. carpio ♀)*



Results and Discussion

Two 96-h LC_{50} experiments of hybrid carp, based on linear interpolation ($y= 0.0047x - 0.4659$, $R^2=0.9448$, y is cumulative mortality , x is ammonia concentration), determined that the 96-h LC_{50} was 206.38 mg/L TA.

Table 1. The effects of acute high ammonia stress on hybrid carp

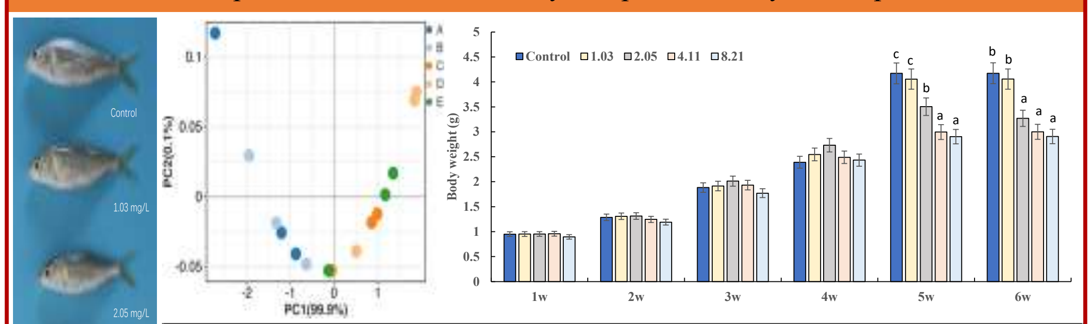
Target TA (mg/L)	Measured TA (mg/L)	Measured un-ionized ammonia (mg/L)	Cumulative mortality (%)
Range Finding Experiment			
0.00	0.01±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
50.00	51.30±1.00^{b}	0.74±0.01^{b}	0.00±0.00^{a}
100.00	99.50±1.50^{c}	1.43±0.01^{c}	0.00±0.00^{a}
150.00	151.40±1.20^{d}	2.17±0.02^{d}	10.00±1.00^{b}
200.00	201.10±1.40^{e}	2.88±0.02^{e}	36.67±2.30^{c}
300.00	301.90±0.80^{f}	4.32±0.01^{f}	100.00±0.00^{d}
Modified Range Experiment			
100.00	101.50±1.20^{a}	1.45±0.01^{a}	5.00±0.06^{a}
140.00	140.50±1.50^{b}	2.02±0.02^{b}	23.33±0.21^{b}
180.00	179.90±1.33^{c}	2.58±0.01^{c}	43.33±5.77^{c}
220.00	219.00±1.00^{d}	3.14±0.01^{d}	40.00±5.00^{c}
260.00	261.50±1.67^{e}	3.75±0.02^{e}	76.67±11.54^{d}
300.00	301.10±0.67^{f}	4.32±0.01^{f}	100.00±0.00^{e}

When TA > 1.03 mg/L, FI and growth performance were inhibited, and when TA > 4.11 mg/L, SR decreased.

Table 2. The effect of chronic low ammonia stress on the growth performance of hybrid carp

	Ammonia levels (mg/L)				
	Control	1.03	2.05	4.11	8.21
SR(%)	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	98.38±1.61^{b}	95.40±1.20^{a}
FBW (g)	4.17±0.41^{b}	4.06±0.30^{b}	3.27±0.21^{a}	3.00±0.23^{a}	2.90±0.16^{a}
WGR (%)	541.96±66.63^{b}	521.69±43.03^{b}	400.82±35.60^{a}	355.97±44.68^{a}	389.87±45.51^{a}
SGR (%/d)	4.42±0.24^{b}	4.35±0.17^{b}	3.83±0.17^{a}	3.61±0.23^{a}	3.78±0.22^{a}
CF	3.43±0.07^{d}	3.25±0.05^{c}	3.00±0.13^{b}	2.84±0.01^{a}	2.85±0.06^{a}
HSI (%)	2.18±0.11^{b}	2.16±0.02^{b}	2.08±0.02^{a}	1.88±0.04^{a}	1.87±0.03^{a}
VSI (%)	14.45±0.30^{c}	11.72±0.73^{b}	11.63±0.45^{b}	9.67±0.08^{a}	10.01±0.77^{a}
FI (g/fish)	4.43±0.42^{b}	4.41±0.06^{b}	4.23±0.03^{a}	4.11±0.11^{a}	4.14±0.11^{a}

Chronic low ammonia stress has an impact on the growth and morphology of hybrid carp, as well as the whole body composition of hybrid carp.



	Ammonia levels (mg/L)				
	Control	1.03	2.05	4.11	8.21
Dry matter (%)	0.81±0.02	0.82±0.05	0.83±0.03	0.82±0.01	0.85±0.05
Protein (% wet weight)	63.82±0.01^{b}	63.68±0.81^{b}	62.10±1.35^{a}	62.09±0.66^{a}	61.65±0.39^{a}
Lipid (% wet weight)	27.87±0.16^{b}	27.15±0.32^{b}	25.94±0.34^{a}	26.09±0.67^{a}	26.11±0.53^{a}
Ash (% wet weight)	10.45±0.55^{b}	10.44±0.70^{b}	9.29±0.45^{a}	9.18±0.45^{a}	9.12±0.28^{a}

Free amino acids deficiency impaired growth and urea cycle enzymes, weakening ammonia detoxification. 2.05 mg/L TA is sufficient to cause a decline in antioxidant enzyme activity, liver damage and inflammation.

Table 3. Free amino acid contents in muscle of hybrid carp during 42-days exposure to different levels of ammonia.

	Ammonia levels (mg/L)				
	Control	1.03	2.05	4.11	8.21
Essential amino acid (% of dry weight)					
Arginine	2.68±0.11^{a}	2.80±0.08^{a}	2.98±0.07^{c}	3.19±0.07^{d}	3.43±0.07^{e}
Histidine	16.50±0.37^{c}	16.19±0.56^{c}	15.55±0.81^{b}	13.36±0.22^{a}	13.53±0.11^{a}
Isoleucine	2.53±0.02^{d}	2.49±0.04^{d}	2.34±0.04^{c}	2.28±0.03^{b}	2.18±0.04^{a}
Leucine	2.89±0.08^{a}	2.91±0.03^{a}	3.06±0.06^{b}	3.27±0.06^{c}	3.45±0.06^{d}
Lysine	7.07±0.06^{c}	7.09±0.06^{c}	6.55±0.11^{b}	6.45±0.33^{b}	5.37±0.22^{a}
Methionine	0.14±0.02	0.13±0.02	0.13±0.01	0.13±0.02	0.13±0.02
Phenylalanine	4.26±0.15^{c}	4.35±0.10^{c}	4.07±0.06^{b}	3.98±0.07^{a}	3.88±0.03^{a}
Threonine	3.70±0.02^{c}	3.67±0.03^{c}	3.62±0.11^{c}	3.40±0.05^{b}	3.19±0.07^{a}
Tryptophan	2.82±0.11	2.80±0.05	2.73±0.06	2.73±0.02	2.71±0.05
Valine	4.33±0.21^{c}	4.34±0.28^{c}	3.88±0.10^{b}	3.57±0.11^{a}	3.32±0.07^{a}
Nonessential amino acid (% of dry weight)					
Alanine	11.51±0.25^{c}	11.20±0.24^{c}	11.01±0.11^{b}	9.36±0.02^{a}	9.35±0.28^{a}
Asparticacid	1.31±0.02^{a}	1.36±0.02^{a}	1.45±0.04^{b}	1.59±0.07^{c}	1.63±0.05^{c}
Glutamine	12.21±0.27^{a}	12.35±0.18^{a}	13.37±0.11^{b}	14.14±0.19^{c}	14.96±0.30^{d}
Glycine	17.42±0.28^{c}	17.37±0.19^{c}	17.07±0.09^{c}	16.64±0.35^{b}	14.99±0.12^{a}
Tyrosine	1.44±0.01	1.41±0.02	1.37±0.01	1.31±0.09	1.29±0.05
Serine	1.63±0.03^{c}	1.62±0.04^{c}	1.53±0.05^{b}	1.47±0.05^{a}	1.39±0.05^{a}

Table 4. The serum ammonia content and biochemical composition of hybrid carp during 42-days exposure to different levels of ammonia.

	Ammonia levels (mg/L)				
	Control	1.03	2.05	4.11	8.21
SA (μmol/L)	132.92±8.28^{a}	135.23±20.90^{a}	178.55±17.89^{b}	173.24±19.69^{b}	176.00±0.8^{b}
TP (g/L)	8.39±0.47^{a}	8.19±0.47^{a}	10.35±1.28^{b}	10.39±0.09^{b}	10.24±0.64^{b}
AST(U/L)	20.12±0.22^{a}	20.97±3.40^{a}	27.48±1.21^{b}	32.23±3.75^{c}	33.03±2.80^{c}
ALT (U/L)	19.35±0.98^{a}	18.98±1.35^{a}	22.47±0.78^{b}	26.12±1.66^{c}	26.78±1.57^{c}
TG (mmol/L)	1.86±0.20^{a}	1.88±0.01^{a}	2.60±0.38^{b}	2.87±0.18^{c}	2.84±0.17^{c}
CHOL (mmol/L)	31.76±0.72	30.55±0.47	30.31±0.55	31.40±0.57	31.35±0.47

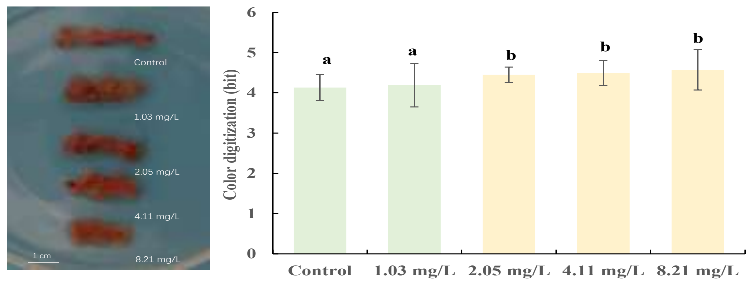
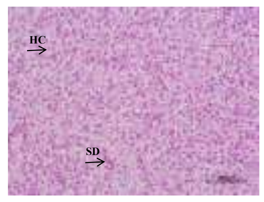


Fig 1. The liver color of hybrid carp on final day representatives from chronic ammonia exposure growth study.



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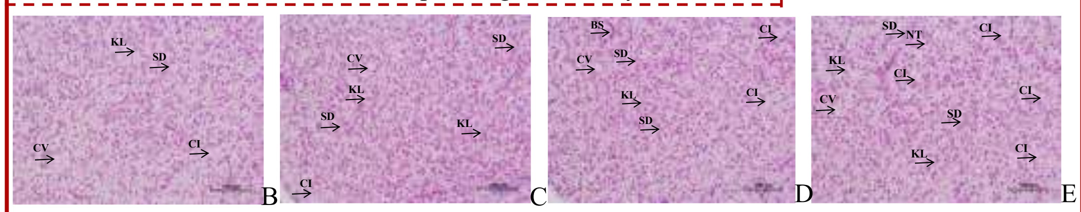

Fig 2. Liver histology of hybrid carp during 42-days exposure to different levels of ammonia

Table 5. The liver antioxidant enzyme activity and inflammatory response and urea cycle enzyme activity of hybrid carp during 42-days exposure to different levels of ammonia.

	Ammonia levels (mg/L)				
	Control	1.03	2.05	4.11	8.21
Oxidative damage index					
T-AOC (U/mg protein)	1.44±0.16^{c}	0.85±0.01^{b}	0.86±0.01^{b}	0.74±0.09^{a}	0.74±0.10^{a}
SOD (U/mg protein)	14.15±0.28^{c}	13.42±0.92^{b}	11.69±0.72^{a}	11.16±0.12^{a}	11.64±0.65^{a}
CAT (U/mg protein)	13.22±0.44^{c}	11.96±0.09^{b}	11.61±1.02^{b}	11.93±0.67^{b}	9.4±0.84^{a}
MDA (nmol/mg protein)	0.80±0.06^{a}	0.88±0.06^{a}	2.04±0.05^{b}	2.00±0.36^{b}	3.99±0.13^{c}
Inflammatory factor					
IL 1 (pg/mg protein)	104.47±4.47^{a}	104.14±5.21^{a}	112.00±3.02^{b}	124.93±4.20^{c}	141.75±2.74^{d}
IL 8 (pg/mg protein)	210.89±1.50^{a}	208.31±3.42^{a}	235.22±4.07^{b}	230.49±0.96^{b}	239.41±1.91^{c}
TNF α (pg/mg protein)	90.3±1.07^{a}	91.93±2.10^{a}	106.70±4.26^{b}	119.52±0.78^{c}	127.44±1.95^{d}
Key enzymes of the urea cycle					
ARG (U/ mg protein)	325.33±11.68^{e}	319.11±17.33^{d}	285.41±13.25^{c}	269.33±11.09^{b}	241.12±15.98^{a}
OTC (U/ mg protein)	812.55±15.78^{a}	809.33±16.38^{a}	822.98±11.29^{b}	825.44±15.66^{b}	839.98±13.29^{c}
ASS (U/ mg protein)	909.12±25.12^{a}	910.45±19.45^{a}	935.44±21.36^{b}	941.68±22.01^{c}	981.12±19.89^{d}
ASL (U/ mg protein)	45.99±9.12^{e}	41.38±7.89^{d}	35.45±7.66^{c}	31.32±6.35^{b}	27.86±6.12^{a}

Long-term low ammonia lowered intestinal microbiota richness/diversity, increased harmful bacteria (e.g., *Levisonia*), while elevated beneficial bacteria (e.g., *Cetobacterium*) may be an ammonia tolerance strategy.

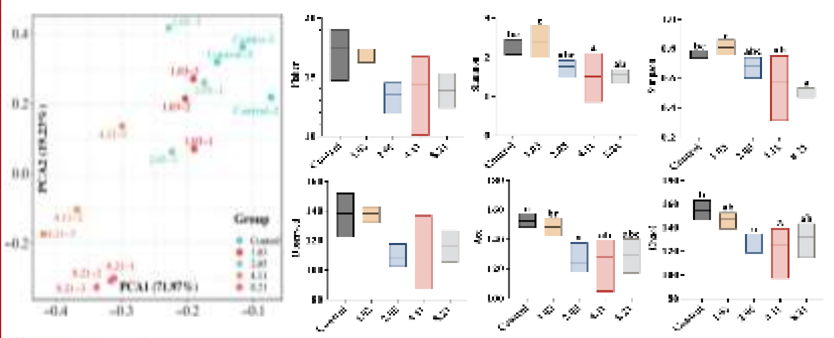

Fig 3. PCA based on weighted UniFrac of the intestinal microbiota, and the difference in alpha diversity of the intestinal microbiot.

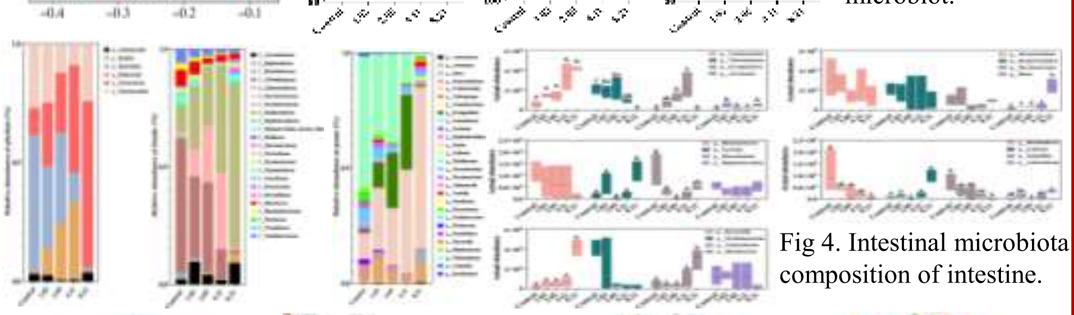

Fig 4. Intestinal microbiota composition of intestine.

Fig 5. LEfSe was used to analyze the difference in microbial abundance between 5 groups.

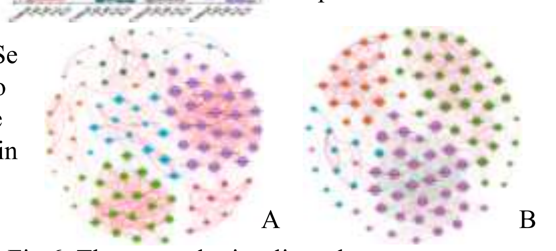

Fig 6. The network visualizes the genus-genus interactions during the operation of ammonia stress.

Conclusion

The estimated 96-h LC_{50} of ammonia is 206.38 mg/L TA. We recommended against exposing hybrid carp to ammonia concentrations exceeding 1.03 mg/L TA. Long-term low ammonia exposure increases the relative abundance of *Cetobacterium*, which may be a self-protection mechanism in carp intestines against ammonia toxicity.

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