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EFFECTS OF ORAL ADMINISTRATION OF β-N(3-hydroxy-4-pyridone) -α-amino PROPIONIC ACID (Mimosine), IN CARPS (Cyprinus carpio L.)

Der Einfluß von verfüttertem Mimosin auf die Gesundheit von Karpfen (Cyprinus carpio L.)

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1. INTRODUCTION

Leucaena leucocephala meal is an economical and nutritious feed source and is now widely used in feeding trials for many farm animal (TER MEULEN et. al., 1979).
The incorporation of Leucaena meal in the feeds of livestock is known to be limited mainly by the contained toxic amino acid mimosine. The compound induces various adverse reactions in livestock and experimental animals (TER MEULEN et al., 1979). Estimation of the consumed amounts of the alkaloid represents an important parameter for evaluating the toxicity due to the consumption of Leucaena meal (TER MEULEN and EL-HARITH, unpublished).
In the literature there is at least one report showing the feasibility of improving the growth of tilapia (Tilapia mossambica) with Leucaena leaf meal and it was claimed that the crude protein contetn of tilapia increased proportionately with increasing levels of Leucaena leaf meal (PANTASTICO and BALDIA, 1978).
This study is designed to investigate the adverse reactions which arise from the ingestion of mimosine, extracted from Leucaena leucocephala, in growing carps.

2. EXPERIMENTAL

2.1. Animals and diets

Four groups of young carps (Cyprinus carpio L.) of 18 animals each were used in this experiment. The basic diet was the same for all the 4 groups and was made from fish meal, Alfalfa green meal and mineral mixture in the percentages of 70, 19 and 1 respectively. The 4 diets differed in their content of mimosine and maize starch as follows:

group	A (control)	B	C	D
% Mimosine	0	2	4	6
% Maize Starch	10	8	6	4

The analysis of these 4 diets showed that they were very similar and contained in average 93% dry matter in which the percentages of crude protein, crude fat, crude ash, crude fibre and N.F.E. averaged 44.0, 4.9, 13.9, 5.0 and 32.2 respectively.

2.2. Experimental protocols

The animals of each group were kept in a 125 liter aquarium adjusted at 22 — 23 °C with a 12-hr alternate light/dark cycle. A part of the water (60 Liters) was exchanged every day with fresh water and the whole water was exchanged once a week. The daily feed offered, which amounted to about 1% of the body weight, was supplied in 8 portions daily with an interval of $1^1/_2$ hours between each meal. This feeding pattern was performed using automatic feed dispensers.
The animals of each group were weighed weekly. The main experimental period was 6 weeks preceded by a 2 weeks adaption period to mimosine containings diets in which small amounts (20 g) of the corresponding feed for each group were daily fed.

2.3. Methods

Crystalline mimosine was extracted from the seeds of Leucaena leucocephala according to a modified Yoshida method (YOSHIDA, 1944, TER MEULEN, 1980 — unpublished). The analysis (Weender) of diets and fishes was performed according to the standard procedures.

3. RESULTS

In this short study the amounts of consumed Mimosine were in the range of 12 — 14, 24 — 26 and 30 — 31 milligrams per animal and day for animals of groups B, C and D respectively. When these values of consumed mimosine were expressed in grams per kg body weight and day the figures: 0.20, 0.40 and 0.60 were obtained for the groups: B, C and D respectively (Table 1). Nine animals of group D died in the second and third day of the experiment and the experiment was carried on with only 9 animals in group D.

Table 1: Mean Mimosine intake by Carps fed on Mimosine containing diets

Absolute intake in mg per animal and day					Relative intake in g per kg body weight and day			
Group	A	B	C	D	A	B	C	D
% dietary Mimosine	0	2	4	6	0	2	4	6
Week 1.	0	12.2	14.2	39.5	0	0.19	0.39	0.67
2.	0	12.9	26.0	30.9	0	0.20	0.40	0.60
3.	0	13.4	26.1	31.0	0	0.20	0.41	0.61
4.	0	13.4	25.3	30.4	0	0.20	0.40	0.60
5.	0	13.6	25.1	30.3	0	0.20	0.40	0.60
6.	0	13.5	25.4	30.1	0	0.20	0.40	0.61

The body weight record showed that the body weight increase was adversely affected in all the animals receiving mimosine diets. This adverse reaction was linearly related to the amount of consumed mimosine. Whereas the body weight of fishes of group D were adversely affected starting from the first week of the experiment, those of groups C and B were significantly affected as from the fourth and fifth weeks respectively (Tables 2, 5).

Table 2: Body weight record of Carps fed on Mimosine containing diets (in g/fish – mean + S. D.)

Group	A	B	C	D
Start	61.2 ± 12.2	60.9 ± 18.1	60.6 ± 14.0	66.2 ± 16.5
week 1.	63.5 ± 13.2	64.3 ± 19.2	64.9 ± 16.5	51.5^{+} ± 8.5
2.	68.8 ± 14.3	66.8 ± 20.1	65.3 ± 13.7	51.8^{+++} ± 8.8
3.	70.6 ± 14.4	66.8 ± 19.1	63.3 ± 13.7	50.6^{+++} ± 8.4
4.	73.6 ± 20.7	68.2 ± 21.3	62.8^{+} ± 13.1	50.5^{+++} ± 8.4
5.	77.8 ± 15.5	67.6^{+} ± 18.8	63.4^{+++} ± 13.7	50.1^{+++} ± 8.8
6.	82.6 ± 16.6	68.9^{+} ± 17.9	64.5^{+++} ± 14.2	49.1^{+++} ± 8.2

Mean values which differ significantly from the control value in the same horizontal column are marked either with + = P 0.05 or +++ = P 0.005 (student's T-test).

The fishes of groups B, C and D receiving mimosine containing diets had normal kidneys and liver weights relative to the control values, but their body weight without the internal organs as well as their gonadal weight were remarkably reduced (Table 3).

Analysis of the rest of body of fishes (without the internal organs) showed that consumption of mimosine had caused slight reduction of the body dry matter, protein and fat. The ash content on the other hand was remarkably increased in fishes receiving mimosine containing diets relative to the control values (Table 4).

The mean feed intake, increase in body weight and protein efficiency ratio (PER) are shown in Table 5. Only the PER of group A was above one, those of groups B and C were less than one and it did not make sense to calculate that of group D because the fishes of group D actually lost weight.

Table 3: Mean body organs' weights of carps fed on Mimosine containing diets

	Fresh Weight in g per animal				Dry Weight in mg per animal			
group	A	B	C	D	A	B	C	D
Gonads	0.26	0.18	0.15	0.07	113	75	65	24
Kidneys	0.67	0.60	0.55	0.65	114	98	91	94
Liver	1.89	1.51	1.43	1.15	383	326	347	369
Rest of Body (Without intestines in g)	71.20	58.62	55.68	41.20	16.17	12.42	12.32	8.52

Table 4: Composition of the Rest of body (without internal organs) of Carps under the influence of Mimosine consumption

Group	A	B	C	D
% dry matter	22.71	21.19	22.11	20.66
% in the dry matter of:				
crude protein	62.32	61.26	61.44	60.23
crude fat	20.30	17.13	17.84	18.69
ash	10.26	14.61	13.40	16.06

Table 5: Mean feed intake, increase in body weight and protein efficiency ratio (PER) of carps fed on Mimosine

Group	A	B	C	D
Feed intake (g dry matter per fish over the whole expt. period)	28.21	26.70	25.77	23.69
Increase in body weight (g per fish over the whole expt. period)	21.44	7.95	3.89	-17.11
PER	1.73	0.68	0.34	-

$$\text{Protein efficiency ratio (PER)} = \frac{\text{body weight increase in g}}{\text{consumed protein in g}}$$

4. DISCUSSION

The intensity of feeding in our experiment (1% of the body weight) is far less than the optimal feeding intensity for carps which may amount to 3% of the body weight. Hence the restricted growth of the control fishes (Tables 2, 5) in our experiment could be mainly attributed to this low feeding intensity. It's worth mentioning here that we were forced to feed our fishes at a level of 1% of the body weight because the results of a preliminary experiment had shown that all the fishes receiving mimosine containing diet did not consume more feed than that equivalent to the mentioned level.

The results of this study shows all the three dietary applications of mimosine are harmful as far as the growth of the fishes is concerned. Consumption of mimosine at a level of 0.60 g/kg body weight is apparently highly toxic as half of the group receiving this dose of the alkaloid died and the rest did not grow at all; in fact they lost weight. However loss of weight may not be attributed only to mimosine intoxication; it is highly likely that the fishes rejected parts of their feed and it is known that carps have highly developed taste sense. In this experiment we had no means of detecting the rejected feed rests.

The reason for the remarkable reduction of the gonadal weight of fishes receiving mimosine is not clear; however the reduced gonadal weight may be a reflection of the clear depression of the growth rate of fishes consuming mimosine.

It was interesting that administration of mimosine at a level equal to or exceeding 0.20 g/kg body weight and day has caused a remarkable increase of the ash content of carps at the expense of the contained protein and fat. This fact indicates that mimosine intoxication may be responsible for the reduced incorporation of protein and fat. Similarly consumption of mimosine at the metioned levels has a clear deleterious effect on the protein efficiency ratio; this again indicates the adverse effect of mimosine on the protein assimilation and utilisation.

PANTASTICO and BALDIA (1978) reported that Leucaena leaf meal when incorporated in the diet of tilapia at a level of 20% was readily acceptable and there was no visible symptoms of toxicity. Since the mimosine content of the Leucaena meal used in the mentioned report was not known and the species of fish was different from ours it is not easy to compare the results of our study with those obtainded by PANTASTICO and BALDIA. However it is highly unlikely that the consumed amounts of mimosine in that study exceeded 1% of the diet as the mimosine content in Leucaena meal rarely exceeds 4% in the dry matter of the material.

It is clear from the present study that administration of mimosine in carps at levels equal to or exceeding 0.2 g/kg body weight is harmful and causes retardation of growth. The conclusion reached here is very comparable to that reached in studies with other animal species (eg. rabbits or chicken) found recently (TER MEULEN and EL-HARITH), unpublished). Furthermore it is important to estimate how much is 0.2 g mimosine/kg body weight and day in terms of incorporated Leucaena material in the diet of carps in order to predict which levels of application of the Leucaena material are harmful to the animals. If we consider, for example, the figures of 2.13 and 3.34 g mimosine per 100 g (dry matter) of a Thailand grown samples of leaves and seeds (TER MEULEN and EL-HARITH, unpublished) and a feeding intensity of 3% for carps, it is possible to do the calculations as detailed in the following:

Consumption of **0.20 g mimosine/kg body weight** and day is harmful; i.e. **0.02 g mimosine/100 g body weight** and day is harmful.

	Leucaena leaves	Leucaena seeds
The amount of Leucaena which contain 0.02 g mimosine:	$= \frac{0.02 \times 100}{2.13}$	$= \frac{0.02 \times 100}{3.34}$
	= 0.939 g	= 0.599 g
if a 100 g fish is fed 3.0 g of feed per day the percentage of the Leucaena material in the feed:	$= \frac{0.939 \times 100}{3.0}$	$= \frac{0.599 \times 100}{3.0}$
	= 31.27%	= 19.97%

Hence Leucaena leaves and Leucaena seed when incorporated in the diet of carps should be applied at dietary levels of less than 30 and 20% respectively provided that a feeding intensity of 3% of the body weight is applied. Similarly if we consider that the protein content in Leucaena leaves and seeds amounts to 26.1 and 33.4% in the dry matter of the same Leucaena material (TER MEULEN and EL-HARITH, unpublished) then the percentage of protein which could have been applied in the present ration (containing 44% protein) when 31.27% or 19.97% of Leucaena leaves or seeds (respectively) were used, can be calculated as follows:

Leucaena leaves	**Leucaena seeds**
$31.27 \times \frac{26.1}{100} \times \frac{100}{44} = 18.55\%$	$19.97 \times \frac{33.4}{100} \times \frac{100}{44} = 15.16\%$

Therefore the contribution of the Leucaena material to the total protein in carps rations should be less than 18% from Leucaena leaves and less than 15% from Leucaena seeds. Bearing this sort of calculation in mind one can predict the harmful level of Leucaena material incorporated in the feed of carps.

5. CONCLUSION

The amino acid mimosine is toxic to carps when consumed at levels equal to or exceeding 0.2 g/kg body weight and day. By means of estimating the consumed amount of the alkaloid it is possible to predict the harmful levels of Leucaena material incorporated in the feed of carps.

6. ABSTRACT

In a 6 weeks experiment, carps (Cyprinus carpio L.) received mimosine at dietary levels of 0.2, 0.4 or 0.6 g/kg body weight and day. It was found that mimosine is toxic to carps when administered at levels equal to or exceeding 0.2 g/kg body weight and day. Administeration of mimosine at 0.6 g/kg body weight and day was highly toxic to carps and led to mortality.

Zusammenfassung

Während eines 6wöchigen Versuches erhielten Karpfen (Cyprinus carpio L.) Mimosin in einer Menge von 0,2; 0» bzw. 0,6 g/kg Körpergewicht und Tag. Das Ergebnis war, daß Mimosin für Karpfen toxisch ist, wenn 0,2 g oder mehr pro kg Körpergewicht und Tag verabreicht werden. Eine tägliche Gabe von 0,6 g Mimosine pro Körpergewicht und Tag war für die Karpfen tödlich.

7. REFERENCES

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