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IMPACT OF FISH MEAL REPLACEMENT BY UNCOUNENTIONAL INGRADIENTS ON PERFORMANCE OF NILE TELEPIA

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ABSTRACT

The effect of partial or total replacing fish meal with dried rumen content (RC) and or Azolla meal (AM) in diets of Nile tilapia of initial weight (7.35g ± 0.23) was investigated in 12-week experiment. Eight experimental diets were formulated to be isonitrogenous and isocaloric. Beside the basal diet, each of RC and AM were incorporated at levels of 5,10 and 15%. A combination of 5% RC and 5% AM (RC-AM) was served to replace 10% fish meal. The control group achieved the significant highest final body weight gain, protein efficiency and food conversion ratio followed by the group fed 10% RC. Azolla incorporation failed to improve any of the performance parameters. The group fed 15% AM was the worth regarding performance parameters, CP and EE retention of final body composition and dietary CP digestibility. The group fed a combination of 5% RC and 5% AM sustained intermediate pattern among treated groups with improved in FCR (4.05). The results indicate some benefits from replacing dietary 10% fish meal with either 10% RC or a combination of RC-AM on Nile tilapia performance as well as reducing feed cost.

INTRODUCTION

Intensification of fish culture has made it essential to develop suitable feeds to be used either as supplementary diets in ponds or as a complete diet in fish reared in tanks. Estimated over 50% of operating cost in intensive aquaculture is related to the expensive dietary protein content depending on fish meal. In Egypt, for economical and practical reasons, aquafeeds should be dependent on locally available protein sources, essentially those consider unsuitable for direct human consumption. Many trials have been directed toward either totally or partially replacing fish meal with either uncounventional sources from animal or plant origin in tilapia diets (**El-Garhy 1994; Saleh, 1994; Hady and Essa, 2000; Shiomi and Kitoh, 2001 and Abou and Micha, 2007).**

Animal by-products have been proven to be highly useful dietary ingredients. The chemical analysis of dried rumen content (RC) revealed a suitable proportions of crude protein which varied from 9.8% (**Eleraky, 19991)** to 25.9% (**Shebata et al., 1984)** with potentiate its use in fish feeding. Dried rumen content

recorded best weight gain and feed conversion when fed at 16% dietary level, which inceasing the level up to 24% adversely affected both parameters of Nil tilapia (**Eleraky, 9991)**. On contrary, **Omar et al. (1993)** concluded that RC could be fed at higher levels up to 30% in Nile tilapia diets with no adverse effect on growth performance and carcass composition. Moreover, **Hassan (1989)** suggested that with increasing the level of dried ramen liquor all EE, ash, CF and NFE contents of common carp were increased but gross energy and feed cost were decreased.

Azolla is a small cosmopolitan aquatic fern that contains obundant nutrients as crude protein which ranged from 23 to 30% on dry matter basis (**Lumpkin and Plucknette, 1982; Nwanna and Falaye, 1997; Hady and Essa, 2000 and Alalada and Iyayi, 2006).** Many authors found that Azolla meal (AM) is to be incorporated in the diet of various species **(Liu, 1989; Saleh, 1994; Nwanna and Falaye, 1997; Shiomi and Kitoh, 2001 and Alalade and Iyayi, 2007). Santiago et al. (1988)** achieved a positive action of dietary AM incorporation at different levels on Nile tilapia performance replacing fish meal. On the other hand, **Almazen et al., (1986), Abdel Fattah and Abdel-Aziz (1990); Hady and Essa (2000) and Shiomi and Kitoh (2001)** found that the growth performance parameters were negatively coorelated with increasing either dried or fresh Azolla level in ***O.niloticus*** diets. From the above contemplation, the objectives of this study are to evaluate the possible usage of some non-conventional cheaper dietary sources namely, dried rumen content (RC) and Azolla meal (AM) to replace partially or totally expensive fish meal in Nile tilapia diets aiming at lowering the

MATERIALS AND METHODS

The current study was carried out at the department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University of the experimentl diets compared to basal diet was calculated considering the local prices.

Sources and nutritive value of rumen contents (RC) and Azolla meal (AM): The rumen contents were collected from normal clinically healthy slaughtered cattle from Moneib slaughter house, Giza governorates. The collected rumen contents were prepared after **Abdel - Rahman (1995).** Azolla meal was obtained from Faculty of Veterinary Medicine, Alexandria University. The proximate chemical analysis of RC and AM (Table 1) were determined according to standard methods of **AOAC (1980).**

Diets and experimental design:

Eight experimental diets were formulated to fulfill Nile tilapia requirements (**NRC, 1993)**. The rumen contents or Azolla meal were incorporated in fish diets at levels of 5,10 and 15% for groups RC_1,; RC_2; RC_3; AM_1, AM_2 and AM_3 to replace partially or totally fishmeal, respectively. A combination that contained 5% RC and 5%. AM was fed for fish in Rc-AM group. The fish

fed on basal diet (BD) was kept as a control group. The formulated diets were isnitrogenous and isocloric and calorie protein ratio was kept as constant as possible (Table 2). Two hundred and forty Nile tilapia fingerlings were acclimatized to laboratory conditions for 2 weeks and fed on the basal diet before the start of 12 weeks experiment.

There were a total of 8 treatments each of 2 replicate (15 fish/ replicate) Fish were stocked into 16 glass aquarium (120L) equipped with air pum and declorinated water. Dissolved oxygen, ammonia, nitrite and nitrate were determined according to American Public Health Association **(APHA, 1992).** At start and at- 2 weeks interval, the fish were weighed and the lengths were measured . Fish were fed twice daily at a rate of 3% of the body weight, which was close to the maximum intake during the acclimatization period. Feed was withhold on the day of weighing and feed intake was a djusted accordingly every $_2$ weeks. Total gain, feed/gain, protein efficiency ratio (PER), and feed conversion ratio (FCR) were calculated according to **Inca et al. (1982)**. Specific growth rate (SGR) was calculated according to **Siddiqus et al. (1988)** . Before stocking, 15 extra fish and at the end of the experiment, 3 fish per replicate were randomly selected, weighted, then freeze to determine the initial and final gross body composition, respectively **(A.O.A.C. 1980)**. While dressing percentage was determined on final fish according to **Hardy** and **Sullivan (1988**) . Digestibility coefficient of the experimental diets were determined using on internal indicator (crude fiber) according to **Jones and Desliva (1998).**

Statistical analysis:

The results were subjected to the single factor analysis of variance (ANOVA) according to **Snedecor and Cochran (1969).**

Table (1): Proximate chemical analysis % of rumen content (RC) and Azolla meal (AM) on dry matter basis.

Subject	CP	EE	CF	Ash
RC	13.10	3.67	31.89	11.84
AM	12.8	0.10	8.77	19.22

Table (2): Ingredients and calculated analysis of the experimental diets

Ingredients %	BD	RC_1	RC_2	RC_3	AM_1	AM_2	AM_3	RC-AM
Fish meal (71.75 Cp%)	15.0	10.0	5.0	0.0	10.0	5.0	0.0	5.00
Soybean meal (44.05 Cp%)	38.0	46.25	49.0	56.00	46.50	56.25	67.15	57.00
Yellow corn	18.0	9.00	3.75	2.0	8.25	2.90	1.80	6.25
Wheat bran	23.5	24.0	18.50	9.0	23.0	18.50	8.0	14.8
RC	0.00	5.00	10.00	15.00	0.0	0.0	0.0	5.0
AM	0.00	0.00	0.00	0.00	5.0	10.0	15.0	5.0
Linseed oil	1.50	1.75	2.75	3.25	2.5	3.35	4.05	2.95
Mineral mix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin mix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated Analysis (%)								
CP	32.99	32.98	32.97	33.01	32.97	32.97	32.97	32.99
EE	4.31	4.12	4.73	4.91	4.66	95	5.16	4.78
CF	5.67	7.74	9.31	10.73	6.56	7.05	7.19	7.95
ME Kcal/Kg diet[3]	3403.75	3401.63	3401.71	3406.99	3402.44	3401.60	3421.7	3402.03
P/E ratio[4]	96.92	96.81	96.92	96.89	96.90	96.92	96.35	96.97
Lysine	2.09	2.11	2.14	2.18	2.11	2.15	2.17	2.14
Methionine	0.60	0.56	0.52	0.50	0.54	0.49	0.45	0.51

1. The mineral mixture was prepared by mixing 27% limestone (38% Ca), 41.5 disodium phosphate, 25% Ramical trace mineral and 6.5% sodium chloride. Each kg contained Ca, 102.9; P, 90.2g; Fe, 25000mg; Cu, 2000mg; Mn, 60000mg; Zn, 40000mg; Sc, 100mg.
2. Each kg of vitamin premix contains Vit.A, 67500000 IU; Vit. D3, 1.500.000 I.U, Vit.E, 2.000IU; Vit.K, 1.000mg; Vit.B1, 1.000mg; Vit B2, 2.000mg; Vit B6, 1.000mg; Vit.B12, 5mg; Vit C, 10.000mg; D- Ca-panto then ate 5.000mg, Nicotinic acid 10.000mg; Folic acid 150mg, Biotine 5mg; Na, 825mg, Mg, 10gm; K, 10gm and Ca, 20gm.
3, ME and P/E ratio were calculated according to Soltan (1990).

Table (3): Impact of feeding different levels of rumen contents (RC) and/or Azolla meal (AM) on Nile tilapia performance.

Criteria	BD	RC_1	RC_2	RC_3	AM_1	AM_2	AM_3	Rc-AM
AV. Initial weight (g)	7.71^{a} ±0. 26	7.40^{a} ± 0.23	7.61^{a} ± 0.33	7.33^{a} ± 0.25	7.42^{a} ± 0.24	7.16^{a} ± 0.26	7.11^{a} ± 0.29	7.08^{a} ± 0.26
AV. Final weight (g)	14.69^{a} ±0.98	11.90^{ab}± 0.56	12.89^{ab} ± 0.86	10.78^{b} ± 0.52	12.26^{ab} ± 0.61	10.58^{b}± 0.50	10.05^{b}± 0.599	12.04^{ab}± 0.83
AV. Total gain (g)/fish	6.95^{a} ±0.54	4.50^{bc} ± 0.19	5.27^{ab} ± 0.09	3.45^{bc} ± 0.24	4.84^{bc} ± 0.17	3.42^{bc} ± 0.21	2.93^{c} ± 0.12	4.95^{b} ± 0.24
% increase in weight gain	100	64.75	75.83	49.64	69.64	49.20	42.13	71.22
AV. Initial length (cm)	7.87^{a} ±0.11	7.78^{a} ± 0.08	7.84^{a} ± 0.12	7.82^{a} ± 0.10	7.83^{a} ± 0.08	7.67^{a} ± 0.03	7.67^{a} ± 0.10	7.77^{a} ± 0.09
Av. Final length (cm)	9.68^{a} ±0.21	9.09^{ab} ± 0.14	9.23^{ab} ± 0.20	8.75^{b} ± 0.10	9.22^{ab} ± 0.14	8.67^{b} ± 0.15	8.53^{b} ± 0.16	9.03^{ab} ± 0.21
Increase in length (cm)	1.81	1.31	1.39	0.93	1.39	1.00	0.86	1.26
Av. Total feed consumed (g)/fish	23.28^{a} ±0.19	20.57^{ab}± 0.75	21.47^{b} ± 0.52	19.79^{ab}± 0.52	21.43^{b} ± 0.60	18.93^{c} ± 0.52	18.52^{c} ± 0.52	20.06^{b} ± 0.15
Av. Total protein consumed (g) fish	7.67^{a} ± 0.05	6.78^{bc} ± 0.25	$7.0\ 8^{ab}$ ± 0.17	6.53^{b} ± 0.16	7.06^{ab} ± 0.20	5.63^{c} ± 0.04	6.09^{c} ± 0.52	6.61^{b} ± 0.06
*Protein efficiency ratio (PER)	0.9^{a} ± 0.06	0.67^{ab} ± 0.05	0.75^{ab} ± 0.03	0.53^{b} ± 0.04	0.69^{ab} ± 0.01	0.55^{b}± 0.04	0.48^{b} ± 0.02	0.75^{ab} ± 0.03
Feed conversion ratio (FCR)	3.36^{d}± 0.23	4.58^{bc} ± 0.37	4.07^{c} ± 0.16	5.91^{ab} ± 0.24	4.42^{bc} ± 0.03	5.63^{b} ± 0.49	6.23^{a} ± 0.23	4.05^{c} ± 0.16
AV. Specific growth rate (SGR)	0.34^{a} ± 0.02	0.25^{ab} ± 0.01	0.28^{ab} ± 0.01	0.20^{b} ± 0.01	$0. 25^{a}$ ± 0.01	0.21^{b} ± 0.01	0.18^{b} ± 0.01	0.27^{ab} ± 0.01
Av. Condition factor	1.26^{a} ± 0.04	1.60^{a} ± 0.01	1.67^{a} ± 0.01	1.62^{a} ± 0.02	1.58^{a} ± 0.02	1.64^{a} ± 0.01	1.62^{a} ± 0.05	1.62^{a} ± 0.01

-Values are means ± SE

A,B,C…etc. means in the same raw with different superscripts are significantly different at P<0.01.

a, b,c,…etc, means in the same raw with different superscripts are significantly at at P<0.05. * Proteins efficiency ratio = weight gain (g)/ protein (g) intake

Table (4): Whole body gross composition (% on DM basis) and dressing % of Nile tilapia fed different experimental diets.

parameter	initial fish Composition (%)	Final fish composition%							
		BD	RC_1	RC_2	RC_3	AM_1	AM_2	AM_3	RC-AM
Dry matter	$19.41^{c}\pm$ 0.69	$25.15^{ab}\pm$ 0.65	$24.04^{b}\pm$ 0.45	$25.23^{ab}\pm$ 0.67	$24.97^{b}\pm$ 0.52	$25.98^{ab}\pm$ 0.59	$26.11^{ab}\pm$ 0.81	$27.33^{ab}\pm$ 0.89	$27.49^{a}\pm$ 0.77
Crude protein	$60.0^{c}\pm$ 1.7	$65.20^{ab}\pm$ 1.1	$64.90^{ab}\pm$ 1.4	$67.00^{ab}\pm$ 1.9	$62.20^{bc}\pm$ 1.6	$65.60^{ab}\pm$ 0.85	$64.4^{ab}\pm$ 1.5	$62.90^{bc}\pm$ 1.42	$63.00^{bc}\pm$ 1.8
Ether extract	$13.64^{a}\pm$ 0.5	$9.58^{b}\pm$ 0.82	$11.29^{b}\pm$ 0.45	$9.15^{bc}\pm$ 1.0	$11.07^{b}\pm$ 0.52	$8.48^{bc}\pm$ 0.85	$7.43^{bc}\pm$ 0.79	$7.22^{b}\pm$ 0.83	$7.11^{bc}\pm$ 0.61
Ash	$14.88^{b}\pm$ 1.1	$19.55^{a}\pm$ 1.4	$17.70^{a}\pm$ 1.5	$16.55^{a}\pm$ 1.8	$17.27^{a}\pm$ 1.7	$17.79^{a}\pm$ 1.5	$17.25^{a}\pm$ 1.4	$21.38^{a}\pm$ 2.1	$16.05^{a}\pm$ 1.9
Dressing (%)	-----	$90.05^{a}\pm$ 0.51	$89.9^{a}\pm$ 0.43	$89.36^{a}\pm$ 0.64	$86.96^{a}\pm$ 1.70	$89.51^{a}\pm$ 1.29	$88.71^{a}\pm$ 0.71	$87.90^{a}\pm$ 1.10	$87.72^{a}\pm$ 0.38

- Value are means ± SE
- a,b,c....Values within the same raw with different superscripts are significantly different at $P<0.05$.

Table (5): Apparent digestibility coefficient of dry matter, crude protein and ether extract of the experimental diets fed to Nile tilapia.

Group	Digestibility %					
	DM	%*	CP	%	EE	%
BD	97.28	100	91.93	100	90.07	100
RC_1	97.00	99.71	92.72	100.86	88.09	97.90
RC_2	97.01	99.72	94.33	102.61	97.90	108.69
RC_3	96.21	98.90	89.92	97.81	90.14	100.1
AM_1	97.21	99.93	93.22	101.4	91.79	101.91
AM_2	97.58	100.31	92.04	100.12	98.63	109.5
AM_3	98.80	101.64	67.63	73.57	92.89	103.13
RC-AM	97.38	100.10	91.08	99.08	98.56	109.42

* Percent of increase or decrease regarding control.

RESULTS AND DISCUSSION

The results of proximate chemical analysis of two unconventional dietary sources namely RC and AM used in the preparation of the experimental diets are presented in table (1). The RC results of CP, EE and ash content came on average with that reported by **El-Yassin et al. (1991) and El-Tahan (1996)**. The obtained CF level (31.89%) came nearly similar with the results of **Shebata et al. (1984) and Adel - Rahman (1995)** . The higher CF-levels of RC reported in the results of other workers (**Eleraky, 1991 and El-Tahan, 1996)** might be related to several factors such as type of diet which had been fed to the animal, time of sampling as well as retained materials from previous feeding (**Abdel - Rahman, 1995).**

The results of chemical analysis of AM showed marked reduction in CP and EE contents as compared to the results of **Abdel-Halim et al. (1998), Saleh (1994), Hady and Essa (2000) and Alalade et al. (2007).** The obtained CF level was almost similar to that obtained by **Santiago et al (1988) and Shaban (1999)**. The discrepancy in the results of the proximate chemical analysis of AM with some studies might be attributed to the species of Azolla predominated in the sample examined (25 different Azolla strains existe, **Antoina et al. (1986)** and/or seasonal variation as well as culture techniques (**Chen and Huang, 1987).**

Performance Results:

The results of the impact of partially or totally substituting fish meal with RC and/or AM on Nile tilapia's performance are presented in table (3). The means initial body weight as wll as mean initial length were insignificantly differed between treatments indicated complete randomization process. The BD-group fed the basal diet exhibited significantly highest body weight gain followed by the RC_2-group (10% RC). The average specific growth rate (SGR) showed insignificant reduction in the groups fed 5 and 10% RC compared to the control (0.25 & 0.28 vs. 0.34), while feeding 15% RC significantly reduced all examined performance parameters. These findings were in a partial agreement with those obtained by **Eleraky (1991)** who found a significant reduction of Nile tilapia's gain with the increase of dietary RC, Nonetheless, at higher dietary level of 16%, no adverse effect was detected. The group fed 15% AM (AM_3) sustained the lowest final weight (10.05g), weight gain (2.93g), PER (0.48), FCR (6.32) and SGR (0.18) among all treatments. These results agree with the work of other authors **(Almazen et al., 1986; Saleh, 1994; Abdel Halim et al., 1998 and Shiomi and and Kitoh, 2001).** On contrary, **Abou and Micha (2007)** found no negative effect on the growth performance and production of ***O.niloticus*** fingerlings (15.5g) reared in wetland pond when fed 10 and 20% Azolla compared to Azolla free diet. The negative effect of complete substitution of fish meal with 15% Azolla meal on Nile tilapia's performance especially PER suggested that Azolla meal might be deficient in specific amino acids (lysine and methionine) and /or the presence of high – levels of neutral detergent fiber and high ash content

(Buckingham et al. 1978). The group fed a combination of 5% RC and 5% AM replacing 10% fish meal sustained intermediate pattern regarding Nile tilapia's performance parameters. The aforementioned results elucidated that some sort of compensation was existed between different dietary protein sources (fish meal, 5%; RC, 5% and AM, 5%) regarding amino acids which was not as optimum as needed for expression of growth potential (**Chambers, 1990).**

Whole body composition results:

The results of whole body composition and dressing percentage of Nile tilapia fish fed different experimental diets are presented in table (4). It was observed that with advancing in age body DM, CP and ash contents increased while EE contents decreased. The recorded values for Nile tilapia gross body composition were within the normal values recorded by **Siddiqui et al. (1988); El-Huseiny et al. (1993) and Essa et al. (1995).** The final DM and EE content showed significant increase in all groups compared to the initial composition. This result indicated that there was an inverse relationship between body moisture and lipid content, a relation, which had been previously reported (**Jauncey, 1982).** Incorporation of 10% RC insignificantly increased CP content as compared to the BD-fed group (0% RC). The positive effect of incorporating 10% RC to replace 10% fish meal protein on increasing the CP and decreasing the EE contents of final fish body composition is coincided with the performance results (weight gain and PR) represents in table (3). Body EE content was insignificantly increased by RC feeding (5 and 15%) and insignificantly decreased by feeding RC (10%), AM (5, 10 and 15%) and RC-AM combination compared to control as reported by other workers, respectively **(Eleraky, 1991. ; Abdel-Fattah and Abdel- Aziz, 1990 and Abdel –Halim et al. 1998).**

The Azolla feeding at higher levels (15%) was accompanied with a reduction in both CP and EE values and an increase in ash content in final gross body composition of the fed fish. Additionally, these results were correlated to the performance results of AM_3 group which retained the lowest gain values, table (3). Similar reports corroborate these findings **(Hady and Essa, 2000 and Shiomi and Kitoh, 2001).** Moreover, **Abdel-Fattah and Abdel-Aziz (1990)** detected a negative correlation between fat content of ***O.niloticus*** body and the dietary Azolla levels. Nonetheless, an improvement effect on performance had been achieved by feeding 10% sun dried Azolla meal in complete carp diet compared to the control followed by 20% Azollo level (**Saleh, 1994).** This discordances in results might be related to the nature of feedstuff which underwent substitution by Azolla (plant protein, soybean meal vs. animal protein, fish meal) or to the difference in the species of used fish (carp vs. Nile tilapia). Regarding the dressing percentage results of Nile tilapia in different groups showed no significant difference due to dietary

treatments. Similar results were recorded by **Soltan (1990)** and **Hady and Essa (2000).**

Digestibility results:

The results of digestibility of the experimental diets fed to Nile tilapia using internal indicator (CF) are presented in table (5). The DM digestibility seemed to be slightly affected by the feeding of either RC or AM. Meanwhile, the EE digestibility corresponded similarly except for the RC_2, AM_2 and RC-AM groups (108.69, 109.5 and 109.42 percent increase over control). **Saleh (1994)** reported that EE digestibility was increased when AM replaced fish meal in carp diets. In regard to CP digestability, there was no prominent changes compared to control, except at the highest level of AM (15%) that induced minimum CP digestibility (67.63 vs 91.93%) . It is worthnoting that the adverse effect of feeding AM at 15% to replace completely fish meal protein was corroborated with the aforementioned results of performance (Table 3) as well as whole body composition (Table 4) for such group. **Hossein and Jauncey (1989)** emphasized that substitution of fish meal by plant protein had decreased apparent protein digestibility. The failure of AM to exhibite a positive trend an digestibility and growth when totally replaced fish meal might be essentially related to the lack of certain amino acids (methionine, lysine and tryptophan)

compared to the requirements for such amino acids reported for ***O.niloticus*** (**Buckingham et al. 1978; Jauncey 1982 and Santiago et al., 1985)** . Moreover, the nature of CF in Azolla, might itself be hardly digested and might envelop other nutrients such as protein and carbohydrate from digestive enzymes **(Hepher, 1988).** Interestingly, Azolla strain showed different preference by Nile tilapia which prefer ***A.filliculoids*** rather than ***A.pinnata*** **(Antoina et al., 1986),** in the current study, ***A.pinnata*** appeared to be more dominant. Feeding a mixture of plant and animal protein (RC-AM group) resulted in higher growth rate than feeding a sole protein-loosed diet (**Sitasit and Sitasit, 1977).**

Conclusively, the results of the current study emphasize the incorporation of either rumen content (10%) or the combination of rumen content-Azolla meal (5% -5%) to substitute 10% of fish meal protein in Nile tilapia diets aiming at reduction of feed cost.

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