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EFFECT OF INCORPORATION OF FISH SILAGE INTO DIETS ON GROWTH PERFORMANCE AND BODY COMPOSITION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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ABSTRACT

The potential value of fish silage as a protein source for freshwater fish was assessed by chemical analysis and by incorporation of silage in exprimental diets for *Oreochromis niloticus* . Fish silage was prepared from a mixture of Nile tilapia unedible parts.. Sulphuric formic silage was prepared by addition of 1.5% concentrated sulphuric acid and 1.5% concentrated formic acid to the minced mixture of whole Nile tilapia bodies. The silage was stored for 24 weeks. The chemical composition of fish silage was determined and some of the changes occuring in protein during storage were described. Three diets were formulated in which fish meal was replaced by fish silages (50,75 and100% silage). Diets were fed to experimental groups of tilapia for 13 weeks. A significant difference in growth performance and protein productive value were noted between tilapia fed on 100% fish silage and other treatements; however feed convertion ratio and protein efficiency ratio showed no significant differences between all treatments. The carcass composition parameters were affected by silage in diets. Cost benefit analysis showed that low profit index and high inclusive cost were obtained by the control diet.

INTRODUCTION

The demand for animal protein in Egypt is expected to increase progressively with each increase in humnan population. Nile tilapia has been called opportunistic omnivore, at it can consume a variety of food, living or dead materials of animal or plant origin (Stickney, 1997). Proteins are the major nutrients required for growth of fish and makes up most of the body structure (Jauncey and Ross, 1982). Fishing operations yield includes considerable waste materials such as bycatch fish, unmarketed fish and filleting scrap. Unfortunately, the lack of processing facilities in many lesser developed countries often leads to the paradoxical situation of wastage accurring where the need for protein is greater. Although priority should be given to the direct use of fish for human consumption, there are often considerable quantities of fish wastes available for animal feeding. In Egypt, as well as at many isolated parts, in the developing sociaties where supplies of fish waste may be small and irregular, it is uneconomical either to transport the waste to a fish meal plant or to build a small local factory. Fish ensilaging may be a suiTable alternative method of preserving fish waste in these circumstances. Acid preserved fish silage is a liquid product prepared by adding acid to fish or fish waste and liquefaction is caused by proteolytic enzymes, present naturally in the fish (Backhoff, 1976). The addition of acid stimulates enzymic proteolysis which help in dissolving bones and prevents bacterial and fungal spoilage (Wingall and Tatterson,1976). Feeding trials have shown that fish silage can replace fish meal in the diets of salmon (Jackson *et al.*, 1984) and carp (Venujopal and Keshavanath, 1984).There are few reports on trials with ruminant animals and poultry (Raa and Gildberg, 1982). The suitability of including fish silage in diets for tilapia was therefore investigated. Whole fish silage prepared acid insilage method was studied to examine changes during storage with particular attention being paid to protein quality. Fish growth response was also monitored when fish silage based diets were offered.

Therefore, the present work is performed to study the effect of replacement of fish meal by fish silage on growth performance , efficiency of feed utilization and economical feasibility of Nile tilapia (*O. niloticus*).

MATERIAL AND METHODS

The present work was conducted in Fish Research Laboratory in the Department of Animal Production, Kafr El-Sheikh Faculty of Agriculture, Kafr El-Sheikh University, during year 2006.

1-Experimental units:

A total number of 180 fingerlings of *Oreochromis niloticus* with an average initial weight of 13.60g were used in this study. The fish were divided into 12 similar groups in glass aquaria (80 x 35 x 40 cm) containing equal amount of water (80 liters) in each, representeing four dietary treatments each in triplicates. The aquaria facilities of the wet lab were used for performing this experiment. Each aquarium was supplied with compressed air through air pump for water areation. Dechloronated tap water was used for replacing about one third of the total water volume in each aquarium daily after the removal of fecal wastes. Water temperature was controlled thermostatically by automatic heaters and was measured two times daily using a thermometer. Water temperature was kept at the range between 27-28 C^{0} during the experimental.

2-Experimental fish:

All fish were taken from a private tilapia hatchery located at El-Hamool area in Kafr El-Sheikh Governorate. The fish were transported in plastic conteiners filled with water and oxygen to the wet Lab, Faculty of Agriculture Kafr El-Sheikh. All fish were kept for two weeks in a fiber glass tank before their distribution into the experimental aquaria for adaptation on the new environment. The fish were then randomly divided into equal groups in the experimental aquaria (15 fish/aquarium). The fish were kept one week in the aquaria before begining of the experiment. The fish were fed on a control diet containing 25% protein at a rate of 3% of total biomass during the acclimatization period. Three experimental aquaria were used to test every experimental dietary treatment.

3-Experimental diets:

Fish silage was prepared using trash fish of unmarkeTable size, which is unused for human consumption. The fish were collected from the local market in Kafr El-Sheikh city and well washed, minced and homogenized. One and half percent from each of conc. sulphuric and conc. formic acid were added to the homogenized fish mixture according to Jackson *et al.*(1984). The fish mixture was transferred thereafter to plastic bags and stored at room temperature for 24 weeks. The chemical analysis of the produced fish silage after 24 weeks storage period is reported in Table (1).

Before formulation of the experimental diets, fish silage was neutralized by adding 1.6% calcium hydroxide to raise the silage pH from 4.1 to 5.2. Fish silage was mixed with the other ingredients to formulate 4 diets, containing, 0, 50, 75 and 100% fish silage to replace fish meal.

The ingredients of the experimental diets were ground in a hammer mill and mixed well together. Thereafter, water (25%) was added to the mixture which was allowed to pass through a mincing machine to produce 2mm pellets in diameter. The wet pellets were dried in an oven and stored in the refrigerator till use. Composition of the experimental diets tested is illustrated in Table (2), while the proximate analysis of the diets is shown in Table (3).

4-Experimental procedure:

The experiment lasted for 13 weeks after start. During the experimental period, fish were fed the experimental diets at a rate of 3% of the live body weight daily and the feed was offered twice daily at 9.0 a.m and 3.0 p.m. The fish groups were weighed weekly and the amount of the feed was adjusted according to the actual body weight changes. Water samples were taken from each aquarium to determine water quality parameters. Light was adjusted by a timer to provide 14: 10 h photoperiod daily. One third of water volume was changed daily and the whole water volume was totally changed every week.

5-Growth and feed utilization parameters:

Average weight gain (AWG), average daily gain (ADG), specific growth rate (SGR % d), feed/gain ratio, feed conversion ration (FCR), protein efficiency ratio (PER) and survival rate were calculated according to the following equations:

a) Average weight gain (g/fish) (AWG):

$$WG = W2 - W1$$

Where: W1 = the initial weight (g)
W2 = the final weight (g)

b) Average daily gain (ADG)

Average daily gain (ADG) was estimated according to the following formula

$$ADG = \frac{W2 - W1}{T}$$

Where: W1 = The initial weight (g)
W2 = The final weight (g)
T = Experimental period (d)

c) Specific growth rate (SGR)

Specific growth rate (SGR) was estimated according to the following equation:

$$SGR = \frac{Ln.\,W2 - Ln.\,W1}{Period\,(days)} \times 100$$

Where: Ln = Natural Logarithm $(log)^{-10}$
W1 = Mean initial weight (g)
W2 = Mean final weight (g)

d) Relative growth rate (RGR)

The relative growth rate was calculated according to the following equation

$$RGR = \frac{W2 - W1}{W1} \times 100$$

Where: W1 = The initial weight
W2 = The final weight

e) Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry feed intake (g)}}{\text{Live weight gain (g)}}$$

f) Protein efficiency ratio (PER)

$$PER = \frac{\text{Live weight gain (g)}}{\text{Protein intake (g)}}$$

6- Proximate analysis

Proximate analysis for silage, experimental diets, and whole fish bodies were carried out for moisture, ash, protein and fat according to the methods described by A.O.A.C. (1990). Nitrogen free extract (NFE) was calculated by differences.

7- Water quality parameters

Samples of water were taken weekly from each aquarium for determination of water temperature using a water thermometer (daily), water pH value using digital pH meter (Orient Research Model 201), dissolved oxygen concentration using an oxygen meter (model 9070). Analyses of NO_2, NO_3 and Hardness were carried out using kits (Hach international Co., Cairo, Egypt). Analysis of alkalinity was performed using a kit (LaMotte International Co., Cairo, Egypt).

8-Economic evaluation:

Economic evaluation of the experimental diets has been calculated by evaluation the feed cost in Egyptian pounds (L.E) needed to produce 1 kg of live weight gain of each experimental fish group.

Feed cost (L.E) = (feed cost/kg) X (food consumption)

Price of one kg gain in weight (LE) (LE/gain "kg") = (feed cost/kg) X FCR

9.Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance . When F-test result was significant, least significant difference was calculated according to Duncan multiple range test (1955).

RESULTS AND DISCUSSION

1-Water quality parameters

Average of the studied ranges of water quality parameters; temperature dissolved oxygen (DO) mg/L, pH, alkalinity, hardness, $NO_3$and NO_2 during the whole experimental period are shown in Table (4). The results revealed that water temperature ranged between 27 to 27.9 Ċ; DO mg./L between 5 to 5.5 ; pH between 6 to 7; alkalinity between 125 to 140 mg/L; hardness between 315 to 330 mg/L; NO_2 between 0.10 to 0.11mg/L and NO_3 between 1 to 1.5 mg/L. These parameters were within the accepTable levels required for normal growth and physiological activities of Nile tilapia (Boyd, 1995; Salem 2006).

2-Effect of incorporation of fish silage in replacement to fish meal on water quality.

As presented in Table (5), the averages of water temperature ranged between 27.0 and 27.8Ċ and dissolved oxygen between 5.16and 5.44, pH between 8.9 and 8.5 , alkalinity 120-138, hardness 250-262, NO_2between 0.2 and 0.4 and NO_3 between 1.15 and 1.43 mg./ L. The results indicate that the studied water quality parameters, except NO_2 were within the permissible levels, however incorporation of fish silage tended to increase these parameters especially at higher incorporation levels. The results are in partial agreement with the findings of Gomaah and EL-naggar (2004), who reported that replacement of fish meal with poultry byproducts meal at 0, 35 or 70% level of fish meal protein in catfish diets reared in earthen ponds, had no significant effects on water quality parameters except total ammonia and conductivity.

3-Effect of different levels of fish silage in the diet on growth parameters of Nile tilapia:

Results in Table (6) revealed that initial weight of Nile tilapia at the start of the experiment was 13.60 g for all groups indicating the homogenity of fish distribution into the experimental groups at the start. Averages of final weight (FW) at the end of the experimental period (13 weeks) were 38.18; (50% fish silage) 40.22; (75% fish silage) 37.81 and (100% fish silage) 41.62g respectively.

Averages of weight gain (AWG) and ADG of Nile tilapia were found to be 24.29; 26.62 ; 24.21 and 28.02 for AWG and 0.27 ; 0.29; 0.27and 0.31 g for ADG, for the control; 50 ; 75 and 100% fish silage groups, respectively (Table 6). Average of RGR% and SGR for the control; 50; 75 and 100% fish silage in Nile tilapia diets were 1.31; 1.19; 1.12 and 1.23 for RGR and 1.81; 1.96; 1.78 and 2.06% for SGR, respectively. These results agree with the finding of El-Gendy (2006) but disagree with the findings of Megahed *et al.* (1997) who reported that no remarkable differences were recorded in final weight among fish groups fed on the pre-heated preserved fish diet or commercial fish meal based diet. However, sulphuric/formic silage fish group recorded the lowest average final weight. They added statistical analyses of specific growth rate figures show that the source of dietary protein has a significant effect on SGR values. In this connection, Salah Al-Din (1995) reported that inclusion of sun dried or over dried fish silage at levels 30; 35 or 40% improved growth performance of growing catfish as compared with the control diet, and the improvement was more pronounced at the higher inclusion levels. Moreover, Fagbenro *et al.* (1994) reported that inclusion of fermented fish silage and soybean meal in replacement for 25; 50 or 75% of fish meal protein in juvenile Nile tilapia diets or *Clarias gariepinus* diets supported body weight for 70 days. Furthermore, Stone *et al.* (1989) reported that incorporation of fish processing wastes silage in rainbow trout diets in replacement with fish meal depressed significantly growth performance compared to fish meal or silage of whole fish bodies.

In general, results in Table (6) indicate that incorporation of fish silage in diets of growing Nile tilapia in replacement to fish meal showed insignificant improvements in growth performance parameters especially at incorporation levels of 50 and 75% and total replacement of fish meal with fish silage which my lead to recommend the substituation of fish meal partially with fish silage in tilapia diets. The substituation of fish meal which represents the most expensive diet ingredient with fish silage will result in redution of diets costs without any adverse effects on fish growth.

4-Survival rate (SR)

Averages of SR of Nile tilapia for the control group and groups fed on diets containing 50, 75 and 100% fish silage were found to be 100, 100,100 and 96%, respectively (Table 7). These results indicate that replacing dietary fish meal with fish silage in Nile tilapia diets seemed to have no drastic effect on fish survival, thus all mortalities observed were due to other reasons and not to the treatments performed, such as aggression or handling of fish.

5-Feed utilization parameters :

Effect of replacing dietary fish meal protein with 50,75 and 100% of fish silage conserved for 24 weeks on feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) of Nile tilapia are presented in Table (7). Results show that averags of FCR during the whole experimental period for the control ,50 , 75 and 100% fish silage in replacement with fish meal protein were 2.57 , 2.79 , 2.71, and 2.52, respectively . Analysis of variance for FCR indicated that the control diet and 100% silage group showed the best FCR with insignifient difference among both groups and the groups fed 50 and 75% fish silage. Results in Table (7) show that the best PER results were obtained by the control and 100% fish silage compared with 50 and 75% silage groups which showed the lowest ($p>0.05$) PER values, however differences in PER recorded among experimental groups were insignificant.

In this connection stone (1989) reported that incorporation of fish silage in diets of rainbow trout was not as efficiently utilized for growth compared to fish meal based diet as was measuring FCR, PER and NPU. Megahed *et al.* (1992) compared the effect of replacing fish meal with raw minced fish or pre-heated preserved fish silage at 43.5% level on feed utilization of Nile tilapia. They reported that the source of dietary protein had a significant effect on PER and apparent net protein utilization

6-Chemical composition of tilapia whole body.

Whole body composition including dry matter (D M %) ; crude protein (CP%) ; ether extract (EE%) ; ash % ; nitrogen free extract (NFE %) and gross energy (kcal / kg) calculated on DM basis as affected by replacing fish silage instead of fish meal in tilapia diets are presented in Table (8) .

Results indicate that the 100% fish silage fed group had significantly ($p<0.05$) higher DM contents in their bodies compared to the control and the 50 % fish silage groups , however differences among the other two group (control, 50% silage) in DM contents were insignificant. On the other hand, the highest CP contents in tilapia whole bodies was obtained by the 50% silage groups (60.96%) and control (60.70%) followed a significant decreasing order by the 100 % silage, control and 75 % silage groups, respectively . As presented in the same Table (8), EE% values were the highest in the control groups (18.16%)

with significant differences compared to the silage fed group where the lowest EE% was obtained by the 100% silage in replacement to fish meal group . On the other hand the group fed on the diets where fish meal was replaced by 100% fish silage had significantly ($p>0.05$) the highest ash contents in their bodies followed a decreasing order by the control; 75 and 50 % silage fed groups , respectively . Averages of NFE contents in whole bodies of Nile tilapia for the control , 50 , 75 and 100% silage groups were 6.29 , 9.51 , 11.11 and 11.01% respectively. The 75 and 100% silage fed groups showed the highest ($p>0.05$) NFE content, compared to the other groups. Gross energy content (kcal / kg dry matter) were the highest in the control group with significant ($p>0.05$) differences among this group and the other two silage groups with 75 or 100%.

In this conection, Olivera *et al.* (1990) reported that incorporation of protein concentrate of cowpea (CPC) at levels up to 50% in Nile tilapia diets affected all body composition parameters. They added that as the concentration of CPC increased in the diet, the carcass water content was reduced. They added that protein content was improved by the inclusion of protein concentrate at substitution levels from 20 to 50%. Ether extract content was significantly higher for fish fed the CPC 50% diet. The lowest fat and highest ash contents were recorded in the fish fed the CPC20% diet. Furthermore, Richter *et al.* (2003), studied the effect of dried moringa leaf meal at inclusion levels of 20 and 30% as alternative protein source for Nile tilapia on body chemical composition. They reported that increasing the level of moringa leaves in the diets increased body moisture, while lipid and gross energy values decreased significantly ($P<0.05$). Crude protein and crude ash contents remained constant in all experimental groups. These results are in accordance with that of Attalla (2001), who reported that inclusion of mixture between fermented fish silage and boiled soybean meal resulted in significant differences in body chemical composition of Nile tilapia fry and fingerlings especially crude protein at 40% replacements level and other parameters of chemical composition at all levels tested. The results of body composition in the present study as affected with replacing fish meal with fish silage are in accordance also with those reported by Siddiqui *et al.* (1989), Conceic *et al.* (1998) and El-Gendy (2006).

As presented in Table (8), the highest DM contents in tilapia whole bodies ($P<0.05$) were recorded by the 100% fish silage fed group followed a decreasing significant order by the 75%; 50% and control groups respectively. Moreover, differences in DM contents of the whole fish bodies among the control and the 50%silage groups were insignificant.

7- Economic efficiency:

The economic parameters of the tested diets are presented in Table (9). The calculation depends on the average price of dietary ingredients during the year 2006, where local market price/ton of fish meal was 8000 LE, soybean meal 1500LE, yellow corn 900 LE, Oil 4000 LE, Vit.&Min. 10000 LE ,and fish silage 500 LE. The calculated figures showed lower cost of one ton of all diets containing fish silage compared to the control. However, the control diet recorded the highest price being 2297.3 LE/ton.The diets containing 0 , 75 % fish silage showed the lowest fish gain compared with the other treatments (50 % and 100 %). Therefore, diets No. 4 and 3 showed lowest cost/Kg gain but the levels of 0 % and 50 % fish silage gave the highest cost/Kg gain, being 5.00, and 4.63 LE respectively. These results agree with that obtained by El-Gendy (2006). Based on the obtained results, the incorporation of fish silage in growing Nile tilapia diets is recommended as replacer of fish meal totally without any adverse effect on growth performance and to reduce the dietary costs.

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Table (1): Chemical analysis of prepared fish meal and fish silage calculated as % on dry matter basis.

Diet	DM%	CP%	EE%	Ash%	NFE%	GE Kcal/kg
Fish meal	92.1	72	8.7	12.6	6.7	5124.5
fish silage (24 week)	23.14	51.47	13.19	17.17	18.17	4867.06

a)NFE= 100-(CP+EE+CF+Ash) b) GE = Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/g DM carbohydrate, protein and fat, respectively (Jobling,1983).

Table (2):Composition of fish silage diets in the experiment.

Ingredient	Control	50% fish silage	75% fish silage	100% fish silage
Fish meal	15	7.5	3.75	-
Fish silage	-	27.6	41.5	55.2
Soybean meal	30	30	34	35
Yellow corn	39.7	19.6	5.45	2.7
sunflower oil	5	5	5	5
Wheat bran	10	10	10	1.8
Vit & min. Mix.	0.3	0.3	0.3	0.3
Total	100	100	100	100

Table (3):Proximate analysis of the experimental diets in calculated as percentages of the dry matter.

Diet	DM%	CP%	EE%	Ash%	CF%	NFE%	GE kcal/kg
Control	97.32	25.16	4.43	14.5	4.01	51.9	3953.22
50% fish silage	96.29	25.63	8.49	13.01	4.70	48.17	4208.16
75% fish silage	96.34	25.87	9.56	14.44	5.06	45.07	4195.14
100% fish silage	98.23	25.77	11.57	17.70	13.32	31.64	3827.88

(DM: Dry matter, CP : Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, GE: Gross energy, GE was calculated using the values 5.6, 9.4 and 4.1 Kcal/g DM for protein, fat and carbohydrate, respectively according to (Jobling,1983).

Table (4): Ranges of physico-chemical parameters measured in fish- rearing-water throughout the experimental period.

Temperature (ċ)	pH value	DO2 Ppm	Alkalinity mg/l	Hardness mg/l	NO2 mg/l	NO3 mg/l
27-28	6-7	5-5.5	125-140	315-330	0.1-0.2	1-1.5

Table (5): Average of water quality parameters for the experimental treatments.

Treatment	Temperature C	pH	DO2 mg/L	Alkalinity mg/l	Hardness Mg/l	NO_2 mg/L	No_3 mg/L
Control	27.0	8.5	5.38	120	250	0.3	1.15
50 % silage	27.0	8.7	5.16	124	255	0.2	1.34
75 % silage	27.8	8.6	5.31	125	260	0.4	1.43
100 % silage	27.3	8.9	5.44	138	262	0.3	1.43

Table (6): Effect of replacement of fish meal protein with different levels of fish silage on growth performance parameters of Nile tilapia

Treatment	Control	50 % silage	75 % silage	100 % silage
IN mean	13.60	13.60	13.60	13.60
± SE	±0.00a	±0.00a	±0.00a	±0.00a
FW mean	38.18	40.22	37.81	41.62
± SE	±0.09b	±1.34ab	±0.80b	±0.68a
AWG mean	24.29	26.62	24.21	28.02
± SE	±0.0.09ab	±1.34ab	±0.80b	±0.68a
ADG mean	0.27	0.29	0.27	0.31
± SE	±0.001b	±0.15ab	±0.008b	±0.007a
RGR mean	1.13	1.19	1.12	1.23
± SE	±0.002b	±0.03ab	±0.002b	±0.01a
SGR mean	1.81	1.96	1.78	2.06
± SE	±0.07b	±0.09ab	±0.06b	±0.05a
Survival rate, %	100	100	100	96

a,b,, means in the same column bearing different letters differ significantly at 0.05 level

Table (7): Effect of replacement of fish meal protein with deferent levels of fish silage on feed utilization parameters of Nile tilapia (mean ± SE).

treatment	FCR	PER	PPV
Control	2.27 ± 0.035a	1.56 ± 0.025a	43.93 ±0.59a
50% silage	2.79 ± 0.158b	1.48 ± 0.078b	40.45 ± 2.07ab
75% silsge	2.71 ± 0.090b	1.48 ± 0.049b	39.55 ± 0.76d
100% silage	2.52 ±0.022a	1.59 ± 0.013a	38.80 ± 1.09c

a, b, c means in the same column bearing different letters differ significantly at 0.05 level

Table (8): Effect of replacement of fish meal protein with some fish silage on whole body chemical composition of Nile tilapia after 40 days

Treatment	Control	50 % silage	75 % silage	100 % silage
DM % mean	26.48	26.53	28.20	28.96
± SE	±0.07c	±0.16c	±0.08b	±0.16a
CP% mean	60.70	60.96	56.91	60.51
± SE	±0.05b	±0.02a	±0.02d	±0.03c
EE% mean	18.16	15.74	17.51	12.19
± SE	±0.25a	±0.08b	±0.15a	±0.35c
Ash% mean	14.85	13.79	14.47	16.29
± SE	±0.20b	±0.06d	±0.05c	±0.01a
NFE% mean	6.29	9.51	11.11	11.01
± SE	±0.30c	±0.51b	±0.16b	±0.39a
GE Kcal/kg mean	5412.5	5329.4	5334.3	5028.2
± SE	±0.72a	±0.47a	±0.17b	±0.36c

a, b, c means in the same column bearing different letters differ significantly at 0.05 level.
DM: Dry matter, CP : Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, GE gross, GE was calculated using the values 4.5, 8.1 and 3.4 KCal/g for protein, fat and carbohydrate, respectively according to Pantha (1982).

Table (9): Data of the economical efficiency due to feeding fish on graded levels of fish silage.

treatments	Feed intake g/fish	Cost (LE) of one ton diet	Total gain g/fish	Feed cost/Kg gain (LE)*
fish silage				
1-control	63.00	2297.3	24.59	5.00
2-50%	74.50	1654.4	26.62	4.63
3-75%	65.80	1341.5	24.21	3.64
4-100%	70.50	1115.3	28.02	2.81

*Feed cost / kg gain (LE) = feed intake x cost (LE) of ton feed / 1000 x total gain.