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Protein rich ingredients from fish waste for sheep feeding

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Fish wastes, including viscera, heads, tails and skins, were ground and mixed with 10% molasses and inoculated with a starter culture made of *Lactobacillus plantarum*. The inoculated mixture was incubated at 25°C for 10 days, for a biopreservation/biotransformation by fermentation. During the fermentation period, changes in nutritional quality and biochemical properties (pH, dry matter, ash, total and volatile nitrogen and lipids) were monitored as well as microbiological determinations, including standard plate count, coliforms and Clostridium. Results indicated that the ph decreased considerably and remained constant at 3.8 after 8 days. Total nitrogen, decreased non protein nitrogen and total volatile nitrogen increased. The microbiological characteristics showed a drastic decrease of coliforms and *Clostridium* counts in 8 days. Two trials and a control were carried out and the final fish waste silage product was used in feeding sheep in two trials of 5 sheep each. The fish silage was added to ground barley and wheat bran* in two proportions respectively (40% barley, 25% fish silage and 35% wheat bran) and (40% barley, 50% fish silage and 10 % wheat bran). The control diet was the conventional feed adopted in the region (40% barley, 60% wheat bran*). The weight gain was followed up for 9 weeks. The results indicated that trial feeding studies with young sheep using formulas containing fish silage showed a net increase in weight above controls as well as a good enhancement of meat characteristics and carcass shape.

Keys words: Fish wastes, molasses, barley, wheat bran, *Lactobacillus plantarum,* fish silage, sheep feeding.

INTRODUCTION

Morocco coastlines are about 3500 km long, and its exclusive economic zone of 200 miles makes it one of the richest fish reservoirs of the world. The annual capture was 475,587 tons in 2005 and 398,916 tons in 2006. Pelagic fish represented 82% of the total coastal capture in 2005 and 83% in 2006. Most of the captured pelagic fish was the sardine with respectively 81% in 2005 and 73% (Office National des Pêches, ONP, 2006). Half of the total production is devoted to industrial transformation (canned, semi-canned, freezing, drying, fish meal etc.), respectively 58.25 and 45.78% in 2005 and 2006, and the remainder is devoted to the local market in fresh (ONP. 2006). The yearly renewable potential of production is estimated at 1.5 millions tons (ONP, 2006).

In Morocco, the fish industry as well as fish markets, discard large amount of fish waste. Drying units use part of the fish waste for fish meal, but this process is expensive, high energy consuming and requires highly qualified engineers and large amounts of fish waste. Therefore, the biotransformation of local fish wastes, which is a low cost process, can be an appropriate method for obtaining benefit from fish waste, protecting the environment and balancing the increasing shortness in the agricultural crops used in animal feed.

Fish wastes are a rich source of protein. Many scientists over the world have investigated fish silage in animal

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Table 1. Diets formula for feeding sheep.

	Barley	Bran of wheat	Waste fish silage
Control group diet	40%	60%	0
1st group diet	40%	25 %	35%
2nd group diet	40%	10%	50%

Table 2. Initial average weight of animals of each group.

Groups	Average weight (kg)
Control	19.4
1st group	21.1
2nd group	2 . .

Animals where weighted every week before the morning meal (Hungry state).

feed and have used fish silage products (Bertullo, 1975; Durairaj, 1976; Jenson, 1977; Disney et al, 1977, Raa et al, 1983, 1989; Grégoire and Boucher, 1988; Machin, 2000; Stone, 1989) However, in Morocco the use of this technique is still limited to research despite the scarcity of animal feed and the large amount of fish waste.

Starting in 1994, Faid et al. (1994, 1995a, 1995b, 1999, 2000) demonstrated various aspects of fish silage techniques and its use in various diets. They studied fermentation as a biological process to preserve fish waste, using combined starter cultures of yeast and lactic acid bacteria. In the present study, fish waste was processed through biological fermentation using *L. plantarum*, and the silage was used as the main source of protein in sheep feed in North Eastern Morocco known as “Hauts plateaux”.

MATERIALS AND METHODS

Fish waste silage preparation

Fish waste was collected from Oujda fish market with *Sardina pilchardus* as the main species and transported to the laboratory (Agronomic Regional Research Center). Fish wastes were immediately ground, mixed and filled into 100 l plastic containers up to 60% of their capacity. Then we added 10% of sugar beat molasses, and mixed thoroughly. After 2 hours, we inoculated each container with a 5% starter culture of *L. plantarum,* grown on MRS Agar (DeMan-Rogosa-Sharpe) (Merck, Germany) for 48 h at 30°C, and then incubated at 25°C. This volume of the starter culture made the product cell concentration around 10^6 CFU/g. The incubation was done at 25°C ± 2°C for 10 days which was required for pH stability.

Chemical determinations

Chemical determination was made for pH, dry matter, fat, ash, total and volatile nitrogen. The pH was determined using a pH-meter (Crison Micro-pH, 2000). The dry matter and Ash were determined using the (AOAC method, 1980). Fat content was determined on the dry matter by the Soxhlet method using hexane as solvent. Total nitrogen (TN) was determined by the Kjeldhal method (APHA, 1989), and the volatile nitrogen using the Conway method (1947).

Microbiological determinations

Dilution preparation

For dilution purpose, each sample of (10 g) was added to 90 ml of sterilized physiological saline in order to obtain a 1:10 dilution. The mixture was blended using an Ultraturax type blender. This blended mixture was then decimally diluted to achieve a 1 x 10^6 –fold dilution. Standard plate count was determinated by pour plating appropriate dilutions (10^{-1} to 10^{-6}) on standard plate count Agar (Biokar, France). Plates were incubated at 30°C for 48 h. Colonies were counted using a colony counter **.** For coliforms we used MacConkey agar. 10 g of the fermented product were added to 90 ml of saline water (8.5 g/l) and four-fold dilutions in tubes containing 9 ml of saline water were prepared. The plates were incubated at 37°C (Leininger, 1976). For Clostridium identification we used Reinforced Clostridium agar. The initial dilution was heat-activated at 80°C for 10 min and immediately cooled in iced water in order to destroy all vegetative forms and to activate spores. 3 tubes containing Reinforced Clostridium agar were inoculated respectively with 1, 2 and 5 ml of the dilution 10 - 1 and incubated at 44°C in anaerobic conditions for 48 h, and black colonies were counted, (method described by Faid et al., 1990).

Feeding trials

In order to test the sheep feeding efficiency of the fermented fish silage, we divided 15 sheep of the Beni Guil breed, having an average age of 3 months, into 3 experimental groups (trials) of five animals each. The first group was the control. Sheep in this group were fed the conventional feed adopted in the region (40% barley, 60% wheat bran)*. The feed was given twice a day; at 8 am and 6 pm. Refused portions where weighted before serving the next, in order to calculate the quantity used. Alfa (*Stipa tenacissima*) was available the whole day for all the groups. Table 1 shows the nutrients for each group. All animals where identified, numbered, weighed and allocated to a group. The Feeding trial lasted 9 weeks, during which time trial groups were kept separated from each other. Table 2 shows the average weight of animals at the beginning of the feeding study.

Statistical analysis

We used Dunnett's test to compare group of means. The two trial groups are pitted against the «Control" group. The method tests the null hypothesis that no group has its mean significantly different from the mean of the reference group. For each pair (control, Group$_i$), the Dunnett's test calculates the value of a statistic « $t_{observed}$ ». This value is compared to a critical value (Vc) read in a "Dunnett's table". For a given pair (Contol, Group$_i$), if t_i observed is larger than this critical value $t_{critical}$, the mean of Group $_i$ is declared significantly different from the mean of the reference group.

RESULTS and DISCUSSION

Chemical parameters

The pH trend in Figure 1 shows the success of the silage fermentation process. After 8 days of fermentation, the microbial load was reduced to low numbers. The pH

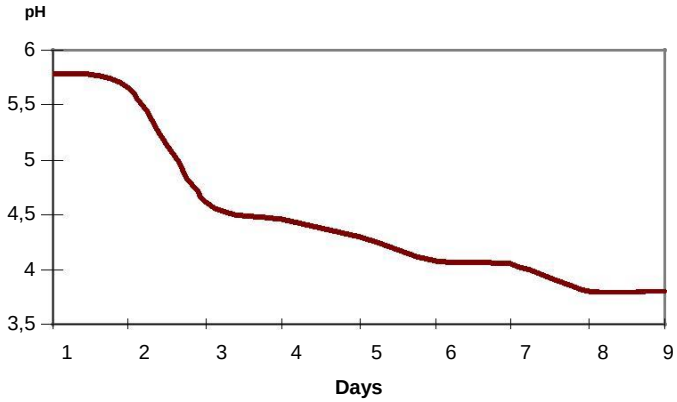

Figure 1. pH evolution during the silage process.

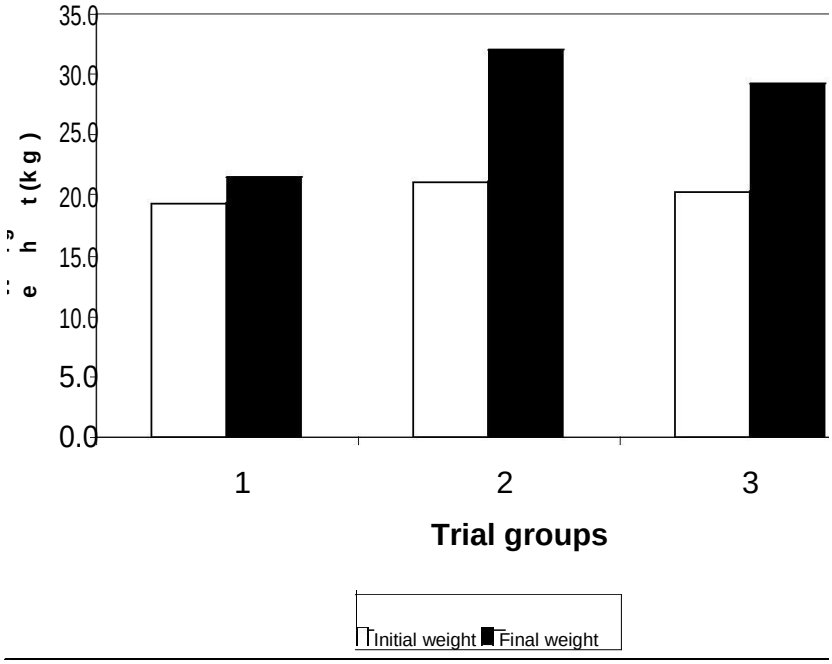

Figure 2. Initial versus final weight of sheep of the 3 trial groups. Legend: 1 = Control group; 2 = First group; 3 = Second group.

dropped below 4, due to the activity of *Lactobacillus* bacteria. This increase of acidity created inappropriate conditions for the growth of most pathogenic organisms (causative agents of diseases), and improved the product hygiene and safety. Fish silage is pasty to liquid, although the chemical analysis reported in Table 3 shows that the dry matter represents 39%, which is high if compared to dry matter of fish wastes. Fish silage must be added to other fodder (animal feed, foodstuff), especially bran in order to be more solid and appropriate for feeding small ruminants. A high level of ash (17.35%) was found in the fermented fish silage due primarily to the solubilization of fish bones and molasses. The content of fat (%) and protein (13.62 %), in the fermented silage were also high which makes fish byproducts an important component of animal feed concentrates and blocks.

During the fermentation process, microorganisms' convert chemical components, which play an important antibiotic role, that confers to the product a great nutritional and hygienic value. During this process, enzymes reduced complex components to simple absorbable ones through an autolysis process, such as soluble nitrogen.

Biological characteristics

The follow up of the product microbiota reveals its hygienic and safety patterns. Table 4 illustrates a great change

Table 3. Chemical composition of raw materials wastes and silage

	Raw material	Fish silage
pH	5.80	3.80
Dry Matter (%)*	34.12	31.67
Proteins (%)*	39.40	13.62
Ash (%)*	15.88	17.35
Fat (%)*	8.41	8.8
Soluble Nitrogen (%)**	3.56	5.73

* : % of silage ** : % of dry matter

Table 4. Microbial counts in raw material and fish waste silage (in cfu/g).

Microorganisms	Raw material	Fish silage
Lactobacillus	$3.2.10^6$	$4.8.10^9$
SPC	$2.3.10^7$	$6.1.10^5$
Total coliforms	$2.0.10^5$	0
Clostridium	$8.5.10^4$	0
Yeast	$1.7.10^2$	uncounted
Fungi	80	

Legend: SPC: standard plate count

in microbial numbers and a drastic destruction of undesirable ones. While the number of lactic bacteria increased from 3.2×10^6 to 4.8×10^8 CFU/gram, SPC (standard plate count) decreased, and coliforms and clostridia were eliminated. The amount of SPC ($6.1.10^5$cell/gram) illustrated the amount of microorganisms tolerant to acidity but not harmful to animal health. These results prove the success of the fermentation process which established unfavorable growth conditions for undesirable and hazardous microorganisms (coliforms, *Clostridium*) . Fungi and yeast are tolerant to acidity, although at this stage they aren't harmful, they could constitute a potential danger if the product was stored for a long period.

Animal feed characteristics

Table 5 presents the chemical composition of the different feed formulations used the dry matter contents for all three formulas was similar. The control and first group had about one-half of the ash content of the second group. The second group had the highest level of proteins and soluble nitrogen, which are the main components in animal fattening diets.

Feeding assays

Bran and ground barley, beside their contribution with additional nutrients such as fiber, were used to confer to the diet feed a solid state, which is appropriate for handling and feeding small ruminants.

Although no vitamins and minerals nor antibiotics had been added to the feed diet formula, observations on animals showed no mortality or symptoms of malnutrition nor any abnormal symptoms (diarrhea, stress, sleepy etc.) Results of the feeding assays showed an obvious increase in weight gain of sheep fed on fish waste silage

Table 5. Chemical composition of the different formula and the control feed.

	Control diet	1st Group diet	2nd group diet
Dry matter	87,31	89,88	86,79
	16,39	18,53	23,15
Soluble nitrogen	0,24	0,86	1,75
Ash	4,94	4,77	8,34

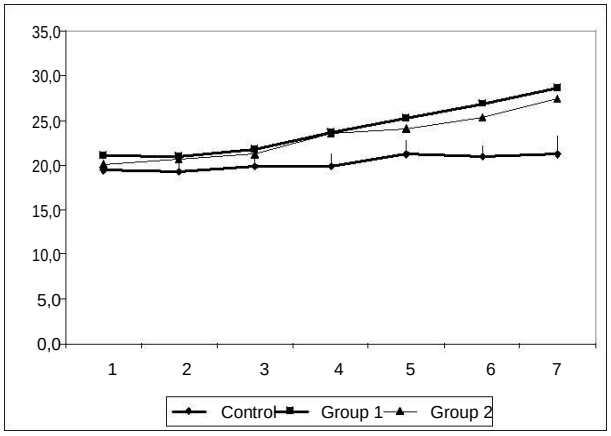

Figure 3. Comparison of weekly weight gain increase pattern of the control with the 2 trial groups of sheep fed on diets waste silage. Dunnet critical value is reported on the control curve.

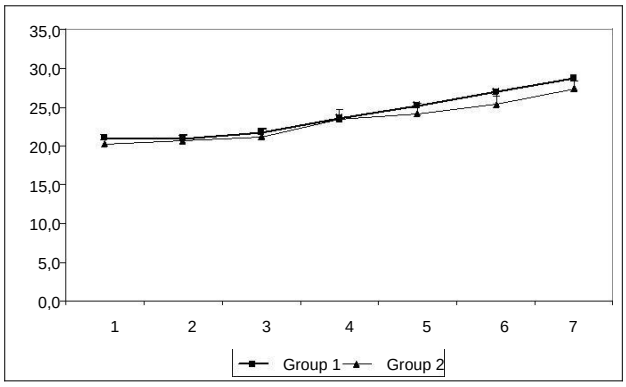

Figure 4. Comparison of the 1st group and 2nd group average weight along with Dunnet critical value data.

supplemented with ground barley and bran of wheat as compared to the control feed (Figure 2). The growth curves Figure 3, established on the basis of weight trend shows that weights of animals were comparable during the first month, but after the 4th week, those fed on diet containing silage increased rapidly. Animals fed with silage diet took more than one month in order to be adapted to such diet, more especially with those of the second group, which was noticed in their daily feed refusal. The weight increase achieved with the 1st and 2nd group diet in 9 weeks requires 6 months with the control diet. Figure 4, shows that the weight gain of sheep fed on the two diets with fish waste silage were similar and the difference is not significant for the whole period.

Further research should be carried out for long feeding period in order to confirm these results.

Comparison of the different formulas proved the potential nutritional quality of diets with fish waste silage as a nitrogen source and probably as a probiotic ingredient for sheep feeding. This fermentation with lactic acid bacteria, made the diet with silage balanced and fit the nutritional needs of the sheep.

At the end of the feeding study; we organized for the local population and breeders an organoleptic meat test which revealed that fish silage enhanced meat quality and carcass shape.

Conclusion

The present study, related to biotechnological techniques for biopreservation of fish wastes and their use in sheep feed, was successful, and reveals that there is a considerable potential for the use of the silage derived from fish offal as a nitrogen source and possibly as a probiotic ingredient for feeding small ruminants. Such a controlled process; if used widely, can contribute to overcoming protein shortage in areas where structural foodstuff is lacking. Nevertheless, further studies should be carried out on aspects related to silage micronutrients components (minerals and vitamins), microbial fermentation, prolonged storage, appropriate diet, feeding period, organoleptic characteristics of resulting meat and safety.

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