

Biotransformation of Ternary Mixture of Organic Industrial Waste into Poultry Feed

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Abstract: The objective of present study was to produce poultry feed with a best quality using ternary mixture of industrial wastes: fish industry waste, sugar industry waste (molasses) and yeast waste after 15 d biotransformation using response surface methodology to design and analyze the experiment data. Changes in the nutritional quality and biochemical properties *viz.*, pH, dry matter, conductivity, total organic carbon, total nitrogen, phosphorus, trimethylamine, protein, fat, carbohydrate, histamine, aflatoxins and minerals were evaluated during a biotransformation period of 15 d. The experimental findings revealed that the formulation including 50% of fish waste, 12.5% of molasses and 37.50% of yeast waste were found to be best quality poultry waste. It was characterized as odorless, with a stable pH, rich in protein, fat and carbohydrate as well as no alterations of bacteria and absence aflatoxins from the fifth day on ward. The nutritional value of the developed poultry fees was studied on a population of broiler chickens by incorporating with barley flour and eggshell. The product possesses a better nutritional quality comparable to commercially feed.

Key words: Fish waste, ternary mixture, biotransformation, poultry feed, response surface.

1. Introduction

The export of fishery products in Morocco is an important first activity for the country's economy. Indeed, Morocco has exported about 641,924 tons in 2015, which corresponds to a value of 19.4 billion MAD. Fishery products are exported to several countries with a predilection for the countries of the European Union (EU) [1]. In Morocco, fisheries are distributed throughout the coast. They are characterized by great diversity and generate waste in considerable quantities [2].

The wastes resulting from industrial fish processing operations include flesh, skin, bones, scales and gelatinous liquid wastes can cause damage to the aesthetics of the environment and bad odors resulting from bacterial decomposition if they are not stored properly or evacuated quickly. For these reasons the

industries concerned are obliged to apply a management strategy, if not, economic and environmental costs will be imposed, to be borne by the industries and also the regions. However, in terms of their composition, this waste is still relatively rich in nutrients and could be recycled as a potential source of protein-rich feed for animals, like livestock, poultry and fish [3]. Recognition of the limited biological resources and increasing environmental pollution have emphasized the need for better and more value added utilization of fish and fish processing wastes [4]. Fish processing wastes constitute around 50% in a fish are not commonly used in human feeding and most often they are disposed on, considering this impact of improper disposal of this residue on the environment and seeking suitable technological alternatives for a nobler use with both economic potential and social application. Novel means of processing are required to convert the underutilized wastes and by-products into more marketable and accepted form. One alternative is

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to produce fish powders or fish protein hydrolysate that may be used as carbon and/or nitrogen source for biomass and metabolite production [5]. The current investigation is aimed primarily at the industrial application of the process. This poses constraints, particularly with respect to the overall cost efficiency of the scaled up process. Low cost and simplicity in operation, by reducing the cost of the material, energy consumption and labour, but maintaining high productivity are some of the important attributes that outline the direction of this investigation. The transformation of this waste into flour is done by drying. This process seems costly and complicated [3].

The objective of the present study was to produce poultry feed from ternary mixture of industrial wastes: fish waste, molasses and brewing yeast, in natural biotransformation process. The response surface methodology was used to design the experiment in an optimality zone, for analyzing data and to generate feed with a high nutritional quality.

2. Materials and Methods

2.1 Initially Nutritional, Chemical and Physico-chemical Analysis

Before the biotransformation, a sample of each

waste *viz.*, fish, molasses and yeast was analyzed. Table 1 shows the results of chemical and biochemical properties of the waste studied.

2.2 Preparation of Mixtures

The three components used to prepare the mixtures were selected in such a way to create a balanced mixture containing all the elements necessary for a favorable biotransformation.

Waste contains the yeast *Saccharomyces cerevisiae*, recovered from the yeast industry is an agent of fermentation that will allow better the biotransformation seen its interesting nutritional and organoleptic characteristics [6, 7]. This component was mixed with industrial waste of sardine (*Sardina pilchardus*), which contains bones, guts and heads, and has been also ground in an ice crusher, and then the molasses, by-product of the sugar industry, was added.

Several fractions of such three components were studied with the aim of identifying better formula, allowing a favorable biotransformation. The experimental design consists of 7-points in the ternary diagram with constrained regions (fish wastes > 50%, molasses > 12.5%) (Table 2).

Table 1 Chemical and nutritional characteristics in dry matter of wastes.

	Fish waste	Molasses	Yeast waste	Method reference
Dry matter (%)	30.00	73.00	33.00	Oven drying 3 g at 60 °C for 24 h
Protein (%)	6.90	0.00	13.60	Bradford method
Carbohydrate (%)	0.00	46.72	12.24	Sulfuric-phenol method
Fat content (%)	3.36	0.73	2.14	Soxhlet method
Phosphorus (%)	0.14	0.002	0.29	NFV18106
Calcium (%)	0.06	0.02	0.01	Flame emission spectrophotometry

Table 2 Compositions in dry matter of initial tests.

Composition N°	Fish waste (%)	Molasses (%)	Yeast (%)	TN (%)	TOC (%)	C/N
1	50.00	50.00	0.00	0.64	24.98	38.87
2	62.50	25.00	12.50	1.13	15.30	13.55
3	87.50	12.50	0.00	3.36	17.55	5.22
4	50.00	12.50	37.50	1.51	15.45	10.23
5	68.75	12.50	18.75	0.69	15.07	21.98
6	68.75	31.25	0.00	0.58	14.65	25.05
7	50.00	31.25	18.75	0.93	17.99	19.33

TN: total nitrogen; TOC: total organic carbon; C/N: carbon/ nitrogen.

Based on a previous study conducted at Laboratory of Biochemistry, Environment and AgriFood LBEA URAC36, of the University Hassan 2 of Casablanca, Morocco, the variability range of the molasses used must be 12.5%-20% and the use of a fraction of fish less than 50% is not recoverable [6]. Fig. 1 shows places of essays.

2.3 Physicochemical Analysis

The pH was determined using a pH-meter Fisher Scientific, Basic AB15 according to Taiek *et al.* [6]. The dry matter (DM) was measured daily by oven drying 3 g at 60 °C for 72 h, three times per day according to Taiek *et al.* [6]. Conductivity and temperature were measured daily using conductivity meter/thermometer HANNA Instruments, EC215 according to Taiek *et al.* [7].

2.4 Nutritional Analysis

The total nitrogen content was determined according to the Kjeldahl method [8]. The rate of phosphorus was determined by spectrophotometric assay according to the French standard NF V18-106 [9]. The total organic carbon was determined by Walkley-Black method [10]. The fat content was determined on the DM by the Soxhlet method using hexane as solvent [11]. The proteins have been

classically and dosed according to the colorimetric method of Bradford after specific marking of proteins by blue of Coomassie [12]. Carbohydrates were determined by the Bertrand method [13].

The dosage of the trimethylamine was performed by the distillation according to the EC Regulation No 2074/2005 for the determination of the TVB-N (basic amines total volatile) [14]. The aflatoxins B1, B2, G1 and G2 were measured by high-performance liquid chromatography with fluorescence detection, according to ISO 16050:2003 standard [15]. The rate of histamine was determined according to No 2073/2005. The exchanged cations were determined by flame emission spectrophotometry (Ca^{2+}) using flame photometer (Digital Flame Photometer PFP7/C, JENWAY®).

2.5 Microbiological Analysis

Microbiological analysis was performed at initial and 15th day. A Columbia blood agar was prepared to determine the presence of *Streptococcus* reflecting proteolytic effects [16]. The presence of *Staphylococcus* (lipolytic marker) was determined in a mannitol salt agar [17]. A MacConkey agar is used to visualize the presence of *Escherichia coli* [18]. The presence of *Salmonella* was determined at a SS agar [19].

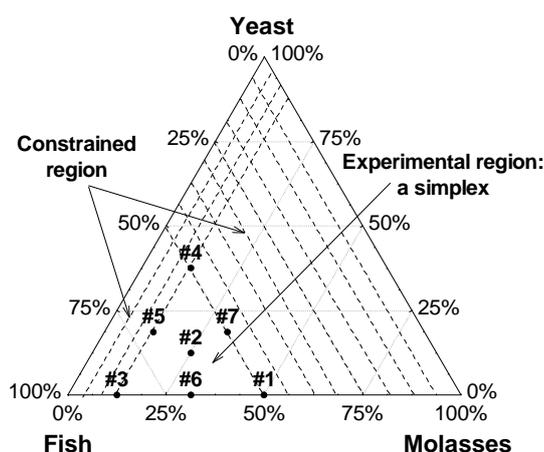


Fig. 1 Simplex-centroid design with 7-points arrangement in the weight fraction ternary diagram with constrained regions (fish wastes > 50%, molasses > 12.5%).

2.6 Data Analysis

The ternary surface response diagram and principal component analysis (PCA) were used to identify factors that have a statistically significant influence on the nutrient quality of the mixtures. These tools were generated by Statistica software® 10 (StatSoft, USA). To optimize the treatment parameters, the three independent variables used in this study were proportion of fish waste, molasses and yeast. After run, according to resulted treatments the various formulas, were prepared and monitored for 15 d by quality control parameters.

2.7 Formulation of Diets

The formula representing the best results of quality control has been mixed with flour of barley to title of 30% and 10% of eggshell (series AB) (Fig. 2).

The second series (series AC) fed by the commercial food, has been used for comparative purposes as a reference to assess the quality of the product developed (Table 3). All series were composed of 10 hens of flesh. The growth curve was established on the basis of weight gain. The daily consumption was measured by weighing.

3. Results and Discussion

3.1 Physical and Textural Properties of Mixtures

At the beginning, all tests formed very heterogeneous thick paste. At the end of the biotransformation process, all mixtures were characterized by a homogeneous appearance, dark color, lumpy structure and development of a pleasant

odor. A physical and textural property of biotransformation product depends on the process conditions such as the biotransformation type, feed moisture, temperature and oxygen uptake rate. Different studies have reported that the product characteristics such as color, appearance odor have an important bearing on the acceptability of the final product [7, 20, 21].

3.1.1 The pH, Temperature, Conductivity and DM

Fig. 3 shows the evolution of the pH, conductivity, results during 15 d of biotransformation of the different compositions.

The pH decreased from an average of 7.09 and 6.67 to 5.70 and 4.93 for the M2, M3 compositions, respectively, after 15 d of biotransformation (Fig. 3), which provides evidence of induction of a low acidity through lactic acid production by lactic acid bacteria. The stabilization of pH in the testing of other compositions was due to the fermentative activity of the yeast *Saccharomyces cerevisiae* [21]. The reported values since yeasts and bacteria involved in the biotransformation have their pH optimum between 5 and 8.5 [7]. The monitoring of pH indicated that after 8 d of testing, all the compositions were mature. The pH stabilization was due to the reduction of activity of microorganisms [22]. A pH marked by a slight acidity was a witness of a favorable biotransformation.

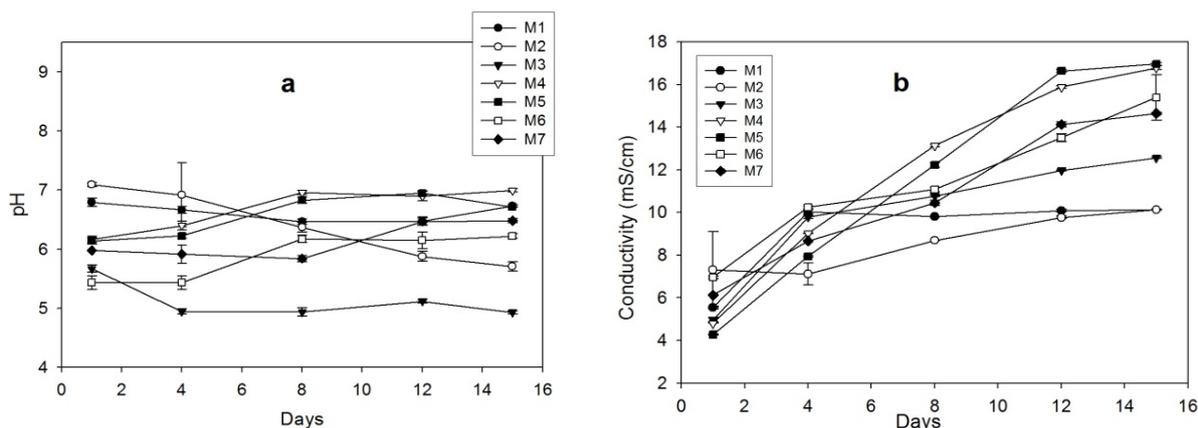
As for conductivity, it increased slightly for all the mixtures for 15 d from a value of 4.27 mS/cm, while M4 and M5 compositions achieved an increase up to 16.78 mS/cm and 16.95 mS/cm, respectively, on the 15th day (Fig. 3).



Fig. 2 Formulation of diet before and after homogenization.

Table 3 Formulation of diets of series AC (100% of commercial food) and AB.

Diets	Parameters	Value
Series AC	Crude protein	18%
	Fat	2.5%
	Carbohydrate	47.62%
	Phosphorus	0.65%
	Calcium	0.95%
Series AB	Best biotransformed mixture	60%
	Eggshell	10%
	Barley flour	30%

**Fig. 3** Evolution of (a) pH and (b) conductivity.

All the mixtures presented a rise in conductivity during the process of biotransformation. The total degradation of carbohydrates may be due by yeast and the release of volatile substances [7-23].

Concerning the recorded temperature, the variations in the temperature were not more than 1.5 °C. It can be considered as insignificant (Fig. 4). This can be explained by the low thickness of the biotransformation mixture in the container as well as the regular mixing applied. The biotransformation process applied in this study therefore allows good practice mastery of temperature.

The DM of mixtures increases during the process (Fig. 4). The increase of the DM in other mixtures was due to the loss of water by evaporation or by the loss of carbon dioxide and ethanol (by evaporation) during fermentation [24].

3.1.2 Quality Control of Bio-transformed Product

The best compositions among the studies are M3, M4 and M5 as shown in the results of the above

parameters: pH, conductivity, DM and temperature (Figs. 3 and 4). This led to develop this study by other analysis (measuring protein, fat content, carbohydrate, phosphorus, total organic carbon and trimethylamine) that will allow us to apply this product in the field of poultry feed.

Table 4 shows that the M3, M4 and M5 compositions had a better evolution concerning these three parameters protein, fat content and carbohydrate. M4 composition contains more than 4% fat, 22% crude protein and 14% carbohydrate. These results meet with the requirements of the first phase of the food need of chicken of flesh in starting phase (1 to 4 first weeks) that are 18% to 23% in crude protein. It should be noted that most of the food energy is found as carbohydrate or fat, as for growing chickens, high fat diets are always the favorite [25]. This suggests the important role of yeast wastes for obtaining the best results facing the stability and the change of hygienic quality of the generated product. All these

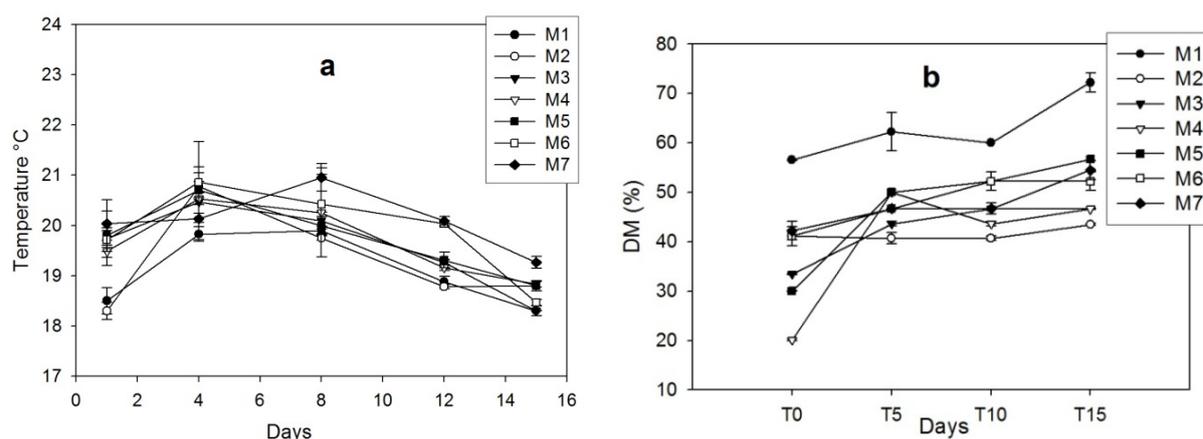


Fig. 4 Evolution of (a) temperature and (b) dry matter (DM).

Table 4 Nutritional analysis in dry matter after 15 d.

Comp.	Fat (%)	Carbohydrate (%)	Protein (%)	P (mg/100 g)	TMA (mg/100 g)	TOC (%)
1	4.53	9.85	17.57	28.40	0.40	25.39
2	3.76	2.32	10.16	20.68	0.63	16.28
3	2.43	8.34	16.43	22.18	5.15	17.69
4	4.49	14.31	22.06	29.03	2.26	17.66
5	2.72	21.83	26.61	36.18	7.64	21.00
6	1.37	7.23	23.92	32.79	7.46	18.92
7	1.65	12.41	18.13	26.13	3.89	26.52

P: phosphorus; TMA: trimethylamine; TOC: total organic carbon.

compositions have a rate of phosphorus that increases during 15 d of biotransformation. This was due to the mineralization carried out by micro-organisms that transforming organic phosphorus in mineral phosphorus [24]. M4, M5 and M6 compositions have a rate as high as the other compositions 29.03, 36.18 and 32.79 mg/100 g, respectively, because they have more sardine wastes and such fish is rich in terms of phosphorus [23]. Therefore, the addition of yeast and molasses to the mixture helps in the conservation of phosphorus. A richer formula in fish waste and molasses allows phosphorus rates to be improved.

The rate of evolution of trimethylamine was between 0.40 mg/100 g and 7.64 mg/100 g for all compositions on the last day. These values proved the stability of our products and demonstrate the mastery of the biotransformation process [26, 27].

All the compositions have a rate of total organic carbon that increases during the process of

biotransformation. In general, the total organic carbon during the simulation of different biotransformation processes decreases. Indeed, it is lost mainly by (CO₂) gas exchange [28]. The increase of total organic carbon may be the result of a development of a microbial flora by the contribution of organic matter. A richer formula in fish waste and molasses allows total organic carbon rates to be improved.

3.2 Statistical Analysis

3.2.1 Principal Component Analysis (PCA)

The graphs in Fig. 5 illustrated the PCA and correlation circle of the data. The biplot showed the grouping of compositions M5 and M4 that were most relevant from the results of analysis. According to the correlation circle, M4 and M5 mixtures were perfectly correlated with the phosphorus, protein and carbohydrate. These products were positively correlated with yeast waste. It has been found that

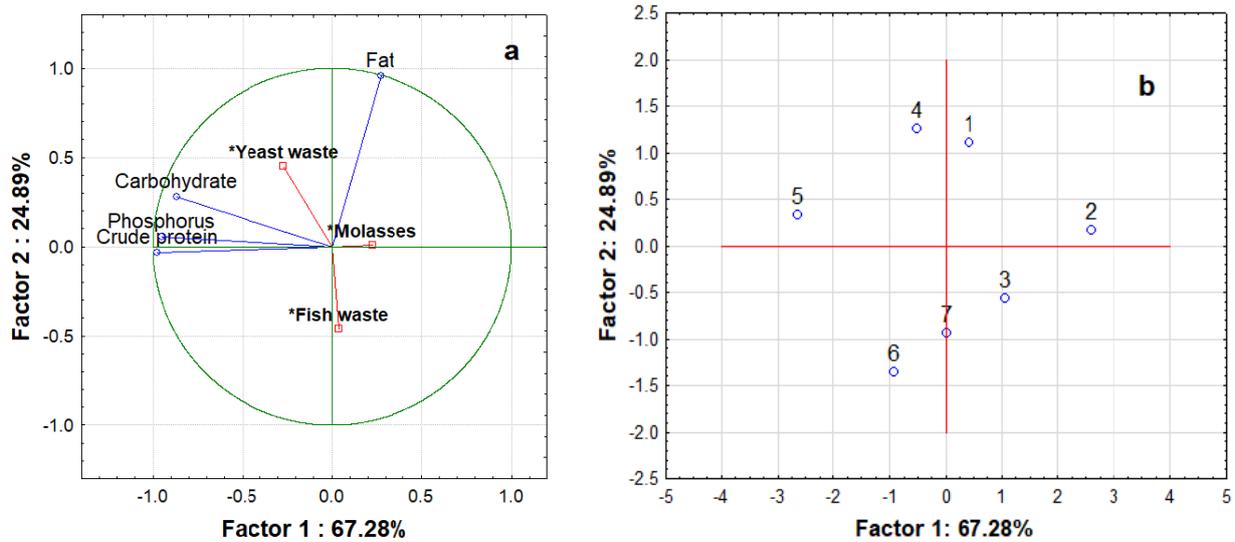


Fig. 5 (a) The biplot consisting projection on PC 1 (67.28%) and PC 2 (24.89%), with 70% of quality of presentation; (b) correlation circle between principal components and original variables.

yeast improved the level of phosphorus, protein and carbohydrate. That contributed with the fish waste to its essential elements to the animal feed. Moreover, the M4 mixture was better correlated with the fat.

The other compositions were also grouped, and anti-correlated with the essential elements, as their quality has been judged bad.

3.2.2 Response Surface Model Estimates

The study tried to translate the potential of protein, carbohydrate, phosphorus and fat to biotransformation through the quadratic model. The obtained results were allowed to establish surface response diagrams (Fig. 6). Combining between these diagrams, the area of interest between mixture M4 and M5 can be delimited in the ternary diagram.

3.3 Microbiological Tests

The results of the bacteriological tests accomplished in the first and last day are presented in Table 5, in order to identify the presence of strain indicator of hygiene and alteration. All tests revealed the absence of *E. coli*, *Staphylococcus*, *Streptococcus* and *Salmonella* at the end of the biotransformation process. Hygiene and inhibition of proteolysis, as well as lipolysis in the mixtures containing yeast, were due to its probiotic activity

[29]. All these tests indicated the importance of yeast wastes in the making of a favorable biotransformation of fish waste, which inhibit the pathogenic bacteria without any degradation of nutritional qualities of the product and without production of a toxic effect.

3.4 Growth Performance of Chickens

Before introducing composition M4 in the poultry sector, specific analysis like determination of histamine, aflatoxins and calcium were needed. Table 6 below brings together the results relevant to the nutritive richness and the safety of the composition M4.

Table 6 shows that, the rate of histamine is 115 ppm and the butterfat is 100 mg/kg. As concerns the mix of aflatoxins B1, B2, G1 and G2, which their absence was revealed in M4, a fact concluding that the product can be used without risk of intoxication by mycotoxins in animal feed. The presence of aflatoxins was less than the allowable limit in feed. According to the ISO 16050:2003 standard the limit of the sum of the aflatoxins B1, B2, G1 and G2 is 8 µg/kg. In developed poultry feed the sum of the aflatoxins B1, B2, G1 and G2 was observed to be less than 0.4 µg/kg [15]. The calcium and sodium requirements of broiler

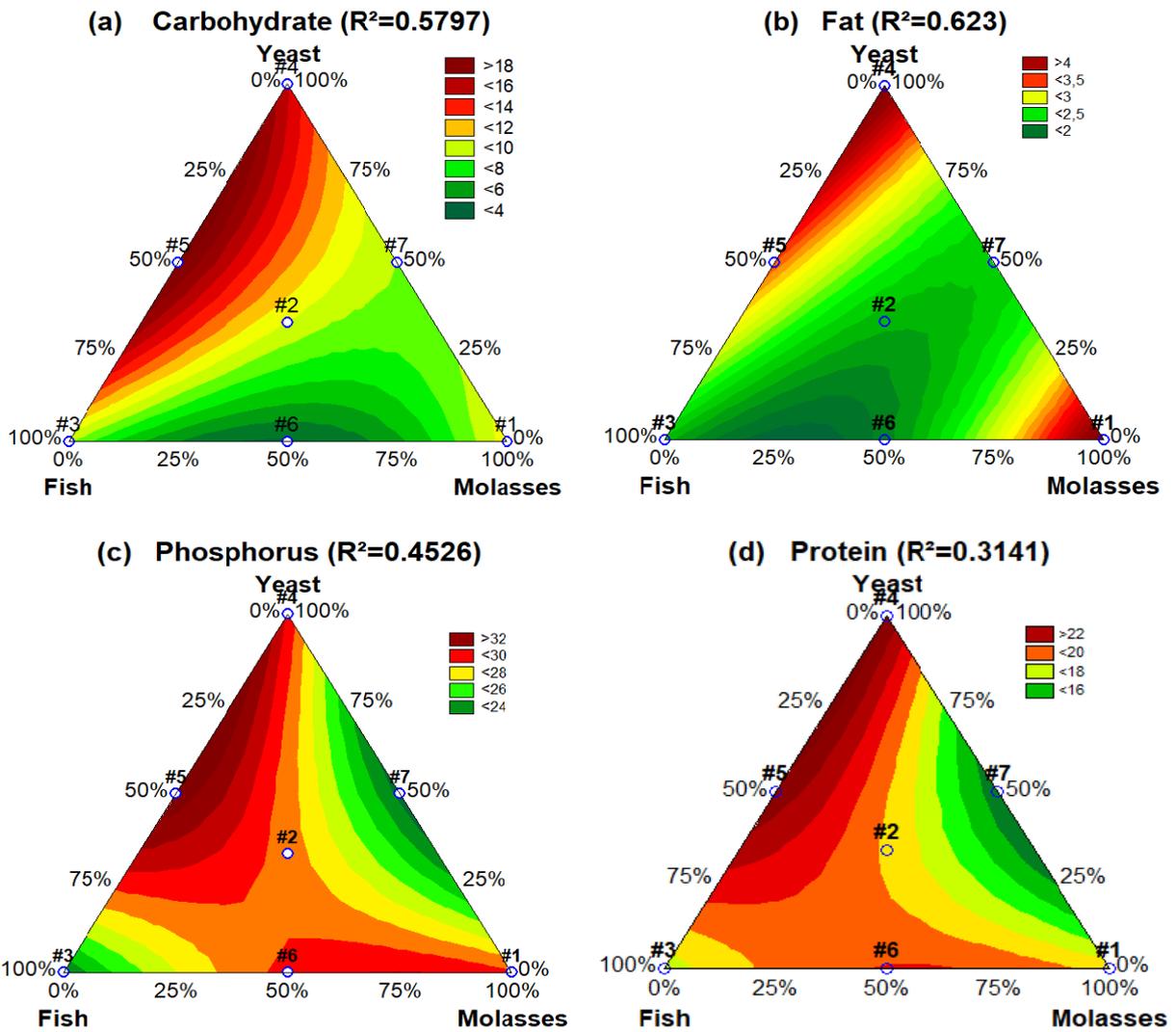


Fig. 6 Ternary response surface diagrams with changes in carbohydrate (a), fat (b), phosphorus (c) and protein (d), concentration calculated from the fitted model equations (quadratic model).

Table 5 Microbiological analysis after 1/10 dilution at T0 and T15.

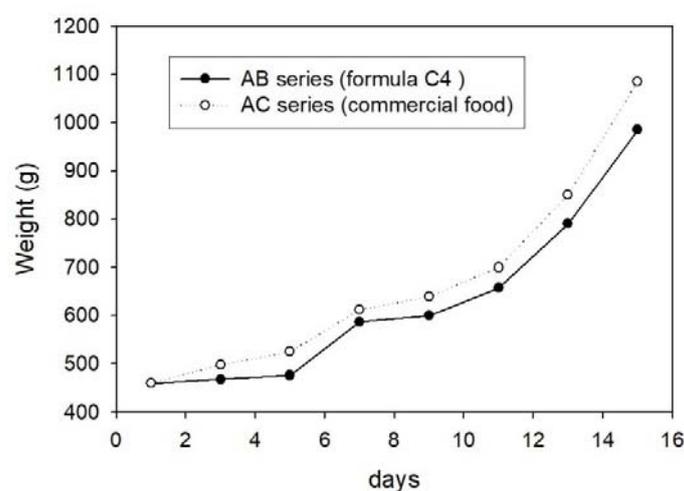
	Day	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Salmonella</i>
M1	T0	+	-	+	-
	T15	-	-	-	-
M2	T0	+	-	-	-
	T15	-	-	-	-
M3	T0	+	-	+	+
	T15	-	-	-	-
M4	T0	+	-	+	+
	T15	-	-	-	-
M5	T0	+	-	+	+
	T15	-	-	-	-
M6	T0	+	-	+	-
	T15	-	-	-	-
M7	T0	+	-	+	-
	T15	-	-	-	-

+: presence; -: absence.

Table 6 Determination of the rate of calcium, histamine and aflatoxins of M4.

Parameters	Composition M4
Calcium (%)	0.3
Sodium (%)	0.16
Histamine (ppm)	115
Aflatoxin B1 ($\mu\text{g}/\text{kg}$)	< 0.5 (LD)
Aflatoxin B2 ($\mu\text{g}/\text{kg}$)	< 0.12 (LD)
Aflatoxin G1 ($\mu\text{g}/\text{kg}$)	< 0.5 (LD)
Aflatoxin G2 ($\mu\text{g}/\text{kg}$)	< 0.12 (LD)

LD: limit of detection.

**Fig. 7** Evolution of the body weight of the series fed by the composition M4 and the food of trade.**Table 7** Zootechnical performance of the two series of chickens fed by the composition M4 and the commercial food.

Parameters	AB Series	AC series
Initial weight mean (g)	460	460
Final weight mean (g)	905	1,085
Average Earning Daily (g/J)	35	42
Food outlet (g)	300	300
Mortality	0/10	0/10

chicken were 0.9% and 0.3%, respectively. Table 6 indicated that the calcium content was insufficient in the composition M4 and for this reason the addition of eggshells and barley flour suggested.

The results of Fig. 7 present the evolution of the body weight and the daily dietary intake of the series of chickens fed by the organic food M4 representing the best results during the process of the biotransformation process as well as the commercial food as compare to reference commercial feed.

The average weight of the chickens fed by M4 (AB series) has evolved from 97% for a daily dietary

intake of 300 g, at the same time the series AC fed by the commercial food presents an evolution of average body weight of 135.86% for a daily dietary intake of 300 g. The average performances of growth were recorded in Table 7. The initial weight and final weight mean, the food taken, mortality, as well as the calculation of average earnings daily, indicated the small differences between the average performances [30].

The zootechnical performance of the two series of chickens fed by M4 (series AB) and the commercial food (series AC) were shown in Table 7. The weight

gained for both series AB and AC was 35 g/J and 42 g/J, respectively. The values of the food outlet and the gains of the corresponding weights to chickens fed with M4 chosen for its best results, attests to the reliability of product towards its nutrient input. The quality of biological product was interesting as the commercial food (Table 7).

4. Conclusions

The influence of three parameters: fish waste, molasses and waste yeast on protein, fat and carbohydrate during biotransformation were investigated and considered in the construction of a complete factorial design.

The formula M4 insuring favorable biotransformation possesses good hygienic properties and interesting nutritional quality for broilers proved even better compared to commercially available and highly marketed feed in Morocco.

Acknowledgments

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