VALORIZATION OF AGRI-FOOD BY-PRODUCTS TO PRODUCE HIGH ANIMAL PROTEIN QUALITY, *ARTEMIA* BIOMASS AT LAB SCALE

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Andrés Salgado-Ismodes
Agricultural and Aquaculture Sciences Dpt., Natural Resources Faculty, Universidad Católica de Temuco, Chile

Italo Salgado-Leu
Agricultural and Aquaculture Sciences Dpt., Natural Resources Faculty, Universidad Católica de Temuco, Chile

Patricio De Los Ríos-Escalante
Biological and Chemical Sciences Dpt,, Natural Resources Faculty, Universidad Católica de Temuco, Chile

ABSTRACT: Human being developing in all society models has generated a variety of by-products that can still be useful into circularity framework. Agro-industrial activity produces huge volumes of by-products. In this regard, poultry feather, grape pomace, rice husk and wheat middling are good examples. For this reason, they were tested as monodiet on *Artemia* biomass production. They were grinded, dehydrated up to 10% wet and then micronized (38 microns). In case of feather, it was prepared as hydrolysed and non-hydrolysed way. A simple quasi-experimental design completely randomized was proposed. Four treatments with three replicates were performed. Ten thousand hatched nauplii were seeded into one liter bottle with filtered seawater aerated constantly. Feeding suspension (50 g/L) were prepared with each monodiet. Artemia was fed on keeping 15 cm of turbidity in each unit. Best biomass increase production, specific grow rate, survival and feed conversion rate were obtained in general by poultry feather and in specific by hydrolysed feather with significant differences. As a consequence, it can be concluded, that agri-food by-product specially poultry feather can be converted in high animal protein quality with easy digestibility through *Artemia* biomass production.

KEYWORDS: *Artemia* biomass, agri-food by-products, high animal protein quality

VALORIZACIÓN DE SUBPRODUCTOS AGROALIMENTARIOS PARA PRODUCIR PROTEÍNA ANIMAL DE ALTA CALIDAD, BIOMASA DE *ARTEMIA* A ESCALA DE LABORATORIO

RESUMEN: El desarrollo humano en cualquier modelo de sociedades ha generado variados tipos de sub-productos que pueden ser utilizables en el marco de la economía circular. Un grupo de ellos son originados en grandes cantidades por la actividad agroindustrial. En el marco del presente estudio, se trabajó con plumas de la crianza de pollos, orujo de vino, harinilla de cáscara de trigo y de cáscara de arroz. En este contexto, se planteó evaluar la producción de biomasa de *Artemia*, alimentadas con estos subproductos agroindustriales. Primero, se deshidrataron hasta alcanzar 10% de humedad y luego micronizaron. Se propuso un diseño cuasi-experimental simple completamente al azar. Se trabajaron cuatro tratamientos con tres réplicas cada uno. Se colocaron diez mil nauplios en botellas con un litro de agua de mar filtrada y con aire permanente. Se preparó una suspensión con cada monodieta (50 g/L) y se suministró manteniendo 15 cm de turbiedad en cada unidad. En general, ambas monodietas de plumas obtuvieron los mejores aumentos de biomasa, tasa de crecimiento específico, supervivencia y conversión alimenticia; y en específico la monodieta de plumas hidrolizada con diferencias significativas. En consecuencia, se concluye, que los sub-productos de la agro-industria pueden ser convertidos en proteínas animales de alta calidad, de fácil digestibilidad, mediante la producción de biomasa de *Artemia*.

PALABRAS CLAVE: *Artemia* biomasa, subproductos agroalimentarios, proteína animal

1 | INTRODUCTION

Traditionally, *Artemia* spp. is known by their cyst products. Its hatched nauplii has been used by Seale since 1933. For many years, nauplii were used as food for initial larvae phase of many species, mostly fish and crustaceans. However, because shortage of cyst supply and its increasing demand, many users have changed to *Artemia* biomass produced mostly outdoor environments (Baert et al., 1996; Baert et al., 1997; Zmora et al., 2002; Van Hoa et al., 2011) and also in indoors (Zmora and Shpigel, 2006). Additionally, since several years ago, *Artemia* biomass started to use inducing ovarian maturation of shrimp, mostly by China, Southeast Asian countries and then, some Latin-American countries (Naessens et al., 1997; Wouster et al., 1998; Balachandar and Rajaram, 2019).

To produce Artemia biomass, live feed is used as several species of microalgae, yeasts and bacteria (single-cell protein) as well different types of inert monodiets coming from agricultural by-products like brans (from rice, wheat, corn, soybean, etc) (Sorgeloos and Persoone, 1975; Sorgeloos, et al., 1980; Sorgeloos et al., 1986; Ownagh, 2015). Production on microalgae has high costs and laborious tasks (Coutteau et al., 1992; Lavens and Sorgeloos, 1996; Naegel, 1999), while inert diets are cheaper. Ogburn et al., 2023 has made a review on agriculture wastes for brine shrimp Artemia production, where it is mentioned a variety of inert raw ingredients used as mono or mixed diets. In this sense, there are several other agricultural by-products that are not used yet, as poultry feather or grape pomace which represent huge quantities of agri-food by-products all over the world.

Related to poultry feather could be represented up 10% of the total bird weight according to Jamdar and Harikumar, 2005 and Lasekan et al., 2013 (Martinez et al., 2015). This ingredient has been rarely incorporated in aqua feeds. Feather is a protein source of poor quality, containing about 85,09% of crude protein as keratin, and is deficient in essential amino acids (lysine, methionine, histidine and tryptophan). Keratin is poorly digestible when raw. This highly polymerized protein contains about 8% cysteine, a sulphur amino acid that makes strong disulphur bonds between each other within the primary structure and contributes to the folding of the chain into secondary structures (alpha-helix and beta-sheet in a ratio of 2:1). While this makes raw feathers light, durable, and unable to stretch (unlike hair), it also makes feather keratin undigestible (digestibility < 5%) (Papadopoulos, 1985; Kornillowicz-Kowalska et al., 2011) (Heuzé, 2020). Nevertheless, protein hydrolysates have been reported as a source of short peptides and certain amino acids that can be absorbed easily, contributing to enhance animal growth. (McCalla et al., 2010). Thanks to hydrolysation process, feathers can be incorporated up levels of 8%. Higher of this value, there is no better results. (Tacon, 1989)

For those reasons described, it is necessary to hydrolyze feather meal in order to transform it into a valuable source of protein in animal feeding (El Boushy et al., 1990). A thorough hydrolysis under controlled conditions under pressure cooking destroys disulphur bonds between amino acids and convert feathers into hydrolised feathers.

Only in Chile is generated 20 248 tons of poultry feather/year (APA, 2020). In the case of grape pomace, in 2019, it was produced 620 815 tons/year (SAG, 2021; FEDNA, 2019). For ricehulk, among 32 and 40 ton/year (Mundoagro, 2020) and for wheat middling, 240 mil ton/year (ODEPA, 2018). The non-utilization of those by-products not only produce loss of potentials revenue but also leads to increasing its cost of disposal.

Based in all reviewed, in order to profit discarded agri-food by-products and considering that Artemia is a very primitive animal, it is proposed to valorize comparing poultry feather, ricehusks, grape pomace and wheat middling, transforming them into a very high-quality protein, easy to digest and with a good amino acid profile through Artemia biomass production at lab scale.

2 I MATERIALS Y METHODS

Artemia GSL strain was used for all treatments. They were incubated using standard techniques (Sorgeloos et al., 1986). After hatch, nauplii were transferred to twelve inverted plastic bottles, with cut-off bottom at a stocking density of 10000 individuals. Each bottle contained 1 liter of aerated and filtered seawater (salinity: 35 g/L). Water was renewed (0,5L) every three days and water temperature was kept constant at 25°C. For this purpose, all bottles were set into thermal bath tanks. Cultures were exposed to continuous light and aeration. Dissolved Oxygen concentration was averaged 6,87 +/-0,22 mg/L and pH 8,26+/-0,05.

Quasi-Experimental design proposed was a simple completely randomized. Five treatments were done: rice hulk (RH), hydrolysed feather (HF), non-hydrolysed feather

(NHF), Grape pomace (GP) and wheat middling (WM). Each one with three replicates.

All monodiets were prepared from raw agri-food by products: rice husk, poultry feather, grape pomace and wheat middling (Table 1). All of them was set into oven(60°C) until moisture reach 10%. Only feather was grinded and pre-treated under steam pressure for 30 minutes at 143°C under 3 atm; then dried for 5 hours at 60 °C. Each ingredient was micronized into a centrifuged mill (Retsch ZM200) and then sieved under 38 microns (Dobbeleir et al., 1979). Feed powder of each ingredient was suspended-50 g/L- into distillated water and kept under aeration and refrigeration. Feed suspension was supplied with graduated pipet until 15 cm of turbidity of culture water and quantity was registered (Bossuyt and Sorgeloos, 1980). After three days, turbidity was checked every hour.

Nutrients	Rice husk	Poultry feather	Grape pomace	Wheat middling
Dry matter	94,06	95,07	94,37	89,55
Protein	3,06	87,86	14,47	16,78
Lipid	0,17	3,18	6,08	4,41
Carbohydrate	34,26	3,69	60,38	67,82
Fiber	30,88	1,09	9,69	5,79
Ash	31,63	4,18	9,38	5,20

Table 1.- Composition of monodiets (%) used as feed

Wet Biomass (g/L) was weighed at beginning and after ten days, final wet biomass was measure again. Calculations of biomass increasing (mg/L and %) was done by differences between final and initial biomass wet weight. Specific growth rate was obtained as Lavens and Sorgeloos, 1991.

SGR = (Ln Final weight – Ln initial weight) / culturing period

Density (ind/L) was measure at initial and end of culturing period. These data were used for survival calculation. Survival (%) = [(Final density – Initial density) / initial density]* 100.

Feed conversion rate (FCR) = Food (g/L dry weight inert diet) / Artemia biomass increasing (g/L)

Normality test (Kolmorov-Smirnoff) was applied. Then, data were analyzed by one-way ANOVA and media comparison test (Tukey) with a probability of 0,05. Minitab v.19 statistical software was employed.

3 I RESULTS

In this study, from five monodiets, (Table 2) the best result was obtained by Hydrolysed feather (HF), in biomass increase (%), survival (%) and feed conversion rate. These comparisons result in a very high statistical difference. Additionally, under means comparison Tukey tests between treatments, in variable biomass increasing (%), all monodiets presented differences between them, one each other. The best results were for HF (3416,7±33,33) and NHF (1191±45,5), observing three folded in favor of HF. Both were highest on the other monodiets tested (wheat middling-WM, grape pomace-GP and rice hulk-RH) showing from 4 to 37-fold for HF and 1,5 to 13-fold for NHF.

Best Specific growth rate (SGR) were obtained by feather, both Hydrolysed (0,31±0,01) and non-hydrolysed (0,30±0,01) without significant differences among them. All the other treatments have significant differences with feathers.

Regarding on survival, HF obtained the highest rate (62,22%), with significative differences in front of the followings (GP: 45,64%; WM: 38,95% and NHF: 30,50%); the lesser value was obtained by RH (22,22%).

The Feed conversion rate also presented the best value for HF (1,48±0,07) without significant differences with NHF (2,55) and WM (3,62) followed by GP (7,9) and in last position, RH (29,41).

4 I DISCUSSION

In all monodiet feed used in this study, it is shown that *Artemia* biomass production can be performed at different values after 10 days trails. Evidences can be observed in Biomass increase, SGR, survival and feed conversion rate.

In regards of Biomass increase, it is evident that HF obtained the highest value with 3416.7 % (=4,1 g/L). It could be said that exist a positive effect to hydrolyse the feather before ingested over non-hydrolysed feather (1191 % = 1,31 g/L). This effect was doubled in front of non-hydrolysed, so hydrolysation process is playing a role of amino acid disponibility for *Artemia*, considering that *Artemia* has a very primitive digestive tract.

Variable	Rice husk (RH)	Hydrolysed feather meal (HF)	Non-hydrolysed feather meal (NHF)	Grape pomace (GP)	Wheat middling (WM)
Initial biomass (g/L)	0,12 ± 0,00	0,12 ± 0,00	0,11	0,16	0,173 ± 0,00
Final biomass (g/L)	0,23±0,01	4,22±0,04	1,42±0,05	0,51±0,08	1,53±0,08
Biomass increase (g/L)	0,11±0,01^{d}	4,1±0,04^{a}	1,31±0,05^{b}	0,35±0,08^{c}	1,36±0,08^{b}
Biomass increase (%)	91,67±8,33^{e}	3416,7±33,33^{a}	1191±45,5^{b}	227,78±12^{d}	800±47^{c}
Specific growth rate (SGR)	0,16589±0,01^{c,d}	0,31±0,0^{1}a	0,30±0,01^{a}	0,15±0,02^{d}	0,25±0,013^{b}

Initial density (ind/L)	10000	10000	10000	10000	10000
Final density (ind/L)	2222±192	6222±509	3000±205	4764±222	3999±133,5
Survival (%)	22,22±1,92^{c}	62,22±5,09^{a}	30,50±2,28^{b,c}	45,64±5,01^{b}	38,95±0,93^{b,c}
Food supply (g/L)	3,26±0,11^{c}	6,07±0,23^{a}	3,34±0,3^{b,c}	2,79±0,8^{c}	4,87±0,89^{a,b}
Conversion factor	29,41±1,23^{a}	1,48±0,0^{7}c	2,55±0,13^{c}	7,9±0,68^{b}	3,62±0,87^{c}

Tabla 2.- Comparative variable results according monodiets fed on Artemia

Naegel (1999) presented biomass production of 0,397 g/L with Nestum and 0,488 g/L for Nestum enriched; Also, Cisneros and Vinatea, 2009 experimented with meals of different sources as soybean, freshwater shrimp, ricebran, alfalfa, fishmeal and a mix of them (20% each). Their best result was obtained with soybean meal (0,37 g/L). Comabella et al., (2004) obtained 0,3 g/L using yeast (*Saccharomyces cerevisiae*). From all monodiets tested in the present experiment, only grape pomace and ricehusk are below of them. As it can be seen, all these results have very lower values in comparison with the result here obtained by HF (4,1 g/L), NHF (1,31 g/L) and WB (1,36 g/L).

There are some experimental works done with different agriculture by-products and different microalgae species, as well. As far as it is known, *Artemia* is a continuous and non-selective feeder, so it can be fed on any particle, live or inert, below 35 microns (Lavens and Sorgeloos, 1996). In this regard, there are experiments fed on micronized ricebran, wheat bran, corn bran and also with microalgaes like *Dunaliella, Tetraselmis, Chaetoceros, Isochrysis*, etc. Vanhaecke and Sorgeloos, (1989) already showed that *Artemia franciscana* performed better with ricebran than microalgae *Dunaliella.* Balchandar, (2018) reported that monodiets are more deficitaries in nutritional contents than a combination diets. In this sense, Lavens and Sorgeloos (1991) suggested that a mixture of two or more ingredients would fill nutritional deficiency. In this issue, presented results of this research observed that highest values can be obtained with HF which three folded over NHF and both are significative higher than WM, GP and RH.

Balachandar (2018) tested growing of *A. franciscana* during 12 days fed on different microalgae (*Tetraselmis, Isochrysis, Chaetoceros, Nannochloropsis, Thalassiosira*) and also ricebran, soybean meal and combination of *Tetraselmis* + *Chaetoceros* + rice bran, obtaining the best growing rate with combination (microalgae + rice brand), followed by rice bran alone. Ownagh, 2015, showed results on *Artemia urmiana* y *A. parthenogenetica* biomass production fed on *Dunaliella salina* as control (1,16 g/L and 1,09 g/L), wheatbran (0,92 and 0,83 g/L), soybean (0,76 and 0,9 g/L) and wheatbran (50%) + soybean (50%)

(0,88 and 1,08 g/L), all these figures corresponding *for A. urmiana* and *A. parthenogentica*, respectively; and *D. salina* was presented in all treatments, combined with all types of inert food. As it can be seen, our results with HFM (4,22 g/L), NHFM (1,31 g/L) and WB (1,36 g/L) surpass Ownagh results.

It is necessary to take into our knowledge that present study was performed with monodiets=only one ingredient, so there are no possibilities to change nutritional contents of each one. Biomass increase results showed a tendency to diminish according to protein content: HF/NHF (87,86%), WM (16,78%), GP (14,47%) and RH (3,06%). This could explain the values produced and again, the objective is to produce high quality protein based on subproducts; it means to profit residual nutrients and energy content in them and its disposal represent a cost besides organic pollution.

Taking into account that feather does not have essential amino acids: lysine, methionine, histidine and tryptophan, the amino acid sequence of a chicken feather is very similar to that of other feathers and also has a great deal in common with reptilian keratins from claws. The sequence is largely composed of cystine, glutamine, proline, and serine (Lasekan et al., 2013).

In this context, it could be that *Artemia* synthesize their amino acids from new, based on it is a primitive animal with this capacity or its essential amino acids requirements can be supplied by bacteria in the culture medium.

SGR is little analyzed in this type of experiments on Artemia biomass production. Highest SGR was recorded for soybean meal in *A. urmiana* (0,34) without significant differences with other treatments (*D. salina*, WB, SM WB/SM) varying from 0,31 to 0,33. (Ownagh et al., 2015). In present study, feather (HF: 0,31 and NHF: 0,30) can be compare with those of Ownagh results. Other treatments WM, GP and RH had below values.

Survival is more consistent for HF (62,22) than others. The following survival rate is for GP with 39,51%, the NHF (33,33%), WM (27,78%) and finally, RH (22,22%); all of them are significantly rather below than HF. Naegel, (1999) obtained survival of 72% fed on Nestum and 79% Nestum + microalgae *Chaetoceros* during 11 days being superior to HF (62%), but considering that Nestum is a feeding formula prepared with special nutrients with highest cost. Balachandar (2018) found best significant survival with microalgae and combined diets mentioned before (from 42,3 to 73,7%). These survival rates are comparable with HF (62,22%). In any case, our HF survival is rather better than *Tetraselmis, Isochrysis, Nannochloropsis, Thalassiosira*, rice bran and soybean meal. It is not the case in comparison with *Chaetoceros* (73,3%) and Tetra+Chaeto+ricebran (73,7%). Ownagh et al., (2015) obtained survivals between 58 to 86% for *A. urmiana* and 66 to 76% for *A. parhenogenetica*. These figures compared with the obtained in this study, only HF can compete with 62,22%.

Respect on Feed Conversion rate, Naegel, (1999) showed a FCR of 1,64:1 for Nestum and 1,17:1 for Nestum enriched in 14 days. Vanhaecke and Sorgeloos (1989), using *D. tertioleca* reached 2,4:1 in 9 days. For this study, it is performed the best FCR of

1,48 for HF followed by 2,55 for NHF, without significant difference. Based on these results, it can be said that feather offers good feed conversion rate. Ownagh et al., 2015 observed very good values of FCR less than 1 (0,17 to 0,23) but they do not offer any explanation about those figures rather impossible to get them. For sure there were another internal generation or supply of some ingredient not controlled.

These results can contribute to a real use of feather meals to transform in a high-quality animal protein and easy digestion for aquatic predators in their larvae, juvenile or adult stages. According to Lavens and Sorgeloos (1996), *Artemia* biomass are used as nursery diets. This means when large *Artemia* biomass is offered instead of freshly-hatched nauplii, predators need to chase and ingest less prey organisms per unit of time to fill their food requirements. There are reports on advantages of using *Artemia* biomass as for instance considerable saving of cyst up to 60% and consequently, a significant reduction of costs. Moreover, Artemia biomass can be consumed directly as omelette as several asian community members (Van Hoa and Sorgeloos, 2020).

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