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Digestibility of different wheat products in white shrimp *Litopenaeus vannamei* juveniles

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ABSTRACT

Dry matter, energy, crude protein and amino acid apparent digestibility coefficients (ADCs) were determined in white shrimp juveniles for six wheat products: hard red winter whole grain meal (HWG), Rayon whole grain meal (RWG), Durum whole grain meal (DWG), hard red winter clear flour (HCF), mixed wheat 2nd clear flour (MCF) and semolina (S). The test diets included 30% of the test ingredients and 70% of a ground commercial diet supplemented with 1% chromic oxide and 1% sodium alginate. Amino acid contents in the ingredients, diets and feces were analyzed, and digestibility was determined by difference in order to minimize the impact of endogenous amino acid losses; crude protein and amino acids ADCs were adjusted for dietary preprandial losses in seawater. In general, nutrients digestibility was far higher in the wheat products than in the fish meal-based reference diet. Dry matter and crude protein ADCs were not statistically different among wheat products (from 84 to 96% and from 88 to 107% respectively). Energy ADCs were significantly higher for clear flours (96% for HCF and MCF) than for whole grain meals and S (from 83 to 86%). Total amino acids (TAA) and essential amino acids (EAA) ADCs, once adjusted for preprandial leaching from the experimental diets, ranged from 81 to 89% and from 58 to 81% respectively, and were statistically comparable among wheat products. Low Thr ADCs appear as a common feature of the amino acids digestibility profiles for whole grain meals, clear flours, or semolina.

1. Introduction

Manufacturing shrimp feeds present unique challenges; shrimp feeds should be stable after immersion in seawater but able to release attractant compounds to guarantee faster ingestion by the shrimp. Wheat meal products, such as gluten, flour and whole grain meal, have been used for many years in shrimp feed manufacture as natural binders and inexpensive energy sources (Hertrampf, 2007). *In vivo* studies have shown significant differences in growth performance of shrimp fed diets containing different sources of wheat products: Shiau et al. (1991) observed higher weight gain in shrimp *P. monodon* fed diets supplemented with straight wheat flour than in those fed diets supplemented with first and second grade clear flours (303% vs. 295–230%) without significant differences among apparent nutrient digestibility coefficients: Cruz-Suárez et al. (1994) found that the inclusion of soft white wheat and cookie-waste meals in feeds for shrimp *L. vannamei* produced higher weight gain than the inclusion of sorghum, millet, rice, corn and pasta meals. In Mexico, shrimp feeds contain wheat products available from local producers or imported from United States and Canada. It will be advantageous from nutritional, ecological and economical points of view to determine if there is a significant difference in nutrient digestibility among these wheat products to formulate less polluting and less expensive feeds. The aim of the present study is to determine apparent nutrient digestibility coefficients of three wheat grains, two clear flours and one semolina meal for shrimp juveniles *L. vannamei.*

2. Material and methods

2.1. Wheat test products

The test ingredients were obtained from the providers of a Mexican shrimp feed manufacturer located in Sonora, Mexico, as grains (hard red winter HWG, Rayon RWG, and Durum DWG), clear flours (hard red winter clear flour HCF, and a mixed wheat 2nd clear flour MCF) or semolina (S) (Table 1). HWG *Triticum aestivum* grain was harvested in California, USA, in year 2005, and RWG *T. aestivum* variety F-89 was harvested in Comondú Baja California Sur, Mexico, in year 2006. RWG variety has strong and elastic gluten, of bread making quality, and its cultivars have expanded widely in Northwest México due to their durable resistance to leaf and stripe rusts (Singh et al., 2005). *Triticum durum* DWG, Jupare variety, was harvested in Valle del Mayo, Sonora, México, in

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Table 1
Chemical composition of the reference ingredient and test ingredients.

	Reference ingredient	HWG	DWG	RWG	HCF	MCF	S
Proximate composition (% DM)							
Crude protein (N × 6.25)	37.3	15.1	14.2	15.5	14.7	16.0	13.7
Crude lipids	9.7	2.7	1.9	2.0	1.1	2.6	2.5
Fiber	3.4	1.1	2.9	2.3	0.5	0.8	0.5
Ash	10.2	1.7	1.8	1.6	0.7	1.2	1.2
NFE	39.4	79.4	79.2	78.6	83.0	79.4	82.1
Energy (KJ/g)	20.2	17.1	16.8	17.2	16.3	17.0	17.1
Aminoacid (g AA/ 100 g DM)							
Arginine	2.06	0.68	0.66	0.67	0.55	0.71	0.55
Histidine	0.80	0.35	0.34	0.35	0.31	0.37	0.32
Isoleucine	1.32	0.49	0.49	0.49	0.50	0.52	0.49
Leucine	2.30	0.98	0.97	0.97	0.97	1.03	0.95
Lysine	1.86	0.41	0.38	0.39	0.31	0.39	0.31
Methionine	0.69	0.23	0.23	0.22	0.22	0.24	0.22
Phenylalanine	1.68	0.68	0.66	0.71	0.71	0.73	0.66
Threonine	1.26	0.43	0.38	0.42	0.38	0.43	0.36
Valine	1.58	0.60	0.63	0.60	0.59	0.66	0.58
$\sum$EAA	13.56	4.85	4.74	4.82	4.56	5.09	4.45
Alanine	1.86	0.52	0.48	0.51	0.44	0.52	0.41
Aspartic acid	2.95	0.75	0.69	0.75	0.58	0.71	0.58
Cysteine	0.43	0.33	0.30	0.33	0.32	0.33	0.28
Glutamic acid	6.09	4.31	4.03	4.58	4.79	4.76	4.23
Glycine	2.15	0.61	0.52	0.60	0.51	0.61	0.45
Proline	2.20	1.46	1.37	1.52	1.63	1.59	1.46
Serine	1.42	0.69	0.63	0.71	0.67	0.71	0.62
$\sum$TAA	30.66	13.53	12.75	13.81	13.51	14.33	12.48

HWG, hard red winter wheat; DWG, Durum wheat; RWG, rayon wheat; HCF, hard red winter clear flour; MCF, mixed wheat clear flour; S, semolina; DM, dry matter; NFE, nitrogen free extract; $\sum$EAA, sum of analyzed essential amino acids; $\sum$TAA, total analyzed amino acids.

year 2006. HCF and S were manufactured by the wheat milling company Altex at San Cristobal mill, Sonora, from the above mentioned HWG *T. aestivum* and Jupare *T. durum* grains. MCF was obtained at San Cristobal mill from the milling tails of medium hard and soft *T. aestivum* grains which had been harvested in Sonora in 2005.

2.2. Feed formulation and preparation

Digestibility in the ingredients was determined following Cho and Slinger (1979) method; the method was set up to determine nutrients digestibility coefficients using 70% of a reference diet and 30% of test ingredient. Reference diet was composed of 98% of a compound reference ingredient plus 1% chromic oxide (Impex Continental, 52-03-05) as an inert marker and 1% sodium alginate (Sigma A-7128, high viscosity) as a binder. The reference ingredient was the same commercial feed elaborated in a feed plant as mentioned by Cruz-Suárez et al. (2009): 45.5% wheat meal, 34% fish meal, 14% soybean meal, 3.5% soybean lecithin, 2.8% fish oil and 0.56% micro-ingredients. This reference diet was a commercial-like, fish meal-based diet (in regard of its main protein source), formulated according to the recommendations by Akiyama et al. (1989), which was first pelleted at an industrial shrimp feed plant, and then ground at our laboratory to obtain a maximum particle size of 500 μm. Wheat grains were ground in a Pulvex 200 grinder to obtain 800 μm particle size and then in a Cyclotec grinder (Tecator, model 1093) to obtain a 500 μm particle size. Semolina was ground in the Cyclotec grinder to obtain a 500 μm particle size. Clear flours were included in the diet without additional grinding because particle size was already below 200 μm. Experimental diets were manufactured as followed: the ingredients (reference mixture alone, or with 30% test ingredient) were mixed for 10 min in a Kitchen Aid mixer, and water (30%) was added and mixed for 15 min. The wet diet mash was passed through a meat grinder (die with 1.6 mm diameter holes) at a rate of 40 min/kg diet, reaching a temperature of 70–75 °C. The spaghetti-like strands were dried in a ventilated oven at 100 °C for 8 min and allowed to cool and dry overnight at room temperature before packing.

2.3. Feeding trial design and feces collection

Experimental animals were obtained from Acualarvas hatchery, Huatabampo, Sonora, Mexico. Prior to the digestibility trial, shrimp were acclimated to the conditions of the bioassay room in 500 L holding tanks. Digestibility trial was carried out at the Programa Maricultura facilities in Monterrey, Mexico, in a closed recirculation artificial sea water system. The experimental facility contains 54 (60 L) experimental fiber glass tanks, each continuously fed with synthetic marine water (Fritz, Dallas, TX) at a flow-through rate of 350 ml per minute. All tanks have a built-in internal recirculation "air lift" system and were fitted with a double bottom, covered with black stocking. The experimental tank array is designed so that possible water quality variations affect all tanks simultaneously. The water quality parameters for this experiment were temperature 29.8(SD 0.7)°C, salinity 26.2 (SD 1.0) g/L, dissolved oxygen 5 mg/L (close to saturation), pH 7.5 (SD 0.3), nitrates 500 (SD 0) mg/L, nitrites 1.0 (SD 0.8) mg/L, total ammonium 0 mg/L; phosphates 20 (SD 9.0) mg/L. Each experimental diet was evaluated in four tank replicates. Nine white shrimp juveniles *L. vannamei* (3.56 SD 0.07 g average initial body weight) were allocated to each 60 L experimental fiber glass tank. Dietary treatments were randomly assigned to the tanks using a four block design (4 replicates). Shrimp were adapted to diets for at least 3 days before feces collection. The shrimp were initially fed at 10% of biomass and the ration was adjusted daily to keep uneaten feed to a minimum. Feces were collected six times a day during 15 days until 12 g feces (wet basis) per tank were obtained. The following feces collection protocol was applied to successive tanks, starting at 30 s intervals: at 08:00 the first tank bottom was siphoned to remove any uneaten feed and molts; at 08:30 the animals were fed and at 09:30 the remaining feed and first feces sample were siphoned and discarded; at 10:30 and 11:45 feces were siphoned, washed quickly in distilled water and stored in a freezer (first and second feces collections). The same process was repeated twice in the afternoon. Feces collected from a particular tank were pooled and stored frozen and then freeze-dried before analyses. During feces collection no mortality was observed.

2.4. Chemical analysis

Moisture (method 930.15), ash (method 942.05), fiber (method 962.09) and crude lipids (method 960.39) were determined in ingredients, diets and feces using AOAC methods (1997). Crude protein (N × 6.25) was determined using Dumas method (AOAC, 1997) and LECO equipment. Gross energy was measured using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Nitrogen free extract was calculated by difference. Amino acid (AA) analyses in diets, test ingredients and feces were performed by wet chemistry at Evonik-Degussa Laboratory using acid hydrolysis and HPLC equipment (Llames and Fontaine, 1994). Chromic oxide in the diets and feces was analyzed using the method by Bolin et al. (1952) as described by Cruz-Suárez et al. (2009). Diet composition analysis (protein, lipids, fiber, ash, energy and amino acids) was done in duplicate. Nutrient losses (dry matter, protein and amino acids) in the experimental diets were determined as followed: 3 g sample of pellets was weighed in a sieve (#40 mesh) that was then fixed in the mouth of a 250 mL plastic bottle, containing 200 mL seawater (the pellets being immersed under the water upwelling through the mesh screen). After agitating the bottles in a water bath for 1 h (30 rpm, 28 °C), the sieves were drained for a few minutes, and then dried before being weighed again. The percentage of nutrients lost by leaching in seawater before diet ingestion (%NL) was evaluated as described by Cruz-Suárez et al. (2007), with the following expression: $\%NL = (N_{diet} * 100 - N_{diw} * (100 - \%LDM)) / N_{diet}$, where N_{diet} and N_{diw} are the nutrient concentrations (crude protein or amino acid, dry basis) in the diet as manufactured or after immersion for 1 h in seawater (35 g/L, 28 °C), and %LDM is the percent dry matter lost from the diet immersed in water. The percent nutrients lost from the ingredient portion in the immersed test diets ($\%NL_i$) was also estimated by calculating the difference between nutrients lost from the immersed test diet and immersed reference diet: $\%NL_i = 100 - [(100 - \%NL_{td}) * N_{td} * (0.7 * DM_{ref} + 0.3 * DM_{ing}) - 0.7 * (100 - \%NL_{rd}) * N_{rd} * DM_{ref}]/(0.3 * N_i * DM_{ing})$, where: $\%NL_{td}$ and $\%NL_{rd}$ are the percentages of nutrient lost from the immersed test and reference diets; N_{td}, N_{rd} and N_i are the nutrient concentrations in test diet, reference diet and ingredient (dry basis); DM_{ref} and DM_{ing} are the dry matter contents of the reference mixture and test ingredient at the moment of mixing. The percentage of water absorption (WA) for the experimental diets was determined in the following manner: A 5 g sample of feed was weighed and submerged for 1 h in 50 ml of distilled water. The wet sample was filtered through a #200 mesh screen and the retained sample was weighed. The percentage absorption of water was calculated with the following formula: %WA = 100*[(Weight of the sample after submersion in distilled water − Weight of the sample before submersion)/Weight of the sample before submersion]*100. Loss of protein, loss of dry matter, loss of amino acid and water absorption capacity was analyzed in three replicates.

2.5. Apparent digestibility coefficients calculations

Dry matter, protein, energy and amino acid apparent digestibility coefficients (ADCs) of diets were calculated using the following equations (Maynard et al., 1981): $\% ADC_{diet} = 100 - 100 * (C_{diet}/N_{diet}) * (N_{feces}/C_{feces})$, where C and N are the chromic oxide and nutrient (dry matter, crude protein, gross energy or amino acid) concentrations in diets or feces (dry basis). ADCs of ingredients were calculated according to Bureau and Hua (2006): $\%ADC_{ingredient} = ADC_{testdiet} + [(ADC_{testdiet} - ADC_{ref.diet}) * (0.7 * N_{ref(as\ is)})/(0.3 * N_{ingr(as\ is)})]$, where: $ADC_{testdiet}$ and $ADC_{ref.diet}$ are apparent digestibility coefficients calculated as shown above; N_{ref} and N_{ingr} are the concentrations of the considered nutrient (dry matter, crude protein, gross energy or amino acid) in the reference mixture and in test ingredient as they were at the moment of the mixing. ADCs of diets and ingredients were also adjusted by taking into account the percent nutrients lost due to leaching (Cruz-Suárez et al., 2009):

Table 2
Formulas and chemical composition of the reference and test diets.

	Reference diet	HWG	DWG	RWG	HCF	MCF	S
Formulas (g kg^{-1}, % as is)							
Reference ingredient	980	–	–	–	–	–	–
Chromic oxide	10	–	–	–	–	–	–
Sodium alginate	10	–	–	–	–	–	–
Reference diet mixture	–	700	700	700	700	700	700
HWG	–	300	–	–	–	–	–
DWG	–	–	300	–	–	–	–
RWG	–	–	–	300	–	–	–
HCF	–	–	–	–	300	–	–
MCF	–	–	–	–	–	300	–
S	–	–	–	–	–	–	300
Proximate composition (% DM)							
Crude protein (N × 6.25)	35.0	29.4	29.2	29.9	29.5	29.8	29.0
Lipids	9.1	7.1	7.1	7.2	6.9	7	7.1
Fiber	3.3	2.7	3.2	3.0	2.5	2.6	2.5
Ash	11.3	8.6	8.6	8.6	8.4	8.5	8.4
NFE	41.3	52.2	51.9	51.3	52.7	52.1	53.0
Energy (KJ/g)	19.5	19.2	19.2	19.3	19.2	19.2	19.2
Aminoacids (g AA/100 g DM)							
Arginine	2.02	1.61	1.62	1.64	1.59	1.64	1.57
Histidine	0.76	0.64	0.64	0.65	0.65	0.65	0.63
Isoleucine	1.30	1.10	1.08	1.09	1.10	1.07	1.05
Leucine	2.28	1.91	1.89	1.92	1.91	1.92	1.89
Lysine	1.83	1.39	1.39	1.40	1.37	1.40	1.36
Methionine	0.67	0.55	0.55	0.55	0.55	0.54	0.52
Phenylalanine	1.56	1.34	1.34	1.39	1.37	1.41	1.39
Threonine	1.23	0.98	0.98	1.00	0.99	1.02	0.98
Valine	1.54	1.31	1.29	1.29	1.30	1.28	1.24
∑EAA	13.20	10.83	10.78	10.92	10.85	10.93	10.64
Alanine	1.81	1.42	1.42	1.44	1.42	1.43	1.40
Aspartic acid	2.89	2.24	2.24	2.29	2.23	2.25	2.20
Cysteine	0.46	0.37	0.36	0.37	0.37	0.39	0.37
Glutamic acid	5.98	5.54	5.41	5.64	5.70	5.70	5.51
Glycine	2.08	1.63	1.61	1.65	1.62	1.63	1.58
Proline	2.20	1.96	1.95	2.05	2.03	2.02	1.95
Serine	1.41	1.15	1.17	1.21	1.18	1.22	1.20
∑TAA	30.02	25.15	24.93	25.57	25.40	25.58	24.86
Diet stability (%)							
LDM	8.2 ± 0.5a	9.1 ± 0.6a	9.3 ± 0.7a	9.5 ± 0.1a	8.6 ± 0.8a	9.3 ± 1.1a	8.2 ± 1.4a
LCP	21.9 ± 0.2a	32.2 ± 0.0de	34.1 ± 0.1e	31.0 ± 1.2 d	25.5 ± 0.1b	28.2 ± 1.1c	29.9 ± 1.8 cd
WA	61.8 ± 4.4a	66.7 ± 9.0a	64.2 ± 11.7a	104.4 ± 8.3b	67.7 ± 5.6a	98.2 ± 21.0b	66.3 ± 16.8a

HWG, hard red winter wheat; DWG, Durum wheat; RWG, rayon wheat; HCF, hard red winter clear flour; MCF, mixture wheat clear; S, semolina; DM, dry matter; NFE, nitrogen free extract; ∑EAA, Sum of analyzed essential amino acids; ∑TAA, total analyzed amino acids; LDM, loss of dry matter; LCP, loss of crude protein; WA, water absorption capacity; letters in same row indicate homogeneous subsets as define by the Tukey´s multiple means comparison test (α = 0.05).

$$\%ADC_{diet.adj} = 100 - 100*(C_{diet}/N_{diet})*(N_{feces}/C_{feces})*(1/(1-\%NL/100));$$
$$\%ADC_{ing.adj} = ADC_{td.adj} + [(ADC_{td.adj} - ADC_{rd.adj})*(0.7*(100-\%NL_{rd})*N_{ref(as\ is)})/(0.3*(100-\%NL_i)*N_{ingr(as\ is)})].$$

2.6. Statistical analysis

Digestibility coefficients were calculated from the four replicated tank feces samples and subjected to a one way analysis of variance among test ingredients, followed by a Tukey's multiple range test ($\alpha = 0.05$, SSPS 16.0, 2007, SPSS Inc., Chicago, Illinois). The same analyses were applied to water absorption and loss of nutrient in test diets. Differences were considered significant when $P<0.05$. Linear regressions of total amino acids vs. crude protein ADCs, loss of protein vs. wheat products characteristics and loss of water absorption capacity vs. wheat products characteristics were tested among the test ingredients.

3. Results

3.1. Chemical composition of the test ingredients and diets

Chemical composition of wheat products and test diets is presented in Tables 1 and 2. Loss of dry matter was similar among test diets (from 8.2 to 9.5%, Table 2) while loss of protein showed significant variations ($P<0.001$) among experimental diets; diets supplemented with whole grain meals showed higher loss of protein (31–34%) than those supplemented with clear flours and S (26–30%). Control diet and diets supplemented with HWG, DWG, S and HCF presented similar water absorption capacity (from 61.8 to 67.7%, Table 2) whereas MCF and RWG diets reached the highest values (98 and 104% respectively). Chemical composition in test diets was close to the expected values calculated from the reference and test ingredients contents.

3.2. Digestibility of test diets

Digestibility coefficients for dry matter (DM), protein, energy and the total of analyzed amino acids ($\sum$TAA) in reference diet were 70, 81, 81 and 84% respectively. Digestibility coefficients in the test diets ranged from 74 to 78% for DM, from 82 to 85% for protein, from 83 to 87% for energy and from 85 to 87% for $\sum$TAA. Gly and Ala were the less digestible amino acids in the test diets (from 70 to 75% for Gly and from 78 to 81% for Ala, vs. from 80 to 91% for the rest of the amino acids).

3.3. Digestibility of the wheat products

Nutrients digestibility values in the wheat products were calculated by difference, which accounts and corrects for the digestibility of the reference diet (70% of test diet). Thus, calculated digestibility coefficients of the wheat product nutrients are dependent of digestibility of the reference diet to a certain proportion. Results of standard calculation are presented in Table 3. DM and crude protein digestibility coefficients were statistically similar between wheat products ($P>0.05$) and ranged from 84 to 96% for DM and from 88 to 107% for crude protein. In contrast, energy digestibility was significantly different among test ingredients ($P<0.001$): HCF and MCF obtained the highest energy digestibility (96%), while HWG, DWG, RWG and S were less digestible (83 to 86%). Amino acid digestibility coefficients were high (from 89 to 102% for total amino acids, Table 3), showing significant differences among test ingredients ($P<0.05$) only for Arg: HCF, RWG and DWG obtained the highest digestibility coefficient for this amino acid (from 100 to 107%) whereas S product presented the lowest digestibility (84%). The digestibility coefficients were adjusted to compensate for the preprandial amino acids losses in seawater (Table 4) by analyzing the amino acid contents of diets immersed for one hour in sea water and using modified mathematical ADCs expressions as suggested by Cruz-Suárez et al. (2009); the result was an important and uneven decrease of amino acid ADCs, depending on the instability of a particular test diet and the solubility of a particular AA, leading to adjusted ADCs in the range of 16-20% (Thr and Ala ADC's in Semola) to 102% (Pro ADC in HCF) instead of the range of 73 to 117% for standard values (Table 3). Individual amino acid digestibility was compared with crude protein digestibility for a particular wheat product: the differences for standard ADCs ranged from −22 to +11 percentage units (Table 5); Lys, Thr, Cys and Ser presented high average differences (more than 5 percentage units), while this variation ranged from −4 to 5 percentage units for the rest of the AA. These differences allowed identifying distinct AA digestibility patterns for whole grain meals, clear flours and semola

Table 3

Dry matter, energy, crude protein and amino acid apparent digestibility coefficients (ADC, %) of wheat products and reference diet consumed by marine shrimp *Litopenaeus vannamei* (mean ± standard deviation, n = 4).

	HWG	DWG	RWG	HCF	MCF	S	ANOVA Probability	Reference diet
Dry matter ADC	85 ± 7a	86 ± 8a	84 ± 9a	96 ± 8a	91 ± 5a	87 ± 5a	0.131	70 ± 2
Energy ADC	83 ± 3a	84 ± 8a	83 ± 2a	96 ± 3b	96 ± 3b	86 ± 5a	0.000	81 ± 1
Crude protein ADC	97 ± 10a	97 ± 3a	99 ± 4a	107 ± 17a	99 ± 7a	88 ± 13a	0.255	81 ± 3
Amino acid ADC								
Arginine	98 ± 8ab	100 ± 13ab	101 ± 1ab	107 ± 7b	91 ± 11ab	84 ± 11a	0.043	84 ± 3
Histidine	99 ± 6ab	98 ± 10ab	101 ± 4ab	106 ± 6b	94 ± 9ab	89 ± 6a	0.074	84 ± 3
Isoleucine	98 ± 7a	99 ± 10a	97 ± 7a	104 ± 5a	90 ± 14a	85 ± 7a	0.082	85 ± 3
Leucine	96 ± 7a	96 ± 9a	95 ± 4a	101 ± 3a	91 ± 12a	88 ± 6a	0.198	86 ± 3
Lysine	103 ± 14a	107 ± 16a	106 ± 5a	117 ± 4a	94 ± 23a	89 ± 13a	0.130	87 ± 2
Methionine	108 ± 10a	108 ± 15a	110 ± 7a	113 ± 7a	94 ± 18a	92 ± 12a	0.106	82 ± 3
Phenylalanine	100 ± 5a	103 ± 11a	104 ± 3a	105 ± 7a	98 ± 9a	94 ± 6a	0.269	83 ± 3
Threonine	89 ± 7a	89 ± 17a	90 ± 7a	94 ± 12a	84 ± 16a	73 ± 16a	0.306	81 ± 3
Valine	97 ± 6a	96 ± 11a	95 ± 5a	102 ± 7a	89 ± 12a	83 ± 8a	0.087	83 ± 3
$\sum$EAA	98 ± 7a	99 ± 12a	99 ± 4a	104 ± 6a	91 ± 13a	86 ± 9a	0.123	84 ± 2
Alanine	101 ± 9a	103 ± 22a	105 ± 5a	112 ± 16a	88 ± 17a	79 ± 20a	0.087	78 ± 3
Aspartic acid	98 ± 9a	99 ± 21a	101 ± 7a	105 ± 19a	86 ± 21a	76 ± 19a	0.189	81 ± 2
Cysteine	82 ± 2a	80 ± 8a	82 ± 3a	85 ± 6a	82 ± 8a	77 ± 5a	0.563	84 ± 3
Glutamic acid	98 ± 2a	99 ± 5a	99 ± 2a	100 ± 3a	97 ± 4a	95 ± 3a	0.464	89 ± 1
Glycine	104 ± 10a	108 ± 30a	116 ± 7a	116 ± 28a	87 ± 16a	79 ± 29a	0.134	70 ± 3
Proline	100 ± 3a	101 ± 7a	103 ± 1a	100 ± 6a	96 ± 5a	93 ± 5a	0.082	82 ± 2
Serine	92 ± 5a	92 ± 11a	95 ± 2a	94 ± 7a	89 ± 9a	85 ± 9a	0.539	81 ± 3
$\sum$TAA	98 ± 5a	99 ± 11a	100 ± 3a	102 ± 7a	93 ± 9a	89 ± 8a	0.181	83 ± 2

HWG, hard red winter wheat; DWG, durum wheat; RWG, rayon wheat; HCF, hard red winter clear flour; MCF, mixed wheat clear flour; S, semolina; $\sum$EAA, sum of analyzed essential amino acids; $\sum$TAA, total analyzed amino acids; letters in same row indicate homogeneous subsets as define by the Tukey's multiple means comparison test ($\alpha = 0.05$).

Table 4
Dry matter, protein and amino acid apparent digestibility coefficients (ADC, %) of wheat products and reference diet, adjusted for the loss of nutrients in seawater before the feed ingestion (mean ± standard deviation, n = 4).

	HWG	DWG	RWG	HCF	MCF	S	ANOVA probability	Reference diet
Dry matter ADC	83 ± 7a	84 ± 9a	81 ± 5a	95 ± 8a	89 ± 5a	86 ± 6a	0.098	67 ± 2
Crude protein ADC	75 ± 13a	69 ± 4a	74 ± 6a	88 ± 22a	85 ± 9a	60 ± 18a	0.080	79 ± 3
Amino acid ADC								
Arginine	81 ± 11a	83 ± 18a	86 ± 1a	93 ± 9a	82 ± 15a	68 ± 14a	0.190	80 ± 3
Histidine	83 ± 8a	80 ± 13a	85 ± 6a	90 ± 8a	83 ± 11a	71 ± 7a	0.167	82 ± 3
Isoleucine	68 ± 9a	66 ± 13a	68 ± 9a	78 ± 6a	69 ± 16a	53 ± 9a	0.086	86 ± 2
Leucine	79 ± 9a	79 ± 11a	78 ± 6a	86 ± 3a	78 ± 14a	69 ± 8a	0.279	86 ± 3
Lysine	63 ± 20a	69 ± 23a	70 ± 7a	81 ± 6a	66 ± 30a	47 ± 17a	0.302	85 ± 2
Methionine	86 ± 16a	86 ± 23a	93 ± 11a	99 ± 11a	79 ± 25a	73 ± 16a	0.400	75 ± 4
Phenylalanine	84 ± 7a	86 ± 13a	85 ± 4a	90 ± 8a	83 ± 12a	74 ± 7a	0.251	82 ± 3
Threonine	46 ± 9a	41 ± 22a	45 ± 8a	48 ± 15a	48 ± 20a	20 ± 20a	0.196	81 ± 3
Valine	62 ± 8ab	59 ± 15ab	63 ± 7ab	72 ± 9b	62 ± 14ab	44 ± 10a	0.055	84 ± 3
$\sum$EAA	72 ± 10a	72 ± 15a	74 ± 6a	81 ± 7a	72 ± 16a	58 ± 11a	0.196	83 ± 3
Alanine	53 ± 13a	47 ± 30a	54 ± 6a	59 ± 22a	56 ± 21a	16 ± 27a	0.096	76 ± 3
Aspartic acid	52 ± 12a	48 ± 27a	53 ± 10a	50 ± 25a	53 ± 25a	33 ± 11a	0.899	80 ± 2
Cysteine	85 ± 2a	81 ± 10a	82 ± 4a	87 ± 8a	83 ± 10a	79 ± 6a	0.690	78 ± 3
Glutamic acid	99 ± 3a	97 ± 6a	98 ± 2a	100 ± 4a	95 ± 5a	93 ± 4a	0.325	86 ± 2
Glycine	82 ± 14a	64 ± 43a	85 ± 9a	89 ± 39a	77 ± 22a	42 ± 37a	0.392	62 ± 3
Proline	100 ± 3a	98 ± 10a	100 ± 1a	102 ± 7a	94 ± 6a	93 ± 7a	0.335	76 ± 3
Serine	73 ± 6a	70 ± 15a	71 ± 3a	73 ± 8a	71 ± 12a	60 ± 12a	0.480	80 ± 3
$\sum$TAA	83 ± 7a	81 ± 14a	84 ± 3a	89 ± 9a	81 ± 12a	71 ± 10a	0.247	81 ± 3

HWG, hard red winter wheat; DWG, durum wheat; RWG, rayon wheat; HCF, hard red winter clear flour; MCF, mixed wheat clear flour; S, semolina; $\sum$EAA, sum of analyzed essential amino acids; $\sum$TAA, total analyzed amino acids; letters in same row indicate homogeneous subsets as define by the Tukey´s multiple means comparison test (α = 0.05).

(Fig. 1), while these patterns appeared more unified when considering ADCs adjusted for nutrients leaching in sea water, with a confirmation of the very low Thr digestibility in all wheat products (Fig. 1). Additionally, a high correlation between crude protein digestibility and $\sum$TAA digestibility coefficient for whole grains and S meals was found ($r^2 = 0.99$, Fig. 2); when clear flours data were included this correlation coefficient was reduced ($r^2 = 0.66$, Fig. 2) due to their higher crude protein digestibility in regard of TAA digestibility.

4. Discussion

4.1. Chemical composition of the test ingredients

Chemical compositions in wheat products evaluated in the present study were close to those previously reported (Carré et al., 2005; Hess et al., 2006). Amino acids contents of the grain meals or processed products, expressed as % of crude protein, were compared to the amino acid profiles of corresponding feedstuffs (wheat grain CP 13%, n = 157; or wheat flour CP13%, n = 50) as reported in Aminodat 3.0 by Evonik-Degussa (Hess et al., 2006); analyzed amino acid contents fitted into the 95% confidence interval calculated from the Aminodat database, with relative differences to the mean Aminodat value of less than 8% for the test grains, and generally less than 8% for the test flours and semolina, except for Arg, Lys and Asp of HCF (−13, −11, −12% respectively) and for Gly of S meal (−11%). Note that with the low values for these amino acids, HCF and Semolina are still inside the flours confidence intervals but would get out of the grains confidence intervals, which shows the precision of such validation of the test ingredients.

Crude protein contents as reported in Table 1 (N × 6.25) were higher than the sum of AAs, for various reasons. First, some AAs were not reported in present study because of small sample size for shrimp feces, which impeded proper analyses for tryptophan, tyrosine, taurine etc.; therefore the sum of total analyzed AAs reported in Table 1 underestimates the sum of total AAs. Secondly, the standard conversion factor (6.25) is too high for wheat products, since correct value is in the

Table 5
Difference between individual amino acid digestibility and crude protein digestibility of the same ingredient (percentage units) (differences between means of four determinations).

	HWG	DWG	RWG	HCF	MCF	S	Average
Arginine	0.82	3.10	2.21	−0.68	−8.46	−3.68	−1.12
Histidine	1.56	0.72	1.93	−1.64	−4.38	1.66	−0.02
Isoleucine	1.65	1.74	−1.81	−3.70	−8.50	−2.88	−2.25
Leucine	−1.33	−0.86	−3.54	−6.34	−8.18	−0.30	−3.42
Lysine	5.56	10.58	7.15	9.47	−4.63	1.45	4.93
Methionine	11.24	10.79	11.35	5.91	−4.49	3.94	6.46
Phenylalanine	3.37	6.34	4.82	−1.99	−1.31	5.96	2.86
Threonine	−8.41	−7.97	−8.81	−13.27	−15.38	−15.01	−11.47
Valine	0.39	−0.51	−3.43	−5.28	−9.99	−4.63	−3.91
$\sum$EAA	0.82	1.93	0.05	−3.24	−7.51	−1.47	−1.57
Alanine	3.60	5.53	6.01	5.19	−10.44	−8.92	0.16
Aspartic acid	1.08	2.49	1.94	−2.22	−12.56	−12.20	−3.58
Cysteine	−14.80	−16.93	−16.99	−22.41	−16.51	−10.91	−16.42
Glutamic acid	1.76	2.16	0.22	−7.43	−2.03	7.17	0.31
Glycine	6.49	10.75	17.18	8.88	−11.58	−9.50	3.70
Proline	3.01	3.90	4.34	−6.91	−2.75	4.93	1.09
Serine	−4.50	−5.08	−3.94	−12.76	−9.51	−2.97	−6.46
$\sum$TAA	1.14	2.01	1.11	−5.23	−5.89	1.00	−0.98

HWG, hard red winter wheat; DWG, durum wheat; RWG, rayon wheat; HCF, hard red winter clear flour; MCF, mixed wheat clear flour; S, semolina; $\sum$EAA, sum of analyzed essential amino acids; $\sum$TAA, total analyzed amino acids.

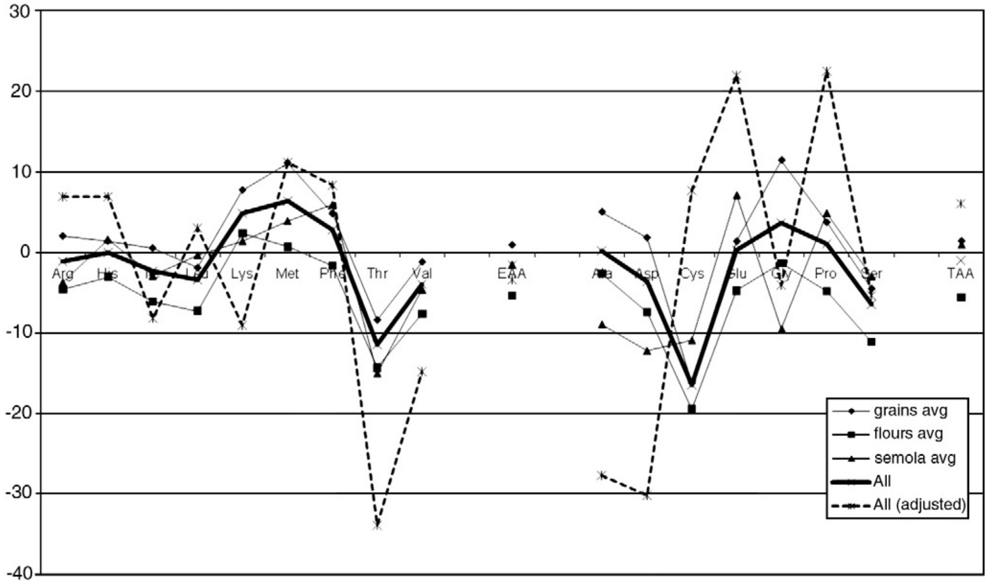

Fig. 1. Mean differences between amino acids and crude protein apparent digestibility coefficients. grains avg., average difference for grains; flour avg., average difference for flours; All, average difference for all wheat products; All (adjusted) average difference for all wheat products, adjusted for nutrient losses by leaching.

range of 5.4–5.6 for wheat flours and 5.5–5.7 for whole grains (Mariotti et al., 2008); for that reason the crude protein contents reported in Table 1 for the wheat products are overestimated. In the case of the compound reference ingredient, standard conversion factor is also too high since correct value for fish or soybean meals, the main protein sources, are in the range of 5.4–5.7 instead of 6.25 (Mariotti et al., 2008); in addition, a small portion of the nitrogen contained in fish meal is from non protein compounds (volatile nitrogenous compounds, biogenic amines…); as a result crude protein content in the reference ingredient was also overestimated. Although it may have been wiser to choose a conversion factor around 5.5–5.6, we have maintained 6.25 as the unique conversion factor for crude protein content estimation in ingredients, diets and feces because it appears both in numerator and denominator inside the digestibility coefficients expressions, and therefore gets eliminated from the ADC calculation, leaving the crude protein digestibility at same value as Nitrogen digestibility.

4.2. Stability in water and nutrient digestibility of the test diets

Diet stability can be affected by binder source, particle size and ingredient composition (Obaldo and Masuda, 2006). Shiau et al.(1991) found small but significant differences in stability between *Penaeus monodon* diets supplemented with different types of wheat flours: diets supplemented with corn starch + carboxylmethyl cellulose and straight flour showed better diet stability than diets supplemented with first grade clear flour and second grade clear flour (6.23–8.97 vs. 9.15–9.50% after one hour immersion in seawater). In present study, although loss of dry matter seems similar among test diets (from 8.2 to 9.5%, Table 2), these small variations correspond to significant variations of the loss of protein ($P<0.001$); diets supplemented with whole grain meals showed higher loss of protein (31–34%) than those supplemented with clear flours and S (26–30%) and this was probably due to the adverse effect of the grain envelopes residues on the pellet structure. Functional and rheological properties of wheat flour are influenced by many factors such as water insoluble pentosans (Van Der Borght et al., 2005), starch damage (Wang and Flores, 2000), non starch polysaccharide content (Campaña-Torres et al., 2005), particle size (Reigh et al., 1990), wheat type and milling process (Wang and Flores, 2000). In the present work, a high positive correlation was observed between gluten index and water absorption capacity, or loss of dry matter ($r^2=0.99$ and 0.80 respectively), while loss of protein did not correlate well with gluten index ($r^2=0.01$). Wang and Flores (2000) concluded that water absorption capacity by wheat meals is affected by particle size, changes in starch quality and changes in molecular weight of protein fractions.

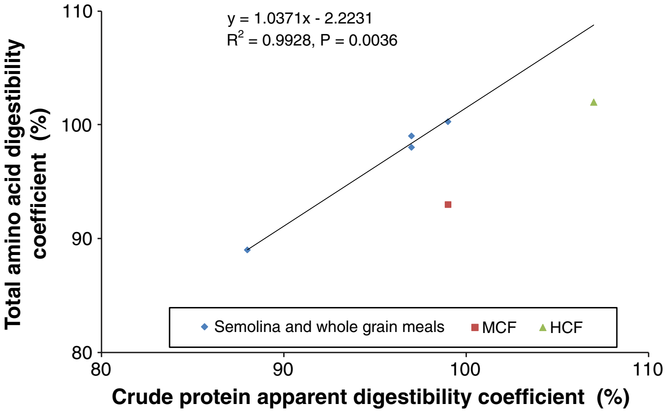

Fig. 2. Relationship between ADCs of crude protein versus the sum of amino acids in semolina and whole grain wheat meals. MCF, mixed wheat clear flour; HCF, hard red winter clear flour.

No significant difference for nutrient digestibility between whole grain meals and clear wheat flours had been reported until present study: Shiau et al. (1991) did not find significant differences in the digestibility of diets supplemented with straight vs. clear flours of wheat; ADCs for dry matter, protein, lipid and carbohydrates ranged from 66.1 to 67.9%, from 79.6 to 80.2%, from 82.5 to 84.9% and 77.4 to 83.5% respectively. Protein and dry matter ADCs observed in present experiment were higher than those reported by these authors; differences could be attributed to the different shrimp species used, or the different reference ingredient.

4.3. Digestibility of the wheat products

4.3.1. Protein, energy and dry matter apparent digestibility coefficients

High protein digestibility coefficients found in the present work agree with values previously reported for red claw crayfish *Cherax quadricarinatus* (Campaña-Torres et al., 2005), red swamp crayfish *Procambarus clarkii* (Reigh et al., 1990) and *L. vannamei* (Davis et al., 2002). High dry matter digestibility coefficients found in the present work contrast to those reported previously for white shrimp: Davis and Arnold (1993) found low dry matter digestibility for whole soft white wheat in *L. vannamei* (≈77%), and Brunson et al. (1997) reported in Atlantic white shrimp *Penaeus setiferus* low dry matter digestibility for wheat middlings, wheat flour and wheat shorts (≈44 to 61%). Low energy digestibility coefficients observed for whole grain meals in present study were similar to that reported by Davis and Arnold (1993) for whole wheat (85%) in *L. vannamei*, but higher than that reported by Brunson et al. (1997) for wheat middlings, wheat flour and wheat shorts (from 47.9 to 66%). Most studies in aquatic organisms have been focused on evaluating the digestibility of nutrients in cereals considering the chemical composition of test ingredients, native versus gelatinized starch and extrusion process (Hansen and Storebakken, 2007) while only few studies have been focused on further aspects: for example Gaylord et al. (2009) observed that digestibility of protein and energy was lower in waxy wheat (53 and 32% respectively) than in soft white wheat (112 and 46%). In terrestrial animals, wheat hardness-related characteristics (Carré et al., 2005), wheat varieties (Carré et al., 2005; Péron et al., 2006), animal genotype (Péron et al., 2006), wheat cultivar (Péron et al., 2006) and particle size (Péron et al., 2005) have been shown to affect energy and lipids digestibility coefficients. In shrimp, no significant variation of nutrients digestibility was observed among wheat varieties; in contrast, a significant reduction of energy digestibility was observed for whole grain meals with respect to clear flours, which could be related to the higher content of fiber and non starch polysaccharides in whole meals.

4.3.2. Amino acid apparent digestibility coefficients

Excellent amino acid digestibility coefficients have been reported for some wheat products: Wilson et al. (1981) reported for catfish an average amino acid digestibility coefficient of 84% for wheat middlings; Akiyama et al. (1989), Yang et al. (2009) and Terrazas et al. (2010) reported for white shrimp average amino acid digestibility coefficients in the range of 85.2 to 102.2% for wheat gluten; Terrazas et al. (2010) reported for white shrimp an average essential amino acids digestibility coefficient of 94.9% for wheat meal. In the present study, amino acid digestibility coefficients were high (from 89 to 102% for total amino acids, Table 3), showing significant differences among test ingredients ($P<0.05$) only for Arg: HCF, RWG and DWG obtained the highest digestibility coefficient for this amino acid (from 100 to 107%) whereas S product presented the lowest digestibility (84%). Apparent digestibility coefficients higher than 100% in shrimp have been previously reported (Carré et al., 2005) and are due to the difference between digestible nutrient contents in test diet and reference diet exceeding the total content of this nutrient in the test ingredient portion of the test diet. It therefore may be due to an overestimation of the content of digestible nutrient in the test diet and/or an underestimation of the content of digestible nutrient in the reference diet. Overestimation of the digestibility in test diet is particularly plausible in shrimp due to their feeding habits: they are slow eaters and they rubble on the pellet like a rodent with their external buccal appendices, provoking high losses of dietary nutrients in seawater before feed ingestion, and an overestimation of diets digestibility, since the lost nutrients are accounted as ingested and retained by the shrimp. If these preprandial losses are higher for the test diets than for the reference diet, the test diet digestibility will be overestimated in comparison to the reference diet digestibility, and the ingredients digestibility, calculated by differences, will be overestimated as well. However, as mentioned earlier, the adjustment of digestibility coefficients for the preprandial losses in seawater led to diminished values, in the range of 16–102% (Table 4), the high bound being still over 100%, which shows that losses in seawater explained only partially the high ADC values found in present study. This situation can be further explained by interactions between ingredients, or variations of fecal endogenous material between reference and test diets (Akiyama et al., 1989; Brunson et al., 1997; Cruz-Suárez et al., 2009): if some factor, responsible for low digestibility of the reference diet or high endogeneous losses, is neutralized, even partially, in presence of the test ingredient, the benefit will be accredited to the sole ingredient digestibility. Finally, the contribution of protein from wheat products to the total of protein in the test diets was low (about 4.1 to 4.5%); small errors in the digestible protein/amino acid determinations for the reference and test diets may therefore appear as important changes with respect to the small wheat protein/amino acids contribution, and lead to digestibility coefficients over 100%.

Another reason to obtain overestimated ADC could be the leaching of nutrients from feces, since nutrients lost from feces will be considered as assimilated. However, no significant losses of protein (5%), carbohydrates (4%) and chromic oxide (4%) have been reported for feces of *L. stylirostris* collected after 15 min, 60 min and 360 min immersion in seawater (Fenucci et al., 1982). In contrast, Smith and Tabrett (2004) observed a significant reduction for protein (5–27%), chromic oxide (9–31%) and ytterbium (4–33) in feces of *P. monodon* collected after 120 to 360 min. These authors concluded that digestibility coefficients maybe overestimated when feces are collected after 120 min. In a study complementary to the present one (unpublished data), feces from shrimp fed the reference diet were collected after 15 and 60 min, and the results confirmed the remarkable stability of shrimp feces in seawater, with no significant differences for protein (cv ≈ 1%), dry matter (cv ≈ 2%) nor amino acids (cv ≈ 1%) contents; therefore we assumed that the impact of nutrient leaching in feces on nutrient digestibility coefficients was minimum.

4.3.3. Individual amino acid apparent digestibility coefficients pattern

AA digestibility patterns as shown in Table 5 and Fig. 1 are useful to estimate the individual AA digestibility of specific ingredients, once their crude protein ($N\times6.25$) or nitrogen digestibility has been evaluated or estimated, which could be made eventually by rapid in vitro techniques or even by NIR spectroscopy. Low *in vivo* digestibility coefficients have been reported in *L. vannamei* for some amino acids by Yang et al. (2009) in wheat gluten (47% for Cys, 77% for Asp) and corn gluten meal (70% for Thr, 34% for Cys), and by Terrazas et al. (2010) in wheat meal (82.4 for Lys). Low digestibility coefficients for some individual AA have also been reported for other aquatic organisms: Wilson et al. (1981) found low digestibility for Met (77%) and Thr (78%) in wheat middlings fed to channel catfish; Aslaksen et al. (2007) observed excellent amino acid digestibility (83 to 88% average) in Atlantic salmon fed diets supplemented with legumes, oilseeds or cereals where Cys and Asp were the less digestible amino acid among diets (<80%). Akiyama et al. (1989) observed lower amino acid digestibility for proline (88.5%) and alanine (86.3%) in several plant-derived ingredients (wheat gluten, soy protein meal, soybean meal and rice bran). Cruz-Suárez et al. (2009) reported excellent total amino acid digestibility coefficients for soy products (92 to 98%) in white shrimp but Cys was less digestible than the rest of amino acids (85–94 vs. 92–102%). In the present study, we found a common pattern of AA digestibility (Fig. 1), which suffered important modifications once adjusted for dietary pre-prandial losses in seawater, a common feature of both standard and adjusted pattern being the low digestibility for Thr, in agreement with some of the above cited studies on vegetable proteins digestibility.

4.3.4. Crude protein vs. total amino acids apparent digestibility coefficients

High correlation between $\sum$TAA digestibility and crude protein digestibility has been reported for some vegetal ingredients. Cruz-Suárez et al. (2009) found a high correlation between $\sum$TAA digestibility and crude protein digestibility (r^2 0.99) in soy products. Yin et al. (2000) observed that crude protein had 5–6% higher digestibility than $\sum$TAA in diets supplemented with wheat flour, wheat middlings and wheat bran, while this difference was of 2% only in diets supplemented with the whole grain meal. Libao-Mercado et al. (2006) found important differences between crude protein digestibility and average amino acid digestibility when wheat shorts were included at 45%. Results of present study confirm that regression of crude protein or nitrogen digestibility to total amino acids digestibility is different for differently processed wheat products (Fig. 2), refined products like clear flours showing a lower ratio AA ADC/N ADC than whole grain meals and semolina.

4.4. Conclusions

Wheat clear flours samples had higher energy digestibility, semolina had lower nitrogen and amino acids digestibility, and clear flour made from hard red winter wheat had higher nitrogen and amino acids digestibility than the other samples tested in present study. However, the six wheat products presented parallel amino acids digestibility patterns; Thr and Cys were the less digestible amino acids when considering standard ADCs, and the low value for Thr ADC was confirmed after adjusting the calculation for preprandial losses of dietary Thr in seawater.

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