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The brine shrimp *Artemia* as a protein source for humans

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Abstract

Artemia nauplii were cultured in raceway systems for 7 or 15 days and were grown on either rice bran powder or whey powder as the sole diet for the entire culture period. Following each culture period, *Artemia* were rinsed with deionized water, drained, and freeze-dried. A commercially obtained adult population of San Francisco Bay (SFB) *Artemia* was also studied.

Individual dry-weight analysis (done prior to freeze-drying) and subsequent proximate analysis showed that *Artemia* is a highly efficient feed converter (up to 40 % efficiency) and contained as much as six times more protein than its culture feed. The essential/total (E/T) amino acid ratio and chemical score exceeded recommended values for infants, children, and adults. Chemical scores for SFB *Artemia* and the rice bran-fed 15-day-old group were above a value of 74 (soy bean) and therefore of high nutritional value. The SFB group was also tested against casein in a protein efficiency ratio (PER) study and found to be slightly superior in quality.

The measures of protein quality used in this study all show *Artemia* to be of high nutritive value. Previous work has indicated that man has already used *Artemia* as food and that modern taste-panel tests on *Artemia* gave quite favorable results.

Therefore, high protein content and quality, ease of production, and good acceptability by consumers makes *Artemia* very attractive as a potential protein source for man as well as the well studied aquacultural species.

Introduction

Geographical collections of *Artemia* have been made from many salines around the world. While there is variation in size, reproductive mode, and biochemical composition, etc., the protein content is consistently very high at 42-60 % of the animals dry weight (Benijts *et al.*, 1975 ; Sorgeloos *et al.*, 1980 ; Tobias *et al.*, 1980).

The relative ease with which *Artemia* can be cultured, along with its high content of animal protein suggest that it might provide a potential source of high quality protein for humans. According to Sorgeloos (1983), millions of hectares of non-arable land exist in the tropical belt, much of which, if properly managed, would be favorable to *Artemia* production. *Artemia* farming in these areas could potentially improve the quality of local diets and also serve as the basis for other industries (*i.e.* solar salt production, fish hatcheries, etc.) to develop, thus aiding local eonomies as well. *Artemia* has been cultured in the laboratory on waste products such as whey powder and micronized rice bran (Dobbeleir *et al.*, 1980 ; Sorgeloos *et al.*, 1980). It was the intent of this research to investigate the value of *Artemia* raised on these materials as a potential human food supplement.

Materials and methods

The *Artemia* used in this study were hatched from Brazilian cysts (Macau) obtained commercially from Aquarium Products (lot no. 10, 1980). The cysts were hatched at 22 °C in 4 l separatory funnels filled with 0.45 µm-filtered, UV-treated seawater from Narrangansett Bay, Rhode Island (30 ‰ S). Approximately 10 cc (4.26 g) cysts/l were aerated for exactly 48 h. Aeration was then discontinued and the hatched nauplii separated from unhatched cysts and other particulates on the basis of their positive phototactic behavior (Persoone and Sorgeloos, 1972).

Culturing of *Artemia*

Preliminary experiments demonstrated that rice bran powder, given at an initial concentration of 0.005 mg/*Artemia* and increased by 0.01 mg/*Artemia*/day, yielded good growth and survival of *Artemia*, provided that 1/3 of the culture medium was replaced with fresh seawater every second day. Whey powder could be given at only 0.025 mg/*Artemia*/day with an increase of just 0.005 mg/*Artemia*/day. The culture medium also had to be changed daily, in order to obtain good growth and survival over the 15-day culture period.

Under these feeding conditions, 48-h nauplii were batch-cultured in 430 l air-water-lift (AWL) operated raceway systems (Bossuyt and Sorgeloos, 1980). In the two raceway systems used, *Artemia* were raised at a population density of one animal/ml of medium under diffuse light. Water temperature (28 °C) was maintained by thermostatically controlled heaters and feed was given twice daily by weighing the material into 300 ml plastic screw capped jars, mixing with some medium and then pouring the contents into the tank.

Starting with 48-h nauplii, *Artemia* were cultured until either the 7th or 15th day, at which time the tank contents were filtered through a 250 µm sieve. The *Artemia* collected on the sieve were then washed in deionized water and kept frozen (-20 °C) under N_2 gas until the time of analysis.

When all culturing was complete, a small sample from each group was taken from the freezer and allowed to thaw. A known number of *Artemia* was then placed onto pre-weighed aluminum pans and put in a drying oven at 60 °C for 24 h. Each pan was weighed to the nearest 1 µg and average individual dry weight (IDW) were calculated.

Calculations

The average individual dry weight (IDW) was used as the basis for extrapolating other growth data in the table. By multiplying the IDW for each group times the population of nauplii inoculated into the AWL-raceways (430 000), the "total theoretical yield" (assuming 100 % survival of the *Artemia* in culture) was determined. By subtracting the total yield of 48-h nauplii from that of each cultured group, an estimate of *Artemia* production or "biomass produced" was obtained. This value was then multiplied by the corresponding percent protein for that group in order to derive the "total protein produced".

Feed conversion efficiency (FCE) was calculated by dividing the biomass produced by the total feed consumed, then multiplying by 100 (Reeve, 1963). Similarly, the protein conversion efficiency was taken as the total protein produced, divided by the total available feed protein, times 100.

ANALYSES OF *ARTEMIA* AND DIETS

All frozen *Artemia* were lyophilized in a Virtis Unitrap 11 freeze-drier for 96 h prior to analysis. For comparison, a commercial sample of adult *Artemia* harvested from San Francisco Bay salterns (SFB) was also lyophilized and analyzed. These were later used to determine the protein efficiency ratio (PER). Whey powder and rice bran powder which were also analyzed, were not freeze-dried as they were already in dry form.

Moisture analysis was performed by placing a known amount of sample into dry, pre-weighed Alundum crucibles, lined with Whatman no. 2 filter paper. The samples were then put in a drying oven at 60 °C for 24 h, cooled to room temperature in a desiccator and reweighed. They were then placed in pre-weighed extraction flasks containing 25 ml ethyl ether and connected to a Baily-Walker lipid extractor. Extraction was continued for 16 h, at which time the Alundum crucibles were replaced in the flasks by glass crucibles and set aside to be used in determining the ash content of the samples. Further distillation allowed recovery of the ether. After evaporating any residual solvent, the crude lipid was fried for 1/2 h, cooled in a desiccator, and weighed.

Fatty acid content was determined on separate samples by extracting the lipid materials using the Bligh and Dyer (1959) technique, as modified by Kates (1972), and methylating the resulting fatty acids with 13 % Boron Trifluoride-Methanol (w/v) (Morrison and Smith, 1964).

Fatty acid methyl esters (FAME) were then injected into a single column Varian Aerograph 1200 gas-liquid chromatography unit operated isothermally at 195 °C and equipped with a flame ionization detector. The temperatures of the injector and the detector were 262 °C and 272 °C, respectively.

Identification of the FAME was made on a 15 % diethylene glycol succinate polyester (DGSP) column, 2.1 m long × 3.2 mm O.D., on a 100-120 mesh Gas Chromosorb W-HP support with a 37.5 ml/min flow of nitrogen as the carrier gas. Identification and quantification of the FAME were made with a Hewlett-Packard 3380 A electronic integrator, programmed with relative retention times of authentic standards. Results are represented as FAME weight percent of the total lipid.

Ashing was determined quantitatively by removing filter paper and samples from the Alundum crucibles used in the moisture and crude lipid determinations, placing them in pre-weighed porcelain ashing dishes in a muffle furnace at 550 °C for 5 h. The samples were then allowed to cool overnight in a desiccator and reweighed.

Protein content was determined by a modified microkjeldahl method of Hiller *et al.* (1948) using an Orion 901 Ionalyzer equipped with an ammonia electrode to detect dissolved NH_3 in the samples. The values obtained (in ppm) were converted to % nitrogen and then to % protein, using the general calculation factor 6.25 for all samples analyzed. Carbohydrate content was then calculated by % difference.

Amino acid analysis was performed by obtaining enough of each material to yield about 10-12 mg of protein which was then acid hydrolized, using the technique reported by Seidel *et al.* (1980). The exchange column used in the present study was packed with Dionex DC1-A (8 % cross-linked) resin.

Since tryptophan is destroyed by this procedure, another method of hydrolysis had to be employed. An alkaline hydrolytic technique which appears to be applicable to intact materials such as those tested here has been developed (Hugli and Moore, 1972), using 4.2 N NaOH. A

modification of this procedure involves the *in vacuo* hydrolysis of about 10-12 mg of protein in 0.3 ml of 4.2 N NaOH and 0.5 ml of pH 4.25 of sodium citrate buffer (the latter is used in place of hydrolyzed starch in order to prevent gel formation in the hydrolysis mixture) at 110 °C for 98 h. The hydrolysis was carried out in the same heavy-walled tubes used in the acid procedure with the exception that Nalgene polypropylene centrifuge tubes (10.9 × 77 mm) were used to line the glass in order to prevent silicate formation during hydrolysis (Oelschlegel *et al.*, 1970).

Following cooling to room temperature, the alkaline hydrolysates were brought to volume with pH 4.25 buffer in 5 ml volumetric flasks containing 420 µl of 6 N HCl which were chilled in an ice bath. The solutions were then run through a Millipore filter (0.45 µm) and analyzed immediately on the amino acid analyzer. Tryptophan peaks were hand integrated and compared to the corresponding peak of the authentic standard amino acid mixture, mentioned earlier in the acid hydrolysis procedure, to which 50 nmoles of tryptophan had been added. The absolute values for tryptophan were then calculated from this comparison. Quantitative amounts of each amino acid were determined by combining the absolute values for tryptophan with those of all other amino acids obtained from the acid hydrolysate of each corresponding sample and are expressed as g of amino acid/100 g of protein. A known amount of tryptophan (Nutritional Biochemicals Corp., Cleveland, Ohio) was also hydrolyzed and analyzed. The amount detected by the amino acid analyzer was then used to obtain a value for the efficiency of the technique which was found to be slightly above 91 %.

A protein efficiency ration (PER) test was performed with 21-day-old male weaning rats obtained from the Charles River Breeding Laboratories Inc. (Wilmington, Massachusetts, USA). The control group was fed a diet containing 10 % protein supplied by casein, while the test group was fed a similar diet except that protein was supplied by *Artemia* (SFB).

Results and discussion

All production data, including biomass production, feed conversion efficiencies, and protein conversion efficiencies, are given in Table I. For whey-fed 7- and 15-day-olds (W-7, W-15) and rice bran-fed 7- and 15-day-olds (RB-7, RB-15), feed conversion efficiency fell within the range of previous studies (Mason, 1963 ; Reeve, 1963).

These results show some very interesting differences with regard to the effect of feed used and the age of the cultured *Artemia* on growth parameters. For example, the IDW and FCE values point out an apparent discrepancy in that whey power appears to be a superior food to rice bran for younger *Artemia*, however, the opposite seems true for older animals. One possible explanation for this lies in the relationship between energy requirements and food availability. Food levels established during the preliminary feeding experiments were based on the maximum amount of either feed which could be cleared from the culture medium in 24 h, as determined by the lack of turbidity. This was then expanded to determine the daily increase which would promote growth without producing some toxic effect during the 15-day period. However, no consideration was given to possible changes in optimum feed concentration as the *Artemia* advanced from one developmental stage to the next. It has been shown that growth increases sharply after the first few days of life as the *Artemia* progress toward adulthood (Mason, 1963 ; Reeve, 1963 ; Johnson, 1980). Therefore, while food levels appear to be adequate for the W-7 group, increases in the feed concentration may not have kept pace with the additional metabolic

TABLE I

Production data for *Artemia* raised on rice bran powder (RB) and whey powder (W) for 7 (W-7, RB-7) or 15 (W-15, RB-15) days and initial data for unfed nauplii (48 h old)

Artemia	Individual dry weight[1]	Total yield[2]	Biomass produced[3]	Feed consumed[4]	Total available protein[5]	Total protein produced[6]	Feed conversion efficiency[7]	Protein conversion efficiency[8]
W-7	0.0417	17.9	17.24	75.2	7.70	10.47	22.9	136.0
W-15	0.148	63.6	62.95	307.4	31.45	38.64	20.5	123.0
RB-7	0.0352	15.1	14.44	150.5	20.60	8.83	9.6	42.9
RB-15	0.512	220.1	219.47	541.8	74.17	110.02	40.5	148.3
48 h	0.0016	0.688	–	–	–	–	–	–

[1] Individual dry weight (g) = $\frac{\text{total weight of } \textit{Artemia} \text{ in Sturdier pan}}{\text{number of } \textit{Artemia} \text{ in pan}}$

[2] Total yield (g) = individual dry wt (in mg of cultured *Artemia*) × 430

[3] Biomass produced (g) = total yield (cultured *Artemia*) - total yield (nauplii)

[4] Feed consumed (g) = total feed consumed in 7- or 15-day period

[5] Total available protein (g) = % protein in feed × feed consumed

[6] Total protein produced (g) = % protein (cultured *Artemia*) × biomass produced

[7] Feed conversion efficiency (%) = $\frac{\text{biomass produced}}{\text{feed consumed}} \times 100$

[8] Protein conversion efficiency (%) = $\frac{\text{total protein produced}}{\text{total available protein}} \times 100$

needs of rapid growth and maintenance later on and became limiting. Conversely, the higher initial levels of rice bran powder (and daily increases) may have been near the saturation level of young larvae. This might cause food particles to move too rapidly through the gut for adequate digestion and absorption to occur and might therefore depress initial growth rates and the FCE. However, as the *Artemia* began to grow more rapidly and feed more effectively, the higher concentration of rice bran powder was probably beneficial in promoting growth and higher feed conversion. Proper feed concentration is a very important consideration because this ensures a sufficient food level, while avoiding excess. Another important food-related consideration is that filter-feeding *Artemia* are not able to effectively ingest particles which settle out of suspension, therefore, adequate circulation of the culture medium is also imperative.

There are several other possible contributors to the peculiar growth pattern shown in Table I. For example, when *Artemia* embryos hatch, the resulting nauplii do not have an effective filter-feeding capability and must depend on their endogenous yolk supply for nourishment. The higher solubility of whey powder in seawater might, in this case, be useful in providing nutrition to the young nauplius (Dobbeleir *et al.*, 1980). Therefore, if ingestion of solubles such as whey powder is more effective than ingestion of particles such as rice bran powder during early development, it follows that whey-raised *Artemia* might grow more quickly in the early stages. This difference in solubility seems to have the opposite effect in later stages when *Artemia*'s feeding mechanism becomes more effective at removing suspended particles from the culture medium. Furthermore, Dobbeleir *et al.* (1980) stated that "except for the first few larval stages, soluble products cannot be efficiently ingested and as such do not support growth in *Artemia*." As the *Artemia* develops then, whey powder probably declines in food value due to its higher solubility, so this could also contribute to the apparent shift in superiority of the two feeds for young and older *Artemia*.

The higher solubility of whey powder is also responsible for supporting microbial growth in the culture medium. The blooms which occurred apparently had a bearing on survival of *Artemia* (with W-15, this was sometimes a problem) and also seemed to be a likely cause for over-estimation of protein conversion and feed conversion efficiency. No attempt was made to sterilize either feed, nor was any antimicrobial agent added to the culture media. There were noticeable differences in gross appearance and growth characteristics of the microbial colonies. The one(s) associated with the rice bran cultures appeared after 8 days of culturing and were detectable as one or two red areas on the tank bottom. These spots seemed to enlarge in a roughly geometric fashion and tended to restrict themselves to their original location until day 14 or 15. This growth in no way seemed to adversely affect the *Artemia*.

With the whey cultures, the situation was quite different. The microorganisms which were roughly the same color as whey, began to appear after only about 4 to 5 days of culturing. They also seemed to disperse in the AWL-raceway and even though the culture medium was replaced with fresh, UV-treated seawater on a daily basis, they grew at such a rate that there was substantial foaming of the culture medium by about day 13. At this time the culture medium probably became toxic to *Artemia*, since a strong odor of ammonia began to emanate from the W-15 tanks and it was at this point that most culture failures occurred. The negative effect of excessive ammonia levels may have been further aggravated by culturing *Artemia* at elevated temperature, where metabolic production has been found to increase (Moffet and Fisher, 1978). The amount and type of microorganisms existing in the four cultured groups therefore bore a relationship to growth and survival of the cultured *Artemia* (Dobbeleir *et al.*, 1980).

In addition to these factors, whey powder is lower in protein and especially in lipid than rice bran. Higher levels of these materials in the latter may have had a positive effect on growth of the RB-15 group since it has been reported that in later *Artemia* development, lipids and proteins are the prime energy source for the animal. Thus, feed type probably contributed to differences in growth performance between feed groups (Von Hentig, 1971 in Johnson, 1980).

Table II shows the proximate composition of all *Artemia* groups and the two culture feeds. These data generally do not show the kinds of differences due to feed consumption or developmental stage that was expected. Presumably, this was due to the brevity of the culture period and that W-15 animals were under stress, therefore being of limited comparative value. There are some interesting differences, however, which may be related to the above parameters. For example, the ash content of all groups (excluding nauplii) and the wild San Francisco Bay (SFB) population are quite similar. Since older *Artemia* contain less lipid, it seems that lipid level in *Artemia* is age (possibly stage) dependent. This trend appears to hold true within and between feed groups but may be more exaggerated in the older whey-fed animals due to lower feed concentration (possibly limiting), decreased suitability as an *Artemia* feed, low dietary lipid content, or environmental stress.

TABLE II

Proximate composition of *Artemia* and the monodiets used in culturing (all values are expressed as % of the dried samples ; abbreviations as in Table I ; SFB = San Francisco Bay adults)

Artemia or diet	Ash	Moisture	Lipid	Protein	Carbohydrates
W-7	11.08	2.85	10.92	60.73	14.42
W-15	9.16	4.81	7.45	61.38	17.20
RB-7	10.01	2.63	11.65	61.14	14.57
RB-15	9.93	6.37	9.47	50.13	24.10
48 h	7.17	6.96	19.40	58.70	7.77
SFB	11.16	4.58	3.37	53.25	27.64
Whey	7.68	4.93	1.30	10.23	75.86
Rice bran	10.77	8.30	6.54	13.69	60.70

Dietary factors do not seem to play any direct role in influencing protein or carbohydrate levels in cultured *Artemia*. In fact, the data seem to point out that stage of development is again quite influential with respect to these two components, especially when comparing RB-15 and SFB adults with other *Artemia* groups. The W-7, W-15, and RB-7 are thought to be at different developmental stages (due to differences in their weights), however, the difference may not be large enough to be reflected biochemically. The RB-15 and SFB groups, on the other hand, are obviously different from the others in terms of morphological characteristics and also protein content. The trend seems to be, at least when comparing adults with sub-adults, that there is a noticeable drop in protein with increasing development in *Artemia*.

Carbohydrate levels likewise show that there is little difference among the W-7, W-15, and RB-7 *Artemia*. When these three groups were again compared to RB-15 and SFB, the developmental effect on carbohydrate levels becomes more readily apparent. From the data in Table II, it is clear that rice bran-raised adults (RB-15) resemble the natural population (SFB) in most respects.

Table III shows the fatty acid patterns for *Artemia* and their feeds. SFB adults and 48-h nauplii illustrate the patterns of a wild adult population and that of the experimental groups prior to batch-culturing in the AWL-raceways. There is some agreement with previous results in that *Artemia* fatty acid levels may be influenced by their dietary intake (Claus *et al.*, 1979 ; Dobbeleir *et al.*, 1980). In some instances (*i.e.* 16:1ω7, 18:0), higher levels in either of the feeds are reflected in the observed *Artemia* patterns ; however, this simple association does not always apply. Several fatty acids, for example 14:1, 22:1, 20:5ω3, appear in the cultured animals without being contained in their feeds. Rice bran contains more lipid than whey power and has a slightly higher percentage of fatty acids which are C_{16} or longer. Furthermore, rice bran also contains higher levels of ω6 and ω3 polyunsaturated fatty acids (PUFA). These fatty acids are important for good growth and maintenance in a variety of marine organisms. If this also applies to *Artemia*, dietary lipid content and quality are probably affecting growth characteristics (Table I) by providing (or not providing) sufficient materials to meet growth and maintenance costs in the developing animal.

Both linolenic and linoleic acid, which have human essential fatty acid (EFA) activity, appear in cultured *Artemia*. The latter fatty acid is most important since it can be converted, in humans, to another EFA, arachidonic acid (Krause and Mahan, 1979). Linoleic acid is contained by all *Artemia* in substantial amounts, especially in the rice bran-raised *Artemia*.

TABLE III

Fatty acid patterns of *Artemia* and their feeds
(abbreviations as in Table I and II)

Fatty acid methyl ester	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
14:0	8.21	14.94	–	–	–	–	–	–
14:1	–	–	10.28	3.40	7.56	13.60	9.25	–
15:0	–	3.08	–	–	–	–	–	–
15:1	–	–	–	1.33	0.77	–	–	–
16:0	22.98	32.51	12.94	10.57	17.16	16.33	16.12	22.72
16:1ω7	–	2.95	14.38	6.32	7.66	16.67	14.69	9.42
18:0	4.08	11.20	9.01	5.82	5.34	10.05	8.47	7.02
18:1ω9	33.23	28.66	26.07	47.10	48.21	29.42	44.35	45.05
18:2ω6	29.17	6.23	11.08	23.03	8.90	3.51	3.44	2.14
18:3ω3 / 20:1	2.30	0.45	10.30	–	1.19	1.35	0.89	9.74
18:4ω3	–	–	–	0.75	1.92	1.12	–	3.91
22:1	–	–	9.21	1.22	0.24	3.97	1.21	–
20:5ω3	–	–	7.01	0.44	1.03	3.96	1.58	–

Table IV illustrates the amino acid patterns of all samples and shows that there is only slight disagreement between values for the cultured groups. An attempt to connect this to differences in the patterns of the two feeds proved inconclusive, as expected. W-7 contained a substantial amount of tryptophan, as did W-15 though only a trace amount of this amino acid was present in whey powder. W-15 also contained a small amount of 1/2 cystine, which was absent in whey powder. Since *Artemia* has been previously reported to be a highly efficient protein converter at the qualitative level (Sorgeloos, 1980), the above observations are probably the result of *Artemia* having ability to synthesize these amino acids or obtain them from bacterial synthesis. In

TABLE IV

Amino acid patterns in cultured *Artemia* and their feeds (in g/100 g protein, abbreviations as in Table I and II)

Amino acid	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
ASP	10.26	10.80	9.75	9.07	8.90	9.82	8.68	10.61
THR	4.64	6.77	4.29	4.03	4.01	3.93	3.97	3.57
SER	5.70	5.61	4.73	3.70	4.14	3.89	3.85	5.42
GLU	14.77	18.25	11.42	11.76	14.47	12.79	11.24	12.66
PRO	5.78	6.30	4.14	5.63	2.94	6.29	5.66	5.80
GLY	6.39	2.24	5.26	5.61	5.61	4.26	5.82	3.40
ALA	7.51	5.16	6.14	5.87	6.60	7.60	5.63	8.47
CYS[a]	0.66	–	–	–	–	–	0.72	0.49
VAL	5.90	7.47	5.39	5.18	5.31	5.09	5.08	5.82
MET	2.74	2.16	2.00	1.70	2.60	1.73	1.59	2.32
ISO	3.97	5.23	5.03	4.73	4.25	4.67	4.60	4.45
LEU	8.52	11.61	6.80	6.96	9.34	7.69	6.93	8.17
TYR	3.49	2.54	3.89	3.66	4.63	3.74	3.73	3.87
PHE	5.35	3.74	4.51	4.23	5.65	4.45	4.25	5.01
HIS	2.64	2.31	0.17	2.34	3.69	2.89	2.34	2.62
LYS	3.72	7.91	7.47	6.54	9.43	7.40	6.61	7.85
TRY	1.24	0.86	11.00	4.91	4.42	2.99	2.83	4.47
ARG	8.27	3.39	7.34	6.93	9.13	6.82	7.23	7.83

[a] CYS was actually read as 1/2 CYS.

attempting to correlate amino acid composition with age, values within the two feed groups were compared. In general, the data do not indicate stage-related differences.

The amino acid values were the basis of the calculated essential/total (E/T) amino acid ratio and chemical score based on reference values (FAO/WHO, 1973) (Table V).

E/T ratios included histidine as part of the overall essential amino acid value for each sample tested. This was done to compare all samples with infant requirements which are higher than those of children or adults (43, 36, and 19 respectively) (Krause and Mahan, 1979). The results reported here indicate that all samples meet the E/T requirement for infants with the exception of rice bran powder which does, however, meet the requirement of both children and adults.

TABLE V

Calculated essential/total (E/T) amino acid ratio and chemical score of *Artemia* and their feeds and protein efficiency ratio (PER) of the San Francisco Bay (SFB) group

	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
E/T	40.67	49.32	50.89	47.69	50.73	46.41	46.99	47.30
Chemical score	67.64	61.71	57.14	48.47	74.29	49.43	66.00	80.29
PER	SFB	3.2						
	Casein	3.0						

When determining the chemical score (limiting amino value) of *Artemia* proteins, amino acid requirements of the human infant were not considered. This is because of the conclusion by the joint FAO/WHO committee that these values should be excluded from any guide to protein scoring that might be developed for older children and adults. Nevertheless, a comparison of the various *Artemia* groups was made with the suggested amino acid pattern for infants (values are presented).

The chemical score in all cultured and SFB-*Artemia* is the one for total sulfur-containing amino acids. It is possible that the scores would be increased if the sulfur amino acids were estimated by the method employing performic acid oxidation as a pre-hydrolysis treatment step (Schram *et al.*, 1954, as modified by Moore, 1963) prior to acid hydrolysis, as described earlier. However, that procedure was not used in this study. It was found that in most cases, the identity of the limiting amino acid did not change as a consequence of comparing observed values with the suggested pattern for infants. However, the chemical score does rise because the suggested value for cystine and methionine in infants is lower than in the FAO/WHO reference pattern. The limiting amino acid does change in the SFB group (threonine) because these animals showed the highest value of all for total sulfur-containing amino acids and the lowest value for threonine, which is required in greater amounts by the infant. The protein evaluation techniques such as those mentioned thus far, are valuable in estimating the worth of test materials like *Artemia* for humans ; however, PER goes further in that it gives some indication of a protein's digestibility. Results of the PER conducted with SFB *Artemia* as the test protein show that it is comparable to casein for digestibility and utilization in rats (3.2 and 3.0, respectively). Other estimations of the biological value of this protein, such as net-protein utilization (NPU) and slope-ratio analysis will clarify still further the ability of *Artemia* to serve as a dietary protein source for humans.

Earlier work has shown that various biotic and abiotic parameters may affect the biochemical composition of marine organisms (Conover, 1978). With *Artemia* it has been reported that biochemical characteristics can be influenced by factors such as geographical origin (Schauer *et al.*, 1980 ; Seidel *et al.*, 1980 ; Tobias *et al.*, 1980), food type (Sick, 1976 ; Claus *et al.*, 1979), food concentration (Mason, 1963 ; Reeve, 1963), and environmental characteristics such as temperature, salinity, or oxygen levels (Morris, 1971 ; Benijts *et al.*, 1975 ; Boulton and Huggins, 1977). For these reasons and others (*i.e.* methods of analysis, stage of development, etc.) strict comparison of data generated by separate investigations can be difficult and somewhat speculative. Within the *Artemia* groups cultured for this research, some of the above factors may be interacting to produce the observed results.

In this study *Artemia* were able to convert rice bran powder to *Artemia* biomass with about a 40 % (2.47:1) efficiency. Reported efficiencies for beef cattle are much lower.

These data show that *Artemia* may potentially be very competitive with common food animals in economy of production for human consumption. Production of *Artemia* biomass and cysts is expected to increase as a result of current projections of aquacultural needs.

There is evidence that *Artemia* has been used as food by man in the past. For example, Indians inhabiting the Great Salt Lake area used to collect and dry *Artemia* to be used as food (Jensen, 1918). The Dawada people of Libya have also consumed dried *Artemia* (Ghannudi and Tufail, 1978 in Sorgeloos, 1980) and sold them to obtain necessary items for their tribe (Bovill, 1968). In addition, modern taste panel tests have been conducted on oriental tempura using whole *Artemia* in the preparation and the results were quite favorable (Helfrich, 1973). In view of the data presented by this and other investigations, it seems possible that in the future the brine

shrimp *Artemia* can be produced as a protein source for humans using cheap feeds (low in protein) and in ponds using fertilizers.

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