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Studies on the functional properties and chemical Composition of commercial shrimp feeds used in Andhra Pradesh, India

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Abstract
Two separate studies were carried out to ascertain the functional properties and chemical composition of shrimp feeds. In the first study five different commercial shrimp feeds (Brand-A, B, C, D and E) were collected from Andhra Pradesh-India. In the second study, five different grades (Grade-1.0, 1.2, 1.4, 1.6 and 1.8mm diameter) of shrimp feed from a particular firm were collected. The results of the first study revealed that Brand-C had the lowest water retention (156.33%). There was a significant ($P<0.05$) difference in bulk density among the brands (786.69-838.61 g/L). Swelling capacity has crossed >100% in Brand-A, B and D. Variations of chemical composition are found to be within the acceptable level. In the second study, as the pellet size increases (1.0-1.8mm), water retention (182.87-158.85%) and dry matter leaching (8.58-3.95%) were decreased, and bulk density (755.06-807.75 g/L) was increased. The chemical composition of feed with varying diameters showed significant variations.

Keywords: Functional properties, Gross energy, pellet size, *Penaeus vannamei,* proximate composition, Shrimp feed

1. Introduction

Andhra Pradesh (AP), with a coastline of 974 kilometers (km) is the leading state in brackish water aquaculture in India. Though all the cultured species are under the family of Penaeidae, *Penaeus vannamei* alone contributes more than 90% of the total farmed shrimp production [1]. *P. vannamei* culture in India was started with 264 hectares (ha) area in AP during the year of 2009-2010, while it was only 19 ha in the rest of the country. The Marine Products Export Development Authority (MPEDA) [2] reported that the production of *P. vannamei* increased from 1655 tonnes (t) to 276077 t in the stipulated period of 2009-10 to 2014-15, respectively in AP, whereas, the total production (77336 t in 2014-2015) in the rest of the country together was still comparatively lower. The growth of aquatic species in general, is influenced by numerous factors. In which, the most important one is feed, that accounts 50-70% of the total production cost. Feed being the central input, assumes paramount importance in commercial shrimp aquaculture. Among the feed quality parameters, functional properties have got a significant role in maximizing the utilistaion of shrimp feed. The shrimp feed industry in India has made rapid strides and quality feeds are produced with modern feed processing mills in the country. The researchers made various efforts in the last two decades to improve the nutritional quality of feed. In addition to the bioavailability of nutrients, knowing the physical/functional quality of feed is also vital to enhance the utilization of feed. Syamsu *et al.* [3] stated that physical or functional properties and chemical or biological composition of feed were significantly affected by the mechanical operation of feed processing. There is a wide information regarding the chemical nature and nutritive value of feed in the published reports and most of the shrimp feed manufacturers declare their ingredients and nutrient content in the feed bag. However, there is not much field study about the nutrient composition actually present in the commercial feeds. Though, the functional properties have been addressed during feed processing, the details are unknown to the end user, in particular farmers. Hence, in the present study an attempt was made to analyze the functional properties and chemical composition of the various commercial shrimp feeds used in AP-India to provide a baseline data. This would be helpful to the farmers for rejecting the feeds, those were prepared with undesirable quality before using itself in a short period of time. This would be economically profit to the shrimp farmers.

2. Materials and Methods

In the first study, five different brands of commercial *vannamei* shrimp feeds (1.6 mm) of leading shrimp feed manufacturers (Brand-A, B, C, D and E) were collected from the shrimp farms in the east coast area of AP, India. Three feed samples of each brand at an interval of 15 days were collected for this study. For the second study, five different grades (Grade-1.0, 1.2, 1.4, 1.6 and 1.8 mm diameter) of shrimp feed from a particular brand have been collected three times at 15 days interval and analyzed. As per the manufacturer's declaration all the feeds contain fishmeal, soybean meal and wheat flour as major ingredients along with squid meal, fish oil, vitamin and mineral mixture as an additive. The basic functional properties such as, water retention, bulk density, sinking velocity, swelling capacity, dry matter leaching and water stability were analyzed for all the feeds along with the proximate composition using a method of AOAC [4]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest [5] method. The nitrogen free extract (NFE) was calculated by a difference [1000 - {moisture (g/kg) + crude protein (g/kg) + ether extract (g/kg) + crude fiber (g/kg) + total ash (g/kg)}] and gross energy of the feeds was estimated according to Jobling [6] using the factor 5.65, 9.45 and 4.00 for crude protein, ether extract and carbohydrates (crude fiber and NFE), respectively.

2.1 Water retention

The feed was immersed in water at the ratio of 1:10 and allowed to stand at an ambient temperature (27±2°C), After 20 min of incubation, the excess of water was removed either by centrifugation (3000 rpm) or using a Buchner filtration with Whatman filter paper No. 3 (5µ). The water retention was examined as per cent water retained per 100 g of feed.

$$\text{Water retention (\%)} = W_{WF} / W_{DF} \text{ X } 100$$

Where, W_{WF} is weight of wet feed (g) and W_{DF} is weight of dry feed

2.2 Bulk density

A known weight (50 g) of feed was placed in a 100 ml graduated cylinder and packed by gentle tapping of the cylinder on a bench top, more than ten times, from a height of 8-10 cm or till getting the constant volume. The final volume of the feed was measured and the bulk density was expressed as g/L.

$$\text{Bulk density (g/L)} = W_F / V_F$$

Where, W_F is weight of feed (g) and V_F is the volume occupied by the feed (L)

2.3 Sinking velocity

A transparent glass pipe with a sealed bottom was used to determine the sinking velocity of feed. The tube was filled up with water and the column temperature has to be maintained at a uniform level during the experiment. The number of pellets was poured one by one and the time taken to settle at the bottom was noticed using a stopwatch. If any pellets remained on the water surface without sinking or adhered to the walls of the tube were not considered. The sinking velocity of the feed was reported as cm/Sec.

$$\text{Sinking velocity (cm/Sec)} = A_P / T$$

Where, A_P is area crossed by the pellet (cm) and T is time taken (Sec)

2.4 Swelling capacity

The diameter of 10 dry pellets from each group was measured using a digital Vernier scale (precision ± 0.01 mm) and they were immersed in water at an ambient temperature for 1 h. After incubation, the diameter of wet pellets was measured and the increase was considered as the swelling capacity of the feed:

$$\text{Swelling capacity (\%)} = D_{WP} / D_{DP} \text{ X } 100$$

Where, D_{WP} is diameter of wet pellet (mm) and D_{DP} is diameter of dry pellet (mm)

2.5 Dry matter leaching

A known quantity of feed (25 g) was immersed in 250 ml of water at an ambient temperature (27±2°C) for 1 h. The un-dissolved feed particles were collected using a Buchner filtration apparatus with Whatman filter paper No. 3 (5µ). The recovered materials were dried at 105°C in a hot air oven overnight or till getting a constant weight and then cooled in desiccators before weighing. The dry matter leaching was calculated as the ratio of dry matter recovered after leaching:

$$\text{Dry matter leaching (\%)} = 100 – [(U_P / D_P) \text{ X } 100]$$

Where, U_P is un-dissolved pellets (g DM basis) and D_P is dry pellet (g DM basis)

2.6 Water stability

Approximately 250 g of feed was spread in a plastic tray (30x25x7 cm) with full of water. A small electronic battery motor with four wings pasted at the centre of the tray to disturb the water as in pond condition. Simultaneously a tray was kept without motor for each feed to know the water stability in a static condition. Every half an hour interval (30, 60, 90, 120, 150 and 180 min), the physical characteristics such as disintegration and cracks on pellets were observed visually.

2.7 Statistical analysis

All the data were subjected to one way analysis of variance (ANOVA) and a multiple comparison of treatments was done using Tukey's test to detect significant difference among the feeds. Prior to statistical evaluation, data were checked for homogeneity of variance after ascertaining the normal distribution pattern of data. The entire data were analyzed using SPSS version 16.0, and statistical tests were performed at $P<0.05$.

3. Results and Discussion

Water retention is a unique parameter for shrimp pellet feeds, which gives very clear idea about the feed stability within 20 min. It is indirectly proportional to the stability of feed. In the first study, though all the feeds irrespective of the brands, were stable >3 h in a static condition, water retention was differed significantly (*P*<0.05) among them (Table 1). Of all the analyzed brands, Brand-C had the lowest water retention of 156.33%, followed by Brand-D and E, while the Brand-A and B had a significantly (*P*<0.05) higher water retention (174.04 and 176.75%, respectively). It is pertinent to note here that higher the water retention lower will be the stability.

Table 1: Functional properties of various commercial shrimp feeds used in the present study (n=3)

Particulars	**Functional properties**					
	WR (%)†	**BD (g/L)ǂ**	**SV (cm/Sec)§**	**SC (%)□**	**DML (%)#**	**WS (h)£**
Brand-A	174.04^{a}	838.61^{a}	5.34^{a}	117.19^{ab}	10.07^{a}	>3 h in a static condition and >90% stable in disturbed condition even after 1 h
Brand-B	176.75^{a}	782.69^{d}	4.96^{a}	111.03^{b}	6.99^{a}	
Brand-C	156.33^{c}	809.28^{b}	6.61^{a}	90.40^{c}	8.09^{a}	
Brand-D	163.96^{b}	807.11^{bc}	8.27^{a}	122.35^{a}	6.03^{a}	
Brand-E	168.47^{b}	795.38^{cd}	6.36^{a}	73.79^{d}	5.31^{a}	
SEM (+)	4.698	28.360	1.140	13.347	4.476	
p-value	<0.001	<0.001	0.122	<0.001	0.330	
CV (%)	1.699	0.869	22.273	4.670	38.148	

Means bearing the same superscript in a column do no differ significantly (*P*<0.05)
†Water retention
ǂBulk density
§Sinking velocity
□Swelling capacity
#Dry matter leaching
£Water stability

In general, bulk density indicates the mass of the materials occupied a volume of the container. But it is also used as a measure of functional property in shrimp feed as it is directly associated with sinking velocity or floatability of the feed. Vijayagopal [7] suggested the values of bulk density of <550 g/L, 550-650 g/L and >650 g/L for floating, slow sinking and sinking feeds, respectively. Among the brands analyzed, Brand-A had a higher bulk density (836.61 g/L) followed by Brand-C and D (809.28 and 807.11 g/L, respectively), while the bulk density of both Brand-B and E was significantly (*P*<0.05) reduced to <800 g/L. Though significant (*P*<0.05) differences were noticed between the brands analyzed in the present investigation, all were agreed with the values suggested by Vijayagopal [7].

As shrimps are the bottom feeders, it is necessitates to prepare a sinking feed with good water stability. The sinking velocity is an important parameter in shrimp feed, which measures that how fast feed pellets are settling at the bottom of the pond. The sinking velocity was found high in Brand-D (8.27 cm/Sec) and was in the range of 5.34-6.61 cm/Sec in Brand-A, C and E, whereas the lowest value was noticed with the Brand-B (4.96 cm/Sec). However, sinking velocity did not significantly differ between each other. In general, the nature of the shrimp feed should be soft and should not be disintegrated after immersing in water. The un-swollen feed particles hindered the growth by reducing feed intake due to the hardness. In the selected brands, the swelling capacity has crossed >100% in Brand-A, B and D and was 90.40% in Brand-C and 73.79% in Brand-E. Though various factors are responsible for obtaining low swelling capacity in Brand-C and E, it would be attributed to the excess usage of the binder or exposure of the materials to a high temperature for more time during the processing. However, processing feed at low temperature and insufficient binder quantity lead feed pellets to swell immediately after immersing in water and disintegrate as soon. This also retards the growth parameters by making unavailability of the feed to the cultured species

The major difference between the aquafeed and other animal feed is water stability, which is defined as the retention of pellet integrity with minimal disintegration and nutrient leaching while in the water until consumed by the animal. Shrimp feeds with few hours' water stability without having any leaching prior to ingestion are ideal, since they are continuous and slow feeders. In the present investigation, water stability of the feed is assessed in two different ways *viz*., static condition and disturbed condition as in culture ponds. In a static condition, the water stability was >3 h irrespective of the commercial feed brands collected and approximately 90% of the feeds were intact even after disturbing the water using a small electrical motor for an hour. This indicates that all the commercial brands used in the present study were prepared in a proper way to meet the required specification. Though both water stability and dry matter leaching are interrelated between each other, a slight difference was noticed between them in the present investigation. The analyzed result showed a nonsignificant difference in water stability among the brands, while dry matter leaching (Table 1) showed a variability. The Brand-A had the highest dry matter leaching of 10.07% compared to others (5.31-8.09%). However, no significant difference was observed among brands analyzed in the present investigation. This result is in agreement with a report of Cuzon *et al.* [8] who suggested that the value of dry matter leaching should not be more than 10% in the crustacean feeds.

Table 2: Chemical composition (g/kg as fed basis) of commercial shrimp feeds used in the present study (n=3)

Particulars	Proximate composition								Gross energy (MJ/kg)□
	Moisture	Crude protein	Ether extract	Crude fiber	NDF†	ADFǂ	NFE§	Total ash	
Brand-A	112.30^{ab}	386.89^{b}	53.41^{cd}	50.17^{a}	504.93^{a}	216.06^{a}	276.13^{d}	121.10^{a}	13.95^{c}
Brand-B	111.67^{b}	391.37^{a}	60.33^{b}	35.87^{b}	361.01^{b}	141.37^{b}	294.83^{c}	105.93^{b}	14.36^{b}
Brand-C	116.43^{ab}	386.20^{b}	51.87^{d}	26.87^{d}	270.43^{d}	115.72^{cd}	307.90^{b}	110.73^{b}	14.02^{c}
Brand-D	105.13^{c}	387.90^{ab}	63.07^{a}	31.23^{c}	314.31^{c}	134.49^{bc}	314.90^{b}	97.77^{c}	14.66^{a}
Brand-E	111.47^{b}	367.33^{c}	54.90^{c}	24.60^{d}	247.58^{e}	105.94^{d}	347.27^{a}	94.43^{c}	14.44^{b}
SEM (+)	0.031	0.029	0.007	0.023	6.367	57.966	0.268	0.045	0.001
p-value	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	2.067	0.582	1.880	5.860	0.978	7.021	2.212	2.646	0.327

Means bearing the same superscript in a column do no differ significantly (P<0.05)
†Neutral detergent fiber
ǂAcid detergent fiber
§Nitrogen free extract calculated by a difference
□Estimated according to Jobiling (1983)

According to New [9], having 100-130 g/kg of moisture is a desirable level for the shrimp feed. The values obtained in the present study were within the specified range irrespective of the brands (Table 2). The optimum protein requirement for *P. vannamei* is varied among the available reports and was 360 g/kg or higher [10], 300 g/kg [11], >320 g/kg [12], 340 g/kg [13]. New [9] recommended that the dietary protein level could be 350-420 g/kg for commercial shrimp feeds. The present result revealed that all the commercial feed brands contained 386.20-391.37 g/kg protein except the Brand-E (367.33 g/kg). The recommended ether extract level in the commercial shrimp feeds is 60-75 g/kg and a maximum of 100 g/kg [14]. The level of ether extract among the brands used in the present study ranged from 53.41 to 63.07 g/kg. However, a substantial difference in the level of ether extract of shrimp feed, collected from different countries, was reported earlier and was 57-65 g/kg in Taiwan [15], 39-139 g/kg in Australia [16], 28-100 g/kg in India [17] and 50-110 g/kg in Sri Lanka [18]. De Silva and Davy [19] stated that very low (20 g/kg) and very high (>100 g/kg) ether extract level is not desirable since oxidative rancidity is very common in the feeds stored for a period of 2-3 months, which spoil the feed and reduces its palatability. The mono-gastric animals, in particular shrimp have the limited capability to digest the fibrous components due to the lack of fiber hydrolyzing enzymes [20]. Shrimp feeds with <30 g/kg of crude fiber is desirable [20] and the same level was maintained in all the commercial brands used in the present study except the Brand-A (Table 2) and it would be due to the inclusion of excess of plant protein sources. A similar trend was noticed for the fiber fractions of NDF and ADF. The NFE content was differed significantly (P<0.05) among the feeds and was in the range of 276.13-347.27 g/kg. Jannathulla *et al.* [21] reported 17.3-18.0 megajoule (MJ)/kg of gross energy in the diet of *P. vannamei* and a slight difference was noticed in commercial shrimp feeds (16.59-24.79 MJ/kg) collected from Taiwan [15], Australia [16] and Sri Lanka [18]. However, the gross energy was in the range of 13.95-14.66 MJ/g in the commercial feed brands collected from the east coast of AP, India in the present investigation.

In the second study, five different grades (Grade-1.0, 1.2, 1.4, 1.6 and 1.8 mm diameter) of shrimp feed from a particular firm have been collected and subjected for all the analysis as in the first study. The statistical analysis (Table 3) revealed that the increasing feed pellet size from 1.0 to 1.8 mm gradually (P<0.05) decreased water retention (182.87-158.85%), which indicates a lower diameter feeds retain more water than the feeds prepared with a higher diameter. The reason would be attributed to the process of less compaction taking place in lower size pellets than the higher diameter pellets, resulting in lower bulk density. The result is in agreement with the findings of Misra *et al.* [22] who stated that the low-density materials had a higher water absorption/retention in extruded pellets and this would be due to the poor gelatinisation during the processing of lower diameter feeds [22]. The present results indicate that water retention should be maintained between 160 and 180% in the feeds with 1.2-1.6 mm diameter, (Table 3). The variation in the pellet diameters had a significant (P<0.05) difference in the bulk density and was gradually increased from 755.06 g/L to 807.75 g/L with increasing feed pellet size (1.0-1.8 mm). The result is corroborated with the findings of Khater *et al.* [23] who reported that the bulk density of pelleted fish feed increased from 630.02 g/L at 1 mm to 696.23, 702.40 and 711.35 g/L at 1.5, 2.2 and 3 mm, respectively. The sinking velocity of the feed is influenced by numerous factors and one among them is pellet length/diameter (L/D) ratio. The L/D ratio was 2.5, 2.9, 2.9, 2.8 and 3.3 for the feeds, prepared with the diameter of 1, 1.2, 1.4, 1.6 and 1.8 mm, respectively. In addition, the processing condition, water temperature and salinity [24] also had a significant effect on the sinking velocity. However, the sinking velocity was not significantly affected by the feed pellet diameter in our study and was in the range of 5.99-8.73 cm/Sec. But in contrary, Chen *et al.* [25] reported that the sinking velocity has increased from 5.6 to 13.9 cm/Sec with increasing feed pellet size of 2 and 10 mm, respectively in the Atlantic salmon feed. Though a significant (P<0.05) difference was observed in the swelling capacity in our study, no specific trend could be noticed due to the variations in pellet size.

Table 3: Functional properties of feeds with varying diameter (n=3)

Particulars	Functional properties					
	WR (%)†	BD (g/L)ǂ	SV (cm/Sec)§	SC (%)□	DML (%)#	WS (h)£
Grade-1.0	182.87^{a}	755.06^{c}	6.12^{a}	84.39^{ab}	8.58^{a}	>3 h in a static condition and >90% stable in disturbed condition even after 1 h
Grade-1.2	176.24^{ab}	789.51^{b}	5.99^{a}	81.57^{b}	8.59^{a}	
Grade-1.4	172.57^{bc}	791.98^{b}	7.03^{a}	92.18^{a}	5.13^{b}	
Grade-1.6	168.47^{c}	795.38^{ab}	6.36^{a}	73.79^{c}	5.31^{b}	
Grade-1.8	158.85^{d}	807.75^{a}	8.73^{a}	91.78^{a}	3.95^{b}	
SEM (+)	9.608	34.158	1.114	13.349	1.151	
p-value	0.001	<0.001	0.191	0.008	0.011	
CV (%)	2.374	0.976	20.288	5.674	22.383	

Means bearing the same superscript in a column do no differ significantly (*P*<0.05)
†Water retention
ǂBulk density
§Sinking velocity
□Swelling capacity
#Dry matter leaching
£Water stability

Of all the analyzed feeds with varying size, Grade-1.6 mm was showed the lowest swelling capacity (73.79%) and was in the range of 81.57-84.39% in Grade-1.0 and 1.2 mm, while it was further increased >90% in Grade-1.4 and 1.8 mm. Dry matter leaching was significantly (*P*<0.05) high in Grade-1.0 and 1.2 mm (8.58 and 8.59%, respectively) compared to those prepared with 1.4, 1.6 and 1.8 mm diameter (3.95-5.31%). The higher leaching with a lower diameter of feeds would be due to the higher water retention/absorption (Table 3) that may be due to binder quality/quantity during the formulation. In addition, the variations in ingredient composition and manufacturing process would also be possible reasons for obtaining such difference in dry mater leaching among the feeds tested. However, all the values were within the value recommended by Cuzon *et al.* [8].

Akiyama *et al.* [14] suggested three levels of protein *viz*., 400, 380 and 360 g/kg from larval to grower stage of shrimp. The average protein content recorded in the present study was gradually (*P*<0.05) decreased with increasing feed pellet diameters (Table 4). This could be due to the higher protein requirement at earlier culture cycle compared to grow-out phase and who also reported to have 10-30% lower protein in grow-out diet than the larval diet. A similar trend was noticed in the total ash content that the lower diameter feed had the high ash content and was gradually (*P*<0.05) decreased with increasing the feed pellet diameter. This may be due to the higher inclusion of fishmeal in lower diameters feeds (1.0 and 1.2 mm) meant for early stages of shrimp, while the inclusion level of fishmeal is gradually decreased, in general, by increasing plant protein sources at higher diameter feeds (Grade-1.4, 1.6 and 1.8 mm) as the requirement of protein decreases as the animal size increases. Jannathulla *et al.* [26] reported higher ash content for fishmeal (189.5 g/kg), while it was in the range of 75.3-78.3 g/kg in plant protein sources. Increased NFE with increasing pellet size is attributed to the proportional changes of crude protein and total ash. However, other proximate principles were not much affected due to the pellet size variations (Table 4).

Table 4: Chemical composition (g/kg as fed basis) of feeds with varying diameter (n=3)

Particulars	Proximate composition								Gross energy (MJ/kg)□
	Moisture	Crude protein	Ether extract	Crude fiber	NDF†	ADFǂ	NFE§	Total ash	
Grade-1.0	107.45^{ab}	423.62^{a}	57.61^{a}	27.16^{ab}	273.35^{b}	116.97^{ab}	250.87^{c}	133.30^{a}	13.91^{c}
Grade-1.2	103.27^{b}	403.50^{b}	55.55^{ab}	23.91^{b}	240.64^{e}	112.55^{b}	287.57^{b}	126.21^{b}	14.06^{b}
Grade-1.4	102.57^{b}	371.07^{c}	54.75^{b}	30.49^{a}	306.86^{a}	131.31^{ab}	340.18^{a}	100.96^{c}	14.48^{a}
Grade-1.6	111.47^{a}	364.61^{c}	54.32^{b}	24.57^{b}	247.28^{d}	105.81^{b}	350.55^{a}	94.48^{d}	14.43^{a}
Grade-1.8	111.40^{a}	365.67^{c}	57.17^{a}	26.83^{b}	270.03^{c}	115.55^{b}	344.50^{a}	94.43^{d}	14.49^{a}
SEM (+)	0.053	0.335	0.008	0.021	0.415	38.607	0.395	0.015	0.001
p-value	0.016	<0.001	0.026	0.021	<0.001	0.047	<0.001	<0.001	<0.001
CV (%)	2.838	1.976	2.050	7.217	0.317	7.023	2.629	1.455	0.348

Means bearing the same superscript in a column do no differ significantly (*P*<0.05)
†Neutral detergent fiber
ǂAcid detergent fiber
§Nitrogen free extract calculated by a difference
□Estimated according to Jobiling (1983)

4. Conclusion

The results of the present study revealed the variability in functional properties and chemical composition of the commercial shrimp feeds available for farmers in India and farmers can use the tool of functional analysis, given in the present study, in addition to the chemical composition to ascertain the real utility to the shrimp.

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