CHAPTER V

DISCUSSION

V.1. Experiment: A

This experiment was designed and conducted using locally available (Cochin) natural feed ingredients. The diet design involved variations in both protein and energy as reported by Ali, (1996); Alava and Lim, (1983); Sedgwick, (1979) and Colvin, (1976). Protein levels ranged from 200 to 500 g kg\(^{-1}\) and energy levels ranged from 413.69 kcal 100g\(^{-1}\) to 470.83 kcal 100g\(^{-1}\) in terms of GE and 260 kcal 100g\(^{-1}\) to 350 kcal 100g\(^{-1}\) in terms of DE in the experimental diets. This was because protein requirement reported for *Penaeus indicus* was 420 – 430 g kg\(^{-1}\) by Colvin (1976) and 350 – 375g kg\(^{-1}\) by Gopal and Raj (1990). Bhaskar and Ali (1984) and Udayakumara and Ponniyah (1984) reported that early post-larval and juvenile *P. indicus* require 400g kg\(^{-1}\) caesin in purified diets for optimum growth. Moreover, in a comparative evaluation of four purified proteins in *P. indicus* Ali (1994) had reported a requirement of 250g kg\(^{-1}\) with albumin and 290g kg\(^{-1}\) with caesin. Thus the diet design in this experiment covered these levels of protein.

In terms of energy and protein interrelationships AQUACOP (1977) estimated an optimum requirement of 330 kcal100g\(^{-1}\) energy and 400g kg\(^{-1}\) protein for *P. monodon*. In *Penaeus merguiensis*, Sedgwick (1979) reported that the optimum protein levels to be in the range of 340 – 420 g kg\(^{-1}\) with an energy content of 290 - 440 kcal 100g\(^{-1}\). Later, Bautista (1986) opined that a twofold increase in body weights could be achieved with diets containing 400-500g kg\(^{-1}\) protein, 50-100g kg\(^{-1}\) lipid and 200g kg\(^{-1}\) carbohydrate with energy values of 285-370 kcal 100g\(^{-1}\) in *P. monodon* juveniles (0.60 - 0.80g). Hajra *et al.*, (1988) observed that at 460 g kg\(^{-1}\) protein 412 kcal 100g\(^{-1}\) GE to be the most appropriate dietary combination in *P. monodon* juveniles (0.5 g) reared in near freshwater conditions (3.5 - 4.5%). Shiau and Chou (1991) reported that, 360 g kg\(^{-1}\) protein and 330-kcal 100g\(^{-1}\) GE combination to be the best in *P. monodon* juveniles (0.82 g) reared in seawater (32 - 34%). However, the only report assessing the optimum energy level in
*P. indicus* is that of Ali (1990) stating that 414.75 kcal 100g\(^{-1}\) as the GE optimum in a purified diet containing 400g kg\(^{-1}\) protein (casein).

In this experiment, GE levels obtained cover a range of 407.68 kcal 100g\(^{-1}\) to 470.83 kcal 100g\(^{-1}\). This includes a lower energy level of 414.75 kcal 100g\(^{-1}\) reported by Ali (1990) with 400 g kg\(^{-1}\) protein and a higher level of 472 kcal 100g\(^{-1}\) recorded with a protein optimum near 428 g kg\(^{-1}\) by Colvin (1976) in *P. indicus*.

The highest final biomass was observed in shrimps receiving diet 5 and diet 2. Even though, diet 5 recorded a 0.92g final biomass gain shrimp\(^{-1}\), when absolute growth rate (AGR) was calculated according to Hopkins (1992), an average daily gain of 0.017 g was observed in both the diets 5 and 2. Similarly, biomass gains were the highest in the aforementioned diets without any statistically significant variations. Highest RGR of 113.95% was found in shrimps fed diet 5 followed by 112.20% in shrimps fed diet 2. PER's were least (1.25) in shrimps fed diet 5 and maximum (3.16) in shrimps fed diet 2 (*P* <0.01). FCR also indicated a similar trend without statistical significance. Highest ADMD coefficient of 98.89% and an APD coefficient of 74.20% were recorded with diet 5. Where as, the highest APD coefficient of 74.58% was obtained with diet 4. The lowest APD coefficient was found in diet 2.

Thus, a complementary reduction in the requirement of protein in feed for shrimps when adequate non-protein energy was available as hypothesized by Sedgwick (1979) holds good in this study. Protein sparing to the tune of 15% with an approximate increase of 30% non-protein energy was clearly evident. This in terms of GE was 21.66 kcal 100g\(^{-1}\) for a protein sparing of 15%. Shiau and Chou (1991) in their experiments with *P. monodon* (average weight 0.81±0.10 g) reported an energy requirement of 330 kcal 100g\(^{-1}\) with 360 g kg\(^{-1}\) protein and 320 kcal 100g\(^{-1}\) for 400 g kg\(^{-1}\) protein; which amounts to a protein sparing of 4% with an increment of 10 kcal 100g\(^{-1}\) calculated GE. This difference of almost two-fold protein sparing ability of *Fenneropenaeus indicus* appears to be due to the propensity of the early juveniles of this species to utilize higher amounts of carbohydrates reported by Ali (1996), using purified diets. PER was found to be significantly higher (*P* <0.01) in the shrimps fed diet 2 and significantly lower with
diet 5. Implications here are (1) diet 2 would have been adequately balanced in terms of amino acids (2) a good quality shrimp diet can be formulated avoiding shrimp meal. An inverse relationship between PER and dietary protein reported by Colvin (1976) was consistent with the present finding and reiterates the fact that dietary protein in excessive quantities may be either unassimilated or used as an expensive source of energy (Sedgwick, 1979). A high APD coefficient in the case of diet 5 could have been due to the excessive catabolism of protein to meet the energy demands. FCR also depicts the same trend with diets 2 and 5 registering similar feed: gain ratios and protein and energy densities below and above optimum leading to elevated FCR’s.

Digestibility of dry matter and protein are two more facets of nutritional responses recorded and perused. However, plummeting of survival rate to a low of 73% in shrimps fed diet 3 may be due to the amino acid imbalance, because the feed was devoid of both shrimp meal and fishmeal. PER obtained with this diet is also indicative of the above, which is in accordance to the report of Colvin (1976).

In quantifying nutrient requirements in fish Zeitoun et al., (1976) and Shearer (2001) had discussed the advantages and disadvantages of polynomial regression analyses to help improve the nutrient requirement estimates. With the present data set, an attempt was made to fit second degree polynomials by regressing the final biomass of shrimps with protein and energy concentrations in the experimental diets.

The equation obtained for protein was \( y = -0.7274 + 0.0859x - 0.0015x^2 \) \( (r^2 = 0.51) \) indicating a optimum growth at 37.14% protein which corresponds to the optimum protein reported by Gopal and Raj (1990) for this species. Similarly, for energy, the equation obtained was \( y = -37.6804 + 0.178462x - 0.000207x^2 \) \( (r^2 = 0.527) \) indicating a optimum growth at 430.95 kcal/100g energy. Being an empirical fit to the growth response of living organisms, the polynomial approach has the advantage of being continuous and is believed to be more accurate than other methods (Zeitoun et al., 1976). Ali (1990) in *P. indicus* reported an optimum requirement of 400g kg\(^{-1}\) protein and 414.75 kcal 100g\(^{-1}\) GE when fed purified diets. The present estimate of 371g kg\(^{-1}\) protein and 430.95 kcal 100g\(^{-1}\) GE by
feeding a diet made of natural feed ingredients indicates a marginally lower requirement of protein (Gopal and Raj, 1990) and slightly higher requirement of energy. Ali (1996) using a series of purified diets in *P. indicus* (initial dry wt: 10 mg) with a fixed lipid level of 70 g kg⁻¹ and varying protein and carbohydrate levels observed increasing trends in live weight gain, FCR and apparent carbohydrate digestibility without an optimum. A protein level of 219 g kg⁻¹ and 534 g kg⁻¹ carbohydrates with a GE of 399.4 kcal 100 g⁻¹ registered maximum weight gain, least FCR and highest carbohydrate digestibility, even though survival rates dropped with diets containing more than 450 g kg⁻¹ carbohydrates. This report is consistent with the present estimate in terms of energy. However, a protein level as low as 220 g kg⁻¹ may be due to the feeding of high quality purified proteins by Ali (1994). The estimated protein requirement of 370 g kg⁻¹ in the present study could be due to the natural sources of protein used in the experimental diets and strengthens the finding of Gopal and Raj (1990) who observed 375 g kg⁻¹ protein optimum. Shiau and Chou (1991) applying the same technique in *P. monodon* reported optimum levels of 320 kcal 100 g⁻¹ in 400 g kg⁻¹ protein diet and 330 kcal 100 g⁻¹ in 360 g kg⁻¹ protein diet which was in agreement with the reported by Bautista (1986) in the same species. However, in *Fenneropenaeus indicus* the animals' capability to derive large quantum energy from non-protein energy constituents established by Ali (1996) was obvious in this investigation where, diet 2 with 570 g kg⁻¹ of non-protein energy constituents performing nutritionally at par with diet 5. Applicability of this result is that, unlike purified diets tested by Ali (1996) all the feed ingredients used for the diet design in this study were natural and location specific. Thus, the results are tangible enough for direct application in hatchery linked nursery systems in the country.

V. 2. Experiments: B1-6

These six experiments were conducted with diet designs modified after Shiau and Chou (1991). The CIM provided the complement of natural feed ingredients such as fish meal, shrimp meal, clam meal and deoiled groundnut oil cake and oil. Chicken egg albumin rated to be the best purified animal protein source by Ali (1994) in *F. indicus* was the other major source of protein incorporated in the CIM.
A natural polysaccharide - starch was used as the carbohydrate source, viz., tapioca flour. Available reports indicate that shrimp are best able to utilize carbohydrates in the form of starch (polysaccharides) rather than monosaccharides (Abdel-Rahaman et al., 1979; Deshimaru and Yone 1978; Andrews et al., 1972; Forster and Beard, 1973; Ali 1988 and Cuzon et al., 2000). Rapid absorption of free glucose (which requires no digestion) results in considerable amount of glucose entering the body tissue before sufficient elevation of the activities of carbohydrate metabolising enzymes. This is proposed to cause a 'negative physiological effect' (Piefer and Pfeffer, 1980) in fishes. Contrarily, starch has to undergo enzymatic hydrolysis and monosaccharides arising from starch hydrolysis appear at the gut absorption sites slower than free glucose. Abdel-Rahaman et al., (1979) reported that the level of plasma glucose in Penaeus japonicus increased rapidly after they were fed a diet containing glucose and remained at high levels for 24 h. In contrast, plasma glucose was found to increase to a maximum level at 3 h and then decrease to a low level when the diet contained disaccharides and polysaccharides. These authors suggested that dietary glucose was quickly absorbed from the alimentary canal and released into haemolymph, resulting in a physiologically abnormal elevation of plasma glucose levels thereby impairing its utilisation as an energy source. Shiau and Peng (1992) also reported that plasma glucose levels in P. monodon fed glucose-containing diets peaked prior to those of shrimp fed dextrin or starch containing diets.

Another possible explanation for the poor growth performance of shrimp fed glucose containing diets is the possible inhibition of amino acid absorption in the intestine due to the presence of glucose (Alvarado and Robinson, 1979). Hokazeno et al., (1979) reported that the presence of 10 mM of glucose reduced the uptake of L-lysine from 26.64 to 12.34% and from 23.24 to 5.4% in the mid-intestine and the posterior intestine, respectively in rainbow trout. However, this interaction has not been studied in crustaceans.

Ali (1993) demonstrated that pure starch imparted significantly superior (P <0.01) growth compared with glucose, fructose, galactose, sucrose, maltose and glycogen in F.indicus. Tapioca flour was used to the extent of 540 g kg⁻¹ in this
investigation. The purpose was dual as reported by Ali (1988). Tapioca flour serves as an excellent binder other than being a good source of energy for shrimp. Hence, the energy variation is primarily brought about in the experimental feeds by varying the incorporation of tapioca flour from 0 – 540 g kg\(^{-1}\). The diets where tapioca flour was less than 100g kg\(^{-1}\) or avoided totally, CMC was incorporated as the binder at 20 g kg\(^{-1}\). (Tables 20, 24, 28 and 32).

Cellulose is used as the filler because, incorporating cellulose as high as 471 g kg\(^{-1}\) did not have any detrimental effects in similar studies reported by Shiau and Chou (1991) and Chuntapa et al. (1999).

Oil used in the CIM is an equal mixture of cod liver oil and groundnut oil. In diet formulations for shrimps major emphasis has been on maintaining an optimum ratio between n-3 type of essential fatty acids generally present in marine oils and n-6 type of fatty acids, most abundant in plant oils (Mercian and Shim, 1994). Grossly this requirement is met by blending cod-liver oil with groundnut oil in the CIM used in this study. Optimal lipid requirement reported by Chandge (1997) in F. indicus is in the range of 8% - 12%. Ali (1990) had reported that a 6% mixture of cod liver oil, prawn head oil, sardine oil and soybean lecithin in the ratio of 1:1:1:1 in the purified diets produced significantly higher growth (\(P <0.01\)), best FCR and high survival in F. indicus weighing 0.075 g. In this investigation, 9% oil was included in the CIM (Tables 9 and 15). This CIM when incorporated at 35 - 68% (Tables 10, 16, 20, 24, 28 and 32) yielded lipid levels of 4.85 to 10.84% (Exp. B1), 5.63 to 9.53% (Exp. B2), 6.51 to 8.40% (Exp. B3), 7.17% (Exp. B4), 7.9% (Exp. B5) and 8.7% (Exp. B6). These variations were mainly due to (1) the ascent in the levels of CIM inclusion to bring about the increase in protein content and (2) in experimental diets where energy increment was not achievable in the formulation from the carbohydrate source (tapioca flour), oil inclusion was resorted to the tune of 1-6% in the diets of experiments B 1-3. Preferential use of carbohydrate over lipid as energy has been demonstrated in shrimp (Ali, 1996 and Cuzon, 2000). Chuntapa (1999) stressed upon the establishment of an appropriate lipid: carbohydrate ratio (L: C) in shrimp diets and reported an L: C ratio of 7:32 (% by weight) in diets of P. monodon. This ratio has been worked out
for all the experimental diets (B 1-6) in this research, which shall be discussed in relation to growth in the succeeding relevant section.

Shrimp are incapable of synthesizing the steroid ring. Many sterols and essential components such as moulting hormones, sex hormones, bile acids and vitamin D, are synthesized from cholesterol. Cholesterol also functions as a component of membranes and in the absorption of fatty acids. Therefore, cholesterol is considered an essential nutrient, which must be provided in the diet (Teshima and Kanazawa, 1971). Akiyama et al. (1992) recommended 0.25 – 0.4% cholesterol in commercial shrimp feeds because many marine invertebrate meals and oils, i.e., squid, shrimp, crab and clam to be excellent sources of cholesterol. However, cholesterol in all the experimental diets contained 0.5% cholesterol based on the report of Chandge and Raj (1997b) in *F. indicus*.

The beneficial effect of phospholipids on growth and survival of shrimp are well documented (Kanazawa, 1983). 1) It is reported that phospholipids containing choline or inositol are most beneficial; 2) phospholipids containing the essential fatty acids are most effective; 3) the position of the fatty acid affects the phospholipids’ effectiveness; and 4) though phospholipids are synthesized by shrimp, the rate of synthesis is slow. It is also proven that an exogenous supply of phosphatidylcholine is required in shrimp feeds. Details of sources, their effectiveness and requirements still remain inconclusive (Russet, 2001). Regarding the requirement of phospholipid in *F. indicus* the only report is by Chandge and Raj (1997b) in larvae to the tune of 4%. Akiyama (1992) recommended a general phospholipid requirement of 2% in shrimp feeds and if lecithin is used the requirement can be brought down to 1%. In this study only 0.5% lecithin was used. This was considering the levels of phospholipids reported by Gill (1998) shown below. These ingredients used in the experimental diets should have contributed to the phospholipid availability excluding the possibility of a diet-induced deficiency.
Approximate Phospholipid Content of Aquafeed Ingredients (modified from Gill, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Phospholipid %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clam meal</td>
<td>1.27</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.14</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.47</td>
</tr>
<tr>
<td>Shrimp meal</td>
<td>1.02</td>
</tr>
</tbody>
</table>

There are only two reports regarding requirements of some water-soluble vitamins, Gopal (1987) and essentiality of vitamins in the Indian White Shrimp *F. indicus* (Reddy *et al.*, 1999), which were inadequate to formulate a vitamin mixture for this species. Therefore, based on a detailed review of vitamins required in crustacean diets by Conklin (1997), a vitamin mixture formulated based on the recommended levels given by Conklin (1997) was used. The composition is detailed as a footnote to Table 10 was used in all the diets in the experiments B1-6.

Similarly, in the case of minerals, Ali (1989) is the only report assessing the mineral requirements in *F. indicus*. Davis and Lawrence (1997) in a detailed review on mineral requirements in crustaceans have opined that the quantitative mineral requirements in most of the species have not been established. However, mineral deficiencies can occur in experiments with semi-purified diets as in this investigation. U.S.P salt mixture No. XIV (1950) whose detailed composition is given as a footnote to Table 10, was incorporated as a safe measure based on the authors’ earlier experience with test diets in shrimp.

The results of the second set of six experiments demonstrated that shrimp fed on diets with 250 g kg\(^{-1}\) to 300 g kg\(^{-1}\) at all energy levels showed a lower growth rate compared with shrimp fed higher protein levels; protein levels below 300 g kg\(^{-1}\) appear to be insufficient for optimal growth.

Colvin (1976) while estimating protein requirement of *F. indicus* tested protein (g kg\(^{-1}\)): GE (kcal 100g\(^{-1}\)) combinations of 213:450, 334:460, 428:470 and 530:480 respectively and found that 428:470 to be the most appropriate combination. Ali (1990) was the next to report that in *F. indicus* with a diet containing 400g kg\(^{-1}\).
protein, 50 g kg\(^{-1}\) lipid and 350 g kg\(^{-1}\) carbohydrate 414 kcal 100g\(^{-1}\) GE as the optimum.

Further, Ali (1996) reported that with 348 g kg\(^{-1}\) protein and 70g kg\(^{-1}\) lipid; maximum growth was at 348 kcal 100g\(^{-1}\) DE (whether estimated or calculated was not mentioned) in \textit{F. indicus}. With the same lipid level (70g kg\(^{-1}\)), and protein levels ranging from 220 g kg\(^{-1}\) to 510 g kg\(^{-1}\) maximum growth was registered at 400 kcal 100g\(^{-1}\). Again, with 348 g kg\(^{-1}\) protein, lipid level ranging from 15 g kg\(^{-1}\) to 178 g kg\(^{-1}\), maximum growth was at 392 kcal 100g\(^{-1}\). This observation of Ali (1996), ascribing the preferential utilisation of carbohydrate as high as 530g kg\(^{-1}\) in a protein deficient (220 g kg\(^{-1}\)) situation was also reported to cause poor survival. In this study, it is observed that in Exp. B1 with 250g kg\(^{-1}\) protein the GE of 409 kcal 100g\(^{-1}\) (Tables 11 and 36) recorded maximum growth and survival. The effect was manifested as poorest growth recorded among the six experiments. Protein sufficiency in formulated feeds in this research is found ensured only in Experiments B3-6. Similar and superior growth resulted (780% RGR), with protein levels of 350, 400 and 450 g kg\(^{-1}\). The potential of manipulating energy levels by altering the inclusion levels of non-protein dietary constituents to reduce protein level to the extent of not having an impact on growth is thus imminent.

In \textit{P. monodon} AQUACOP (1977) estimated that a total dietary energy content of 330 kcal 100g\(^{-1}\) was required for optimal growth at 400 g kg\(^{-1}\) protein. Hajra \textit{et al.}, (1988) reported that a GE level of 413 kcal 100g\(^{-1}\) to be the optimum at 460g kg\(^{-1}\) protein with feeds compounded using natural ingredients and shrimp reared in near freshwater conditions. In their review Cuzon and Guillaume (1997) found that the energy levels in crustacean diets generally ranged from 310 to 410 kcal 100g\(^{-1}\). While attempting to discern the most appropriate range in this work, it is clear that there is a threshold level for protein (350g kg\(^{-1}\) here), which is responsible for optimum growth. GE level of 371 kcal 100g\(^{-1}\) required to sustain this is derived from an L: C (% weight) ratio of 7:27. Bautista (1986) reported that the \textit{P. monodon} (0.60-0.80 g) fed with 300g kg\(^{-1}\) protein and GE ranging from 205-335 kcal 100g\(^{-1}\) had lower growth rates compared with shrimp fed on diets containing 350-450 g kg\(^{-1}\) protein at all energy levels. Shiau and Chou (1991) in their work on \textit{P. monodon} reported that at 400 g kg\(^{-1}\) protein the optimum GE level was
320 kcal 100g⁻¹ and at 360 g kg⁻¹ protein the GE level was 330 kcal 100g⁻¹. In *P. monodon*, Chuntapa *et al.* (1999) documented observations similar to the present study. Low growth at energy levels ranging from 203-339 kcal 100g⁻¹ with protein levels below 330g kg⁻¹. In shrimp fed on diets containing 330 – 440 g kg⁻¹ protein and GE levels ranging from 223 – 459 kcal 100g⁻¹ had greater growth. Further, growth was reported to be similar with 340 g kg⁻¹ protein and GE levels of 223 and 331 kcal 100g⁻¹. At 330 g kg⁻¹ protein with GE of 439 kcal 100g⁻¹ growth rate tended to decrease. However, at 360 g kg⁻¹ protein and 459 kcal 100g⁻¹ GE, growth rate was similar in diets containing 330-440 g kg⁻¹ protein at all GE levels. At 440 g kg⁻¹ protein and GE levels of 263 – 371 kcal 100g⁻¹ growth is again reported to match the levels of growth observed at 330 - 440 g kg⁻¹ protein. Using regression analysis with this data they derived the optimum P/E ratio as 146-150 mg protein kcal⁻¹. This trend is observed in the present work also, however, the GE values corresponding to 350, 400 and 450 g kg⁻¹ protein in the diets where maximum and similar growth was observed were 362 – 371 kcal 100g⁻¹ and P/E ranged from 97-124 mg protein kcal⁻¹. With regression analysis these GE values ranged between 353 – 360 kcal 100g⁻¹ and P/E ranged from 103-125 mg protein kcal⁻¹.

Thus, the optimal protein requirement in *F. indicus* in this study does conform to the earlier reports on this species by Colvin (1976) and Gopal and Raj (1990). The energy requirement even though decreases with an increase in the protein content in the diets as depicted in Figure 43, the protein sparing capability in this species appears to be lower when compared with the report on *P. monodon* (Shiau and Chou 1991). P/E ratio (103-125 mg protein kcal⁻¹) is also lower implying cheaper and more cost effective feeds can be formulated for this species.

L: C as a ratio in feed by weight is another important parameter perused which were 7:27, 7:21 and 8:13 by weight for the diets containing 350, 400 and 450 g kg⁻¹ protein respectively. This ratio of non-protein energy constituents indicates the gross tolerance level of this organism towards unnatural levels of fat and carbohydrates without ignoring the fact that the natural disposition of shrimp in general is towards a protein rich food and environment. The ratio reported for *P. monodon* is 7:32 by weight by Chuntapa *et al.* (1999). Ali (1990) in *F. indicus*
reports this ratio to be 5:35 for the diet, which registered the optimum growth. The current research shows that 7:27, 7:21 and 8:13 to be the appropriate ratios for optimum growth for diets containing 350, 400 and 450 g kg\(^{-1}\) protein respectively. Moreover, these ratios recorded higher growth compared to the work of Ali (1990) who had not tested lipid level beyond 6.25% because his own finding that 6% gross lipid level was optimal. Chandge and Raj (1997\(^a\)) reported a range of 8-12% for the same species. As shown in Table 36 the L: C ratio of 8:48, 6:37 and 9:11 at protein levels of 250, 300 and 500 g kg\(^{-1}\) protein produced sub-optimal growth. This indicated threshold levels of fat and carbohydrate beyond which abnormally high levels of these nutrients indirectly affecting protein deposition (growth).

SGR, PER, FCR FCE and survival are the other nutritional indices which conformed to the optimal values of growth in all the six experiments (B1-6) conducted. Significantly higher values (P <0.05) values for SGR, PER, FCE and significantly least values for FCR support the findings discussed. Varying levels of protein and energy in feed did not impact the body composition of the animals (Tables 13,19, 23, 27, 31 and 35).

The situation when viewed in totality, Exp. A with natural feed ingredients indicated that protein sparing to the tune of 150g kg\(^{-1}\) could be demonstrated with an increment of 21 kcal 100g\(^{-1}\) GE. However, with semi-purified diets (Exps.B1-6) this capability of the animal is not manifested as evident in Table 36 and Figure 43, where the possibility is only to the extent of 100g kg\(^{-1}\) protein, with a GE increment of 9 kcal 100g\(^{-1}\).