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Effect of Shellfish and Cabbage as Nutritive Supplements on Haematological Parameters, Body Weight and Organ Weight of Female Wistar Rats

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Abstract: Malnutrition being a major problem in current times. This research was undertaken to study the dietary impacts of two food sources, Shellfish (Squid) and Green Cabbage to determine their efficiency in tackling malnutrition. 36 female Wistar rats in post weaning stage were divided into 6 Groups, each with a specific diet. The study was conducted for a duration of 20 weeks. Body weight, organ weight and haematological parameters such as Haemoglobin levels, RBC count, WBC count, Differential leucocyte count, PCV, MCV, MCH, MCHC were studied for each Group to understand the effect of the above mentioned food sources as nutritive supplements.

Keywords: Malnutrition, Shellfish, Cabbage, Haematological parameters, Body weight, Organ weight, Nutritive supplements

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Introduction

Inadequate nutrition intake, be it from lack of essential nutrients including protein and specific micronutrients or lack of quantity, is a problem throughout the developing world (Samuelina *et al.*, 2015). Unavailability of sufficient food or incorrect eating can induce a nutritional deficit or malnutrition condition, which is characterized as a pathological condition due to lack of energy, proteins and/or nutrients in varied proportions (Lacerda *et al.*, 2006; Estrela *et al.*, 2014). Malnutrition is one of the major causes of ill health, disability and death (WHO 2013; Samuelina *et al.*, 2015), as globally an estimated 2 billion people suffer from it (FAO 2011; Samuelina *et al.*, 2015). Malnutrition includes imbalances, deficiencies or excess in the intake of nutrients (WHO 2017; Sławomir Lewicki *et al.*, 2018). Malnutrition has been associated with low educational standards, poverty, poor housing and sanitation, large numbers of people living in the same house, and maternity at less than 20 years of age (Olinto *et al.*, 1993; Monteiro *et al.*, 2009; Estrela *et al.*, 2014). Although it is rarely listed as a direct cause of mortality, the World Health Organization (WHO) estimates that malnutrition contributes to more than a third of all infant casualties in the world (WHO 2014; Estrela *et al.*, 2014). Undernutrition can have effects persisting through lifetime of an individual. Physical stunting (defined by the WHO as low height-for-age) can lead to an increased susceptibility to infection, affect cognitive development, impair learning and future productivity of affected people as well as of their future generations. Deficiencies in iodine, iron, zinc and vitamins can cause effects such as anaemia, brain damage and stunted growth. Being underweight makes it difficult to work and much more difficult to fight off infections and recover from illness (Samuelina *et al.*, 2015).

Studies have shown that protein malnutrition can reduce body mass in animals (Bray *et al.*, 2012; Lewicki *et al.*, 2014; Sławomir Lewicki *et al.*, 2018). Protein-energy malnutrition is accompanied by metabolic changes whereby the organism attempts to guarantee an energy supply to very important organs such as the heart and brain at the expense of fat and structural protein consumption (Cahill 1970; Osorio Miguel Parra *et al.*, 1995). The result is emaciation or loss of body weight including the protein mass of some internal organs, the liver in particular (Addis *et al.*, 1936; Osorio Miguel Parra *et al.*, 1995). Hematological parameters are also affected due to malnutrition, in situations of food restriction and protein restriction, respectively (Ferrari *et al.*, 1992; Santos *et al.*, 2004; Estrela *et al.*, 2014). The leucocytes (lymphocytes, monocytes and granulocytes) play a significant role in immune response. Malnutrition is the primary factor causing immunodeficiency worldwide and can alter cell-mediated immune responses in both humans and experimental animals (Katona and Katona-Apte, 2008; Sławomir Lewicki *et al.*, 2018). Protein deficiency is found to reduce IgA immune response in mouse and rat models (McGee and McMurray, 1988; Sullivan *et al.*, 1993; Sławomir Lewicki *et al.*, 2018). Protein-energy malnourished mice had become more susceptible to influenza infection (Taylor, 2013) and were found to have decreased cytokine production (Iyer, 2012). Protein malnutrition affects several parameters in blood. A decrease in MCH and MCHC levels in birds fed with low protein content diet (21% in comparison to 23% of crude protein) has been observed (Mohamed *et al.*, 2012; Sławomir Lewicki *et al.*, 2018). Protein deprivation (from 24% to 13% of crude protein) lead to decline in RBC count and MCV level and increased the MCH and MCHC in common carp (*Cyprinus carpio)* fingerlings (Al-Sraji and Nasir, 2013). A study conducted on rats feeding protein-deficient diet (9.5% of protein) for 80 days found significantly reduced hemoglobin, MCV and MCH concentration and increased MCHC level (Prestes-Carneiro *et al.*, 2006). Protein malnutrition (4.5% of protein) had a negative impact on Hemoglobin, MCV and MCH parameters (Sławomir Lewicki *et al.*, 2018).

Shellfish meat has been recommended in several dietary regimes for its high protein content, low calorific values, lower proportions of saturated fat, significant amount of omega-3- fatty acids, dietary essential amino acids, vitamin B12 and several important minerals such as iron, zinc and copper (Krzynowek *et al.*, 1989; Dong, 2001). Cabbage is rich in antioxidants, which play an important role in health maintainence. They neutralize harmful chemicals called free radicals that cause cell damage in the body. Antioxidants have been strongly linked to the protection from numerous diseases; heart disease, cancer, eye disease as well as regulation of the immune system (Michael *et al.*, 1999; Lynn *et al.*, 2006). Cabbage is a good source of beta-carotene, vitamin C and fiber as well. It is a cruciferous vegetable and has been shown to reduce the risk of some cancers, especially those in the colorectal Group (Zarzour, 2012).

Addressing the problem of malnutrition would lead to beneficial outcomes for mankind and it becomes necessary to find out such food sources which are nutrition wise beneficial and easily accessible to people. This study was undertaken to study the effects of Shellfish and Cabbage as nutritive supplements to deal with malnutrition in female Wistar rats and findings with respect to haematological parameters, body weight and organ weight are discussed here.

Materials and Methods

Female Wistar rats (n=36) in post weaning stage, weighing 55 g to 85 g, were obtained from Bharat Serums and Vaccines, Thane, India. The animals were procured after getting the required approval from Institutional Animal Ethics Committee (IAEC) at Dr.L.H. Hiranandani College, CHM College Campus, Ulhasnagar and were placed at the animal house of the same Institute. The animals were kept in Polypropylene cages with corn cob bedding. Acclimatization period was two weeks. Normal rat feed pellets from Nutrivet Lifesciences, Pune were used for feeding all the animals *ad libitum* during this period. Throughout the experiment period, water was provided *ad libitum.* Two weeks later, rats were randomly divided into six Groups with each Group having six rats. Each Group was fed specific diet as follow:

Rats in Group 1 were fed Normal rat feed pellets *ad libitum* throughout the experiments. In Group 2, the rats were fed Normal rat feed pellets for 75% of their dietary requirements and remaining 25% was Shellfish. Rats in Group 3 were fed Normal rat feed pellets for 75% of their dietary requirements and remaining 25% was Cabbage. Group 4 rats were malnourished as they were on a restricted diet, being fed just 50% of their dietary requirement of rat feed pellets. Rats in Group 5 were also malnourished being fed just 50% of their dietary requirement of rat feed pellets but these were provided with Shellfish as nutritive supplement for the remaining 50% of their dietary requirements. Group 6 rats were malnourished too, being fed just 50% of their dietary requirement of rat feed pellets and these were provided with Cabbage as nutritive supplement for the remaining 50% of their dietary requirements. Feed consumption of Group 1 rats (*ad libitum*) was noted daily and it was used as the basis for calculation of feed requirements for the other Groups.

Shellfish (Squid) and Green Cabbage were brought from local market, cleaned with water, cut into small pieces, weighed and fed to the animals as per their feeding Groups. Body weight was noted every week. The feeding experiments were carried out for 20 weeks after which the rats were sacrificed using CO_2 chamber and blood samples were collected by cardiac puncture in EDTA vials to study haematological parameters. Rats were dissected, organs collected, cleaned with distilled water and fresh weight was noted.

Hematological investigations include Red blood cell (RBC) count, White blood cell (WBC) count, differential count for Leucocytes, Hemoglobin (Hb), packed cell volume (PCV) or hematocrit. RBC and WBC were measured manually using Neubauer counting chamber, PCV was measured by Wintrobe tubes. The differential Leucocyte count was done manually using a thin blood film stained with Leishman stain and hemoglobin concentration was determined by Sahli's method. Using the values obtained for RBC, PCV and haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the standard formulae given in hematological books (Lewis *et al.*, 2006; Hoffbrand and Moss, 2011; Barrett and Barman, 2016; Delwatta *et al.*, 2018).

Statistical Analysis:

On Minitab software, results were analyzed statistically by the use of one way analysis of variance (ANOVA) to determine the differences between the mean values at $P < 0.05$ level followed by Tukey post hoc test. Data expressed as Mean ± SD. Graphs were created using Microsoft Excel.

Results and Discussion

Haematological Parameters:

As shown in Table 1, Haemoglobin (Hb) levels were found to be the lowest for Group 6 and

Table 1: Haematological Parameters of different Groups

Rat Groups	Hb (g %)	RBC x $10^6/mm^3$	WBC $x10^3/mm^3$	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	N (%)	E (%)	L (%)	M (%)	B (%)
Group 1 - Normal Feed	14.48 ± 0.46	8.23 ± 0.42	9.72 ± 0.87	40.44 ± 1.13	49.20 ± 1.61	17.60 ± 0.49	35.80 ± 0.42	23.60 ± 6.07	1.80 ± 0.84	73.40 ± 5.64	1.20 ± 0.45	0.00 ± 0.00
Group 2 - Normal Feed + Shellfish	14.98 ± 0.37	8.45 ± 0.29	9.52 ± 1.22	40.75 ± 1.27	48.25 ± 0.64	17.75 ± 0.44	36.78 ± 0.57	31.33 ± 5.65	1.67 ± 0.82	66.17 ± 5.81	0.83 ± 0.41	0.00 ± 0.00
Group 3 - Normal Feed + Cabbage	14.76 ± 0.25	8.39 ± 0.12	8.82 ± 1.31	41.02 ± 0.89	48.88 ± 0.79	17.56 ± 0.22	35.98 ± 0.22	31.20 ± 14.41	1.60 ± 0.55	66.20 ± 14.36	1.00 ± 0.00	0.00 ± 0.00
Group 4 - Malnourished	14.17 ± 0.44 *	7.81 ± 0.24 *	8.42 ± 1.38	40.47 ± 1.59	51.85 ± 1.55*	18.17 ± 0.49	35.05 ± 0.80*	30.67 ± 6.41	2.33 ± 1.10	65.83 ± 6.71	1.17 ± 0.41	0.00 ± 0.00
Group 5 - Malnourished + Shellfish	14.78 ± 0.40	8.41 ± 0.47*	11.16 ± 1.52*	40.96 ± 1.86	48.76 ± 0.84*	17.60 ± 0.60	36.10 ± 0.89*	25.8 ± 7.92	2.20 ± 1.10	66.80 ± 7.73	1.20 ± 0.45	0.00 ± 0.00
Group 6 - Malnourished + Cabbage	14.00 ± 0.28 *	7.79 ± 0.09 *	7.72 ± 1.63	38.82 ± 0.88*	49.82 ± 0.95*	17.97 ± 0.30	36.08 ± 0.59*	25.83 ± 5.91	2.00 ± 2.10	71.17 ± 5.12	1.00 ± 0.63	0.00 ± 0.00

Data presented as Mean ± SD. *Means are statistically significant at P<0.05 (One way ANOVA followed by Tukey post hoc test). Hb- haemoglobin, RBC- Red Blood Corpuscles, WBC – White Blood Corpuscles, PCV – Packed cell volume, MCV- Mean corpuscular volume, MCH – Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration,N- neutrophils, E – Eosinophils, L- Lymphocytes, M- Monocytes, B- Basophils.

Group 4 and highest for Group 2 and Group 5 both of which had Shellfish included in their diet. On statistical testing, it was found that Hb level of Group 6 is significantly different from Groups 2, 3 and 5 and that of Group 4 is significantly different from Group 2. RBC counts too were lowest for Groups 4 and 6 and highest for Groups 2, 3 and 5. Post hoc testing found that RBC count of Group 6 is significantly different from Groups 2 and 3 and that of Group 4 also is significantly different from Groups 2 and 3. Group 5 RBC count is higher than Group 4 and also statistically significant (Table 1). WBC count was lowest for Group 6 and highest for Group 5.WBC count for Group 5 was significantly different from that of Group 4. PCV was found to be lowest for Group 6 and highest for Group 3 (table 1). Statistically also, PCV for Group 6 was significantly different from Group 3. MCV was found to highest for Group 4 and lowest for Group 2. On statistical testing, MCV of Group 4 was significantly different from Groups 1,2,3 and that of Groups 5 and 6 was significantly different from Group 4. MCH was highest for Group 4, i.e. malnourished rats however, no statistically significant difference was observed (Table 1). MCHC was lowest for Group 4 and it was also found to be significantly different from Group 2. MCHC of Groups 5 and 6 was significantly different from that of Group 4. Neutrophil percentage was highest for Group 2 and lowest for Group 1. Percentage of Eosinophils was highest for Group 4 and lowest for Group 3. Lymphocyte percentage was highest in normal Group i.e. Group 1 and lowest in malnourished rats (Group 4). There was no remarkable change observed in monocyte percentages across 6 Groups. With respect to Differential leucocyte count, no statistically significant difference was observed.

Body Weight:

As depicted in Table 2 and Figure 1, observations with respect to body weight across 20 weeks showed that rats in Groups 1,2 and 3 with normal rat feed had higher weight gain compared to all three malnourished Groups i.e. Groups 4, 5 and 6 that showed slower increase in body weight.

Table 2: Rat body weight (g) during 20 weeks of experimental duration

Rat groups	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**	**Week 13**	**Week 14**	**Week 15**	**Week 16**	**Week 17**	**Week 18**	**Week 19**	**Week 20**
Group 1 - Normal Feed	156±13	181±12	195±15	193±44	229±15	236±20	251±20	258±22	264±21	271±20	281±25	282±25	269±42	287±24	293±26	275±31	275±61	297±20	298±22	294±15	294±17
Group 2 - Normal Feed + Shellfish	132±22	162±24	178±23	191±18	210±20	218±20	235±22	242±25	253±24	260±28	267±27	270±31	265±45	283±34	299±43	303±37	290±36	292±35	280±27	279±30	298±35
Group 3 - Normal Feed + Cabbage	153±10	172±8	183±8	191±10	210±10	222±11	231±10	239±9	249±14	254±12	263±16	269±11	269±19	278±19	280±19	282±16	287±18	288±21	288±20	287±18	283±17
Group 4 - Malnourished	160±5	174±6	181±7	188±9	195±11	205±11	210±8	215±12	215±11	216±12	226±8	229±12	232±13	233±11	237±12	234±14	243±14	247±13	248±14	254±18	254±17
Group 5 - Malnourished + Shellfish	148±14	167±15	175±16	190±12	202±17	207±15	221±18	222±11	232±12	231±13	237±9	242±9	252±14	256±18	253±17	257±22	257±22	263±23	271±25	270±24	281±30
Group 6 - Malnourished + Cabbage	143±15	156±14	165±16	171±14	180±16	190±16	198±22	202±16	211±15	206±16	220±18	224±15	227±15	227±15	227±16	223±17	232±18	238±18	236±20	241±20	248±19

Data are presented as Mean ± SD. Values are rounded up to nearest complete value.

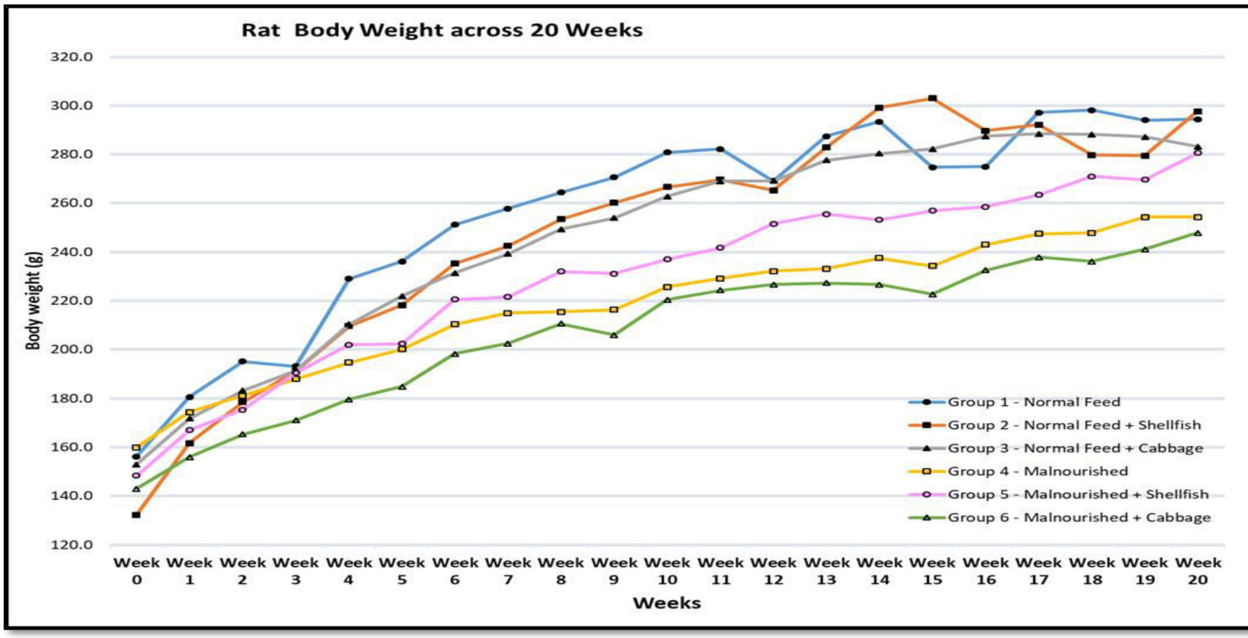

Fig. 1: Rat body weight (g) during 20 weeks of experimental duration.

However, among the malnourished Groups, Group 5 rats which were provided Shellfish could cope up better with respect to body weight gain than the one provided with Cabbage. Among the normally fed Groups also, Group 2 which was provided with Shellfish along with normal feed showed considerable increase in body weight along week 14 to 17 thus giving an indication that Shellfish fed Groups gained weight sooner compared to cabbage fed Groups.

Absolute and relative organ weights:

As illustrated in Table 3, absolute weight of liver was lowest in Group 4 rats and highest in Group 3. Statistical analysis revealed that in Group 4 absolute liver weight was significantly different from that of Group 3 and Group 6 was significantly different from Group 1 and 3. However, no significant difference was observed concerning relative weight of Liver among the Groups. Absolute weight of Kidneys in Group 5 was significantly different from Group 4. Kidneys, with respect to relative weight, did not show any remarkable difference. In case of Heart, neither absolute nor relative weight showed any significant difference. Absolute Brain weight for Group 4 was found to be significantly different from Groups 1, 2, 3. Groups 5 and 6 absolute Brain weight was significantly different from Group 4. For Ovaries and Lungs, neither absolute nor relative weight showed any significant difference. Absolute weight of Spleen in Group 4 was significantly different from Group 3. Relative weight of Pancreas for Group 4 was significantly different from Group 2.

Conclusion

In this study, Shellfish as a nutritive supplement was effective in provoking an increase in haematological parameters such as Haemoglobin levels, RBC counts and WBC counts of malnourished rats in comparison to Cabbage. Both Shellfish and Cabbage fed malnourished rats have shown rise in MCHC and lowering of MCV. Shellfish fed rats were also found to gain body weight sooner in comparison to Cabbage fed rats. Cabbage when provided as an additional nutritive supplement along with feed pellets, led to higher absolute as well as relative weights with respect to liver. Both Shellfish and Cabbage fed mal-

Table 3: Absolute and relative organ weights of different groups

Absolute Organ Weight (g)								
Rat groups	**Liver**	**Kidneys**	**Heart**	**Brain**	**Ovaries**	**Lungs**	**Spleen**	**Pancreas**
Group 1 - Normal Feed	12.25 ± 0.71	2.15 ± 0.12	1.12 ± 0.12	1.94 ± 0.15	0.38 ± 0.12	1.94 ± 0.54	0.76 ± 0.19	0.97 ± 0.53
Group 2 - Normal Feed + Shellfish	11.69 ± 1.91	2.29 ± 0.29	1.05 ± 0.17	2.00 ± 0.07	0.35 ± 0.05	1.88 ± 0.28	0.84 ± 0.12	0.95 ± 0.48
Group 3 - Normal Feed + Cabbage	13.38 ± 1.62	2.15 ± 0.34	1.18 ± 0.14	1.93 ± 0.04	0.36 ± 0.05	2.25 ± 0.71	0.91 ± 0.22	1.31 ± 0.43
Group 4 - Malnourished	10.34 ± 1.71*	1.81 ± 0.24	1.02 ± 0.19	1.74 ± 0.09*	0.34 ± 0.09	2.10 ± 0.72	0.59 ± 0.10*	1.37 ± 0.22
Group 5 - Malnourished + Shellfish	11.90 ± 1.36	2.36 ± 0.52*	1.13 ± 0.14	1.92 ± 0.09*	0.32 ± 0.08	2.13 ± 0.54	0.79 ± 0.28	1.41 ± 0.19
Group 6 - Malnourished + Cabbage	9.69 ± 1.20*	2.04 ± 0.19	1.07 ± 0.08	1.95 ± 0.12*	0.38 ± 0.06	1.70 ± 0.47	0.69 ± 0.12	1.23 ± 0.18
Relative Organ Weight (g)								
Rat Groups	**Liver**	**Kidneys**	**Heart**	**Brain**	**Ovaries**	**Lungs**	**Spleen**	**Pancreas**
Group 1 - Normal Feed	4.17 ± 0.28	0.73 ± 0.06	0.38 ± 0.04	0.66 ± 0.08	0.13 ± 0.05	0.66 ± 0.17	0.26 ± 0.06	0.33 ± 0.18
Group 2 - Normal Feed + Shellfish	3.96 ± 0.66	0.77 ± 0.08	0.35 ± 0.06	0.68 ± 0.06	0.12 ± 0.01	0.64 ± 0.10	0.28 ± 0.03	0.31 ± 0.14
Group 3 - Normal Feed + Cabbage	4.72 ± 0.46	0.76 ± 0.13	0.42 ± 0.04	0.68 ± 0.05	0.13 ± 0.03	0.79 ± 0.21	0.32 ± 0.07	0.47 ± 0.16
Group 4 - Malnourished	4.06 ± 0.53	0.71 ± 0.07	0.34 ± 0.09	0.69 ± 0.07	0.13 ± 0.04	0.81 ± 0.22	0.23 ± 0.04	0.54 ± 0.10*
Group 5 - Malnourished + Shellfish	4.25 ± 0.34	0.83 ± 0.13	0.39 ± 0.05	0.69 ± 0.07	0.11 ± 0.03	0.77 ± 0.21	0.28 ± 0.09	0.51 ± 0.09
Group 6 - Malnourished + Cabbage	3.92 ± 0.47	0.83 ± 0.09	0.42 ± 0.04	0.78 ± 0.08	0.15 ± 0.03	0.68 ± 0.15	0.28 ± 0.04	0.50 ± 0.10

Data presented as Mean ± SD. *Means are statistically significant at P<0.05 (One way ANOVA followed by Tukey post hoc test)

nourished rats have shown higher absolute brain weight compared to malnourished rats.

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