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Growth performance of wistar rats (*Rattus norvegicus*) fed with different formulations of dry pet kibble

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Abstract
A detailed investigation was carried out to study the effect of various iso-nitrogenous and calorific pet food formulations (as kibble) such as C (control), T1 with chicken boneless breast, T2 with whole chicken and T3 with chicken offal meal and its growth performance on Wistar rats (n=6). The pet food intake by Wistar rat in the groups was significantly (P<0.05) higher in control, T1 and T3 than T2. Similarly, the cumulative weight gain was significantly higher (P<0.01) in T2 than the C, T1 and T3, correspondingly, the weekly body weight gain also following the same trend. The treatment group (T2) fed with whole chicken based pet food had a low protein intake and exhibited a higher weight gain followed by T1 and T3 than the C group. Comparatively a higher PER was noticed in T2 than T1, T3 and C group. Hence, it is concluded that the Wistar rat groups fed with whole chicken (T2) based pet food formulation had a higher weight gain, followed by pet food formulated with offal meal and chicken boneless breast than the fully vegetarian based pet food.

Keywords: wistar rat, dry pet food, growth rate, PER, FCR

Introduction
Increased urbanization and an increase in nuclear families across the Indian subcontinent, more people are keeping dogs as pets. As per the industry data, the pet food industry size is pegged at Rs 2,500 crore in India, which has an estimated pet population of about 29 million and is facing a shortage of pet foods (PTI, 2021) [11]. The commercial pet food makers explained a higher taxes, ambiguous business environment, absence of uniform framework and quality standards are the major hindrances for the sector's growth. On an average, India requires 30,000 tones of pet foods and not sufficient to meet demand and quality. At present, India does not have many pet food manufacturing facilities as it is not considered as a profitable business. Moreover, the existing commercial pet food makers explained various hindrances for the sector's growth. India is producing 8.1 million tones of meat, out of which chicken meat is 50 per cent and the offal's are wasted and also cause problem in disposal and these offal' scan be utilized for pet food manufacturing. By considering the demand for the low cost pet food and eliminate the problem of disposing the poultry offal's, the present study is aimed to formulate pet food by using chicken boneless breast, whole chicken and chicken offal meal with control based on vegetarian origin and to assess the growth performance in Wistar rats.

Materials and Methods
The experimental pet food diets were divided as control (C) based on soya, T1 with chicken boneless breast, T2 with whole chicken and T3 with chicken offal meal by adhering the nutrient specifications and recommendations given by the AAFCO (2014) [1] for the adult dog maintenance diet. The quantity of ingredients for the various pet food formulations are presented in Table 1.

Procedure for pet food manufacture
The selected ingredients were weighed individually, according to the formulated level of inclusion with an electronic digital balance. All the ingredients were thoroughly mixed and added 10 per cent of water gradually in the mixer. The mix was extruded using double screw dry extruder model SLG65-III with the screw length 1050 mm, speed 210 RPM, output 80-120kg/h (Manufactured by Jinan saibanuo Technology Development Co., Ltd, China) temperature fixed at 124 ˚C and the prepared food was conveyed through pneumatic conveyer

to the drier, where the product was dried at 80 ˚C for two hours. All the four pet food diets were prepared by the above-same procedure, then cooled and packed in LDPE bags and stored at room temperature (30.16 ± 1.26 ˚C) (Brindha, 2013) [2].

Fig 1: Flow diagram for the preparation of dry pet kibbles

Experimental Design and Diet for Wistar rats

Day old Wistar rats (*Rattus norvegicus*) were purchased from Biogen Laboratory Animal Facility (CPCSEA Reg No.971/PO/RcBiBt/S/2006/CPCSEA), Bangalore, India. Necessary consent has been obtained from ethical committee (Approval No. IAEC/9/VCRI-NKL/2019 Dated 30.10.2019). A randomized block design was adopted and a total of 24 rats with same age group and weight were distributed into four groups. All the groups comprises six rats (n=6, each) with equal number of male and female. All the group of rats were kept individually in separate cages. An identification mark was given to each rat and tags were prepared with respective identification mark and attached to the cages. The Wistar rats were housed in the laboratory animal shed in separate groups (cages) provided with *ad libitum* wholesome clean purified portable drinking water supply. Further, the control and various treatments diets were fed to the Wistar rats after 21 days weaning period with 3 days adaptation period till 45 days. All the control and treatment pet food diets were formulated iso-nitrogen (18 per cent protein) and iso-calorific (3400kcal/g of energy) value as per the AAFCO (2014) [1]. The growth performance parameters of Wistar rats such as the feed intake (g), feed conversion ratio, protein intake (g), weekly body weight gain (g) and cumulative weekly body weight (g) were assessed on 0th week, 1st week (24-31days), 2nd week (32-38days) and 3rd week (39-45 days).

The initial weight of Wistar rat was recorded before the start of feeding trial and is presented in Table 2. The rats in each group were fed *ad libitum* with the respective diets every day. Individual daily pet food consumption rate was recorded. The pet food served to each rat was weighed and recorded accordingly. The split and leftover pet food was weighed every day to obtain an accurate level of the pet food consumed by the rats. The food consumed and leftover feed by the rats was calculated to obtain feed conversion rate (FCR). The FCR was calculated as per the procedure followed by Manjula *et al.*, (2016) [8].

Results and Discussion

The cumulative weekly body weight (g) of Wistar rats fed with control and treatment kibbles are presented in Table 2.The initial body weight of the Wistar rats were maintained uniformly. The cumulative body weight was significantly (P<0.01) differ from first week onwards, however in first week, it was better (P<0.01) in all treatment group, but in second and third week T2 performed better than T1 and T3, over all treatment groups were exhibited better growth (P<0.01) than control group throughout experimental period. The weekly body weight gain (g) for the control was 15.51, 16.33 and 18.17 g and exhibited no significant difference between 0 -1, 1-2 and 2-3 weeks. The weekly body weight gain (g) for the T1 was 23.34, 19.33 and 19.67g and exhibited a significant difference (p<0.05) between 0 -1, 1-2 and 2-3 weeks and for T2 was 26.87, 24.66 and 24.00g, exhibited a significant difference (p<0.05) between 0 -1, 1-2 and 2-3 weeks. Subsequently, T3 had 21.84, 21.66 and 19.67 also revealed a significant difference (p<0.05) between 0 -1, 1-2 and 2-3 weeks. It could be understood that the T2 pet food fed rats had a pronounced weight gain as compared to T1 and T3, since from the first week to third week. The control pet food fed rats received a lower cumulative weekly body weight as compared to the treatments.

The cumulative weekly body weight gain(g) in Wistar rats fed with various pet food formulations are presented in Table 3.The control and treatment kibbles had a marked impact on the growth performance of Wistar rats from first week to third week and exhibited a significant difference (p<0.05) among them. But the treatment groups (T1 to T3) had a well-defined elevated weekly body weight gain. Analysis of results of the weekly body weight gain of Wistar rats in ascending order are T2 >T3 &T2 >T1 >C and inferred that the T2 (whole chicken based pet food) obtained higher weight gain. Higher body weight gain of the Wister rat in treatment groups might be due to higher protein efficiency ratio and more bioavailability. Weight gain of the whole chicken based kibbles was higher; it might be due to bone contain essential minerals for weight gain and higher digestibility and amino acid composition of the whole chicken than offal's/boneless breast. Manjula *et al*. (2016) [8] studied the feed intake and weight gain of *Spirulina* and rice diet in Wistar rats and reported that the feed consumption rate is very low in *Spirulina* diet but gained more weight when compared to stock and rice diets, this might be due to high biological value protein in *Spirulina*. In the present investigation, the findings of cumulative body weight and weekly body gain are in agreement with the findings of Manjula *et al*. (2016) [8].

The feed intake (g) of Wistar rats fed with various pet food formulations are presented in Table 4. The control and treatment group rats on the first week found to have a uniform feed intake and showed no significant (p<0.05) difference among them. In the second week, the control and T1 groups had comparable feed intake and T2 & T3 had a significant increase (p<0.05) thereby exhibited a statistical significance among them. But in the third week, the control and treatment groups noticed a wide variation in the pet food intake and exhibited a (p<0.05) significant difference among them. The rat groups fed with control (C) and poultry offal meal based pet food (T3) found to be similar and also there is a significant variation in the T1 and T2 group rats. Food intake is determined by the body requirements of growth and development (FAO, 2011) [4] as well as the ability of the foods to satisfy these needs. In the present study, there seems to have a selective affinity in choosing the pet food diet (kibbles) among the rat groups.

The feed conversion ratio (g) of Wistar rats fed with various pet food formulations of kibble are presented in Table 5. The feed conversion ratio (FCR) of control and treatment groups had a significant difference (p<0.05) among the them on first week and the T1 and T2 are comparable with each other, followed by a higher FCR has been found in control and the T3 group. Similarly, in the second week the FCR of the control and treatment groups exhibited a significant difference (p<0.05) and a highest was observed in control, followed by T1, T3 and T2 respectively. In the third week the FCR of the control and treatment groups exhibited a significant difference (p<0.05) and the ascending order of the groups were C>T3>T2 and >T1 respectively and the findings indicate that the Wistar rats fed with (T2) whole chicken based pet food had lowest FCR. Better FCR of the whole chicken based kibbles might be due to better bioavailability/balance nutrient present in this diet. Because it contains bone, muscle, fat etc in a balanced way and digestibility might be higher than the offal and veg based kibbles. Similarly, Manjula *et al*. (2016) [8] studied the feed conversion ratio of *Spirulina* and rice diet in Wistar rats and reported that the *Spirulina* utilization was much higher compared with rice diet which is poor in protein content and poor quality of amino acids and *Spirulina* was significantly lower than rice diet indicating better utilization. The current findings with regard to FCR of control and treatment kibbles utilization by Wistar rat groups are in accordance with the findings of Manjula *et al*. (2016) [8].

The protein intake (g) of Wistar rats fed with various pet food formulations of kibble are presented in Table 6. In the first week of feeding trial, the protein intake of the control and treatment groups exhibited a significant difference (p<0.05) among them and a higher was observed in T3 and lower was in control. In the second week the control and treatment groups found to have a significant variation (p<0.05) in the pet food intake. The control and T1 are comparable with each other but a higher protein intake was in T3 than T2 group. In the third week the protein intake of control and treatment groups had a significant difference (p<0.05) and the control and T1 are comparable with each other, similarly T3 is also comparable with control. The treatment group (T2) fed with whole chicken based pet food had a low protein intake of 12.99 g. Onofiok and Nnanyelugo, (1998) [10] reported that the quality and type of protein in a diet can influence food intake. Elijah *et al*., (2017) [3] reported that the protein quality of fortified foods can only be tested using other indices of protein quality but not the intake. The present study, protein intake by the various rat groups are in agreement with the findings of Onofiok and Nnanyelugo, (1998) [10] and Elijah *et al*., (2017) [3].

The protein efficiency ratio (g) of Wistar rats fed with various pet food formulations of kibble are presented in Table 7.The protein efficiency ratio (PER) is one of the commonly used methods of assessing the quality of a protein (FAO, 2011) [5]. A food with a higher PER is deemed superior to a food that yields a lower PER. The PER of control and treatment groups showed a significant difference (p<0.05) among them on first week of feeding trial. The PER of control is 1.57 and the treatments T1, T2 and T3 were 2.41, 2.62 and 1.96 and the highest PER is observed in T2 and the lowest is in control. During the second week, a significant difference (p<0.05) is noticed in control and treatment groups and the control, T1 & T3 groups found to be similar but a higher PER is noticed in T2 group. The PER values in the third week found to have a significant difference (p<0.05) among the control and treatment groups. Moreover, the control, T1 & T3 groups had no significant variation among them but a higher PER value is noticed in T2 group. Plant-based proteins are less digestible than animal proteins due to the different structure of plant versus animal proteins (FAO, 2013) [6]. Most fortified diets had PER similar to the reference protein despite the protein intake for the latter being higher and is probably due to compositing cereals with legumes which yield high protein quality (FAO/WHO, 2002) [7]. The significant difference in PER in the control and treatment pet food samples which slowed down the effective utilization of the proteins and the present results are in accordance with the report of FAO/WHO, (2002) [7] and FAO, (2013) [6].

Table 1: Quantity of ingredients used for the various kibbles formulation in percentage

S. No.	Ingredients	C (Control) Plain (Vegetarian)	T1 Chicken Boneless Breast	T2 Whole Chicken	T3 Chicken offal meal
1	De oiled rice bran	27.2	24.65	24.9	27.8
2	Maize	26.0	24.55	25.2	29.2
3	Rice flour	20.0	20.0	20.0	20.0
4	Soya (CP 48%)	19.2	13.2	14.475	5.775
5	Chicken product	0	10.0	10.0	10.0
6	Tallow	5.0	5.0	3.0	5.0
7	DCP	1.726	1.717	1.272	1.372
8	Methionine	0.203	0.224	0.223	0.194
9	Choline chloride	0.150	0.150	0.150	0.150
10	Trace minerals	0.150	0.150	0.150	0.150
11	Salt	0.1126	0.0365	0.0346	0.0369
12	Additives	0.10	0.10	0.10	0.10
13	Calcite powder	0.0460	0.0115	0	0
14	Threonine	0	0	0.0180	0.0079
15	Antioxidant	0.12	0.25	0.52	0.25
16	Others	0	0	0	0
		100.0	100.0	100.0	100.0

Table 2: Cumulative weekly body weight (g) (Mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibbles

Type of kibble (n = 6)	Weeks (After 21 days weaning period +3 days adaptation period)			
	0^{th} week	1^{st}week (24-31^{st}day)	2^{nd} week (32-38^{th}day)	3^{rd} week (39-45^{th}day)
C (Control)	$47.66^{a} \pm 2.30$	$63.17^{a} \pm 2.09$	$79.50^{a} \pm 2.16$	$97.67^{a} \pm 2.51$
T1	$47.83^{a} \pm 1.62$	$71.17^{b} \pm 1.45$	$90.50^{b} \pm 2.89$	$110.17^{b} \pm 4.25$
T2	$47.8^{a} \pm 1.62$	$74.67^{b} \pm 1.69$	$99.33^{c} \pm 2.23$	$123.33^{c} \pm 3.12$
T3	$47.83^{a} \pm 2.04$	$69.67^{b} \pm 2.38$	$91.33^{b} \pm 2.96$	$111.00^{b} \pm 3.87$
P-Value	1.000^{NS}	0.004*	0.000*	0.001*

Means with at least one common superscript within columndo not differ significantly (**p*<0.05)

Table 3: Cumulative weekly body weight gain (g) (mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibbles

Type of kibble (n = 6)	Age / Week		
	1^{st}week(24-31^{st}day)	2^{nd} week(32-38^{th}day)	3^{rd} week(39-45^{th}day)
C (Control)	$15.50^{a} \pm 0.99$	$31.83^{a} \pm 1.58$	$50.00^{a} \pm 1.98$
T1	$23.33^{b} \pm 0.80$	$42.67^{b} \pm 1.41$	$62.33^{b} \pm 2.80$
T2	$26.83^{c} \pm 1.08$	$51.50^{c} \pm 1.36$	$75.50^{c} \pm 2.59$
T3	$21.83^{b} \pm 0.60$	$43.50^{b} \pm 1.03$	$63.17^{b} \pm 2.20$
P-Value	0.0001*	0.0001*	0.0001*

Means with at least one common superscript within columndo not differ significantly (**p*<0.05)

Table 4: Feed intake (g) (Mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibbles

Type of kibble (n = 6)	Weeks (After 21 days weaning period +3 days adaptation period)		
	1^{st}week(24-31^{st}day)	2^{nd} week(32-38^{th}day)	3^{rd} week(39-45^{th}day)
C (Control)	$55.06^{a} \pm 2.99$	$62.83^{a} \pm 1.33$	$79.56^{c} \pm 2.33$
T1	$52.50^{a} \pm 0.67$	$64.00^{a} \pm 0.75$	$74.83a^{b} \pm 1.13$
T2	$56.28^{a} \pm 1.09$	$67.78^{b} \pm 0.56$	$71.00^{a} \pm 0.52$
T3	$59.33^{a} \pm 0.75$	$72.16^{c} \pm 0.97$	$78.50^{bc} \pm 1.11$
P-Value	0.061^{NS}	<0.001	0.002

Means with at least one common superscript within column do not differ significantly (**p*<0.05)

Table 5: Feed conversion ratio (g) (Mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibble

Type of kibble (n = 6)	Weeks (After 21 days weaning period +3 days adaptation period)		
	1^{st}week(24-31^{st}day)	2^{nd} week(32-38^{th}day)	3^{rd} week(39-45^{th}day)
C (Control)	$3.58^{c} \pm 0.17$	$3.88^{b} \pm 0.19$	$4.39^{b} \pm 0.09$
T1	$2.26^{a} \pm 0.06$	$3.46^{b} \pm 0.34$	$4.02^{b} \pm 0.50$
T2	$2.11^{a} \pm 0.06$	$2.76^{a} \pm 0.08$	$3.01^{a} \pm 0.19$
T3	$2.73^{b} \pm 0.11$	$3.35^{ab} \pm 0.14$	$4.14^{b} \pm 0.36$
P-Value	0.0001	0.011	0.035

Means with at least one common superscript within classes do not differ significantly (**p*<0.05)

Table 6: Protein intake (g) (Mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibble

Type of kibble (n = 6)	Weeks (After 21 days weaning period +3 days adaptation period)		
	1st week (24-31st day)	2nd week (32-38th day)	3rd week (39-45th day)
C (Control)	$9.91^{a}\pm 0.54$	$11.31^{a}\pm 0.24$	$14.32^{bc}\pm 0.41$
T1	$9.96^{a}\pm 0.12$	$11.78^{a}\pm 0.14$	$13.77^{b}\pm 0.21$
T2	$10.29^{ab}\pm 0.19$	$12.40^{b}\pm 0.10$	$12.99^{a}\pm 0.10$
T3	$11.15^{b}\pm 0.14$	$13.57^{c}\pm 0.18$	$14.75^{c}\pm 0.21$
P-Value	0.012	<0.001	0.001

Means with at least one common superscript within classes do not differ significantly (*$p<0.05$)

Table 7: Protein efficiency ratio (g) (Mean ± SE) in Wistar rats (*Rattus norvegicus*) fed with different formulations of kibble

Type of kibble (n = 6)	Weeks (After 21 days weaning period +3 days adaptation period)		
	1st week (24-31st day)	2nd week (32-38th day)	3rd week (39-45th day)
C (Control)	$1.57^{a}\pm 0.07$	$1.45^{a}\pm 0.08$	$1.27^{a}\pm 0.03$
T1	$2.41^{c}\pm 0.06$	$1.64^{a}\pm 0.15$	$1.43^{a}\pm 0.13$
T2	$2.62^{c}\pm 0.07$	$1.99^{b}\pm 0.06$	$1.85^{b}\pm 0.11$
T3	$1.96^{b}\pm 0.07$	$1.60^{a}\pm 0.07$	$1.33^{a}\pm 0.10$
P Value	0.0001	0.007	0.003

Means with at least one common superscript within classes do not differ significantly (*$p<0.05$)

Conclusion

In the existing study the kibbles intake by Wistar rat in the groups are higher in control, T1 and T3 than T2. Similarly, the cumulative weight gain is higher in T2 than the control, T1 and T3, correspondingly the weekly body weight gain also following the same trend. The treatment group (T2) fed with whole chicken based pet food had a low dry matter intake and exhibited a higher weight gain followed by T1 and T3 than the control pet food with vegetarian diet. Comparatively a higher PER was noticed in T2 than T1, T3 and control. Hence, it is concluded that the Wistar rat groups fed with whole chicken based kibbles had a higher weight gain, followed by offal meal and chicken boneless breast based kibbles. Finally, the control pet food with soya had lower weight gain than the other kibbles.

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