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The effect of protein vs carbohydrate rich diet on the behaviour of juvenile Indian free-ranging dogs

Tanima Choudhury

Abstract
Domestic dogs have evolved from the carnivorous wolf. During the process of domestication, the dogs underwent directional selection which targeted the gene Amy2B. This process had enabled dogs to survive on more starch rich diets than their ancestors' carnivorous diet. In India, free-ranging dogs are found mainly in environments rich in carbohydrate based foods but poor in protein foods. Juvenile free-ranging dogs, which are in their growth phase, have higher energy requirements than adults. In our experiment we provided twenty juvenile dogs with either a carbohydrate rich or a protein rich food source and tried to determine which was a better food source for the dogs. We observed that the dogs that fed on the protein foods performed active behaviours at a higher frequency. This result could indicate that despite the dogs' evolutionary change for more efficient starch metabolism, protein is still a better food source for dogs.

Keywords: Juvenile free-ranging dogs, protein foods, starch metabolism, dog domestication

Introduction
Free-ranging dogs (*Canis lupus familiaris*) consist of unconfined dogs that found both in the cities and in rural areas. These dogs are generally stray dogs and are not under direct human supervision. They are present in all kinds of habitats where human settlements are found, from urban settlements to forest fringes [1, 2]. So, they are appropriately called free-ranging dogs [3]. Free ranging dogs are found in numerous countries around the world, including Japan [4], India [5], Mexico [6, 7], Italy [8, 9] and some parts of Africa. These dogs are scavengers, living on the garbage produced by humans, in all habitations [1, 2]. Indian free ranging dogs live in an environment which is rich in starch based foods but poor in protein based foods. So, their diet consists mainly of carbohydrate (rice and wheat based foods) and relatively little protein (decomposing meat, garbage of meat shops etc.) [10]. They rarely hunt and so they do not get the chance to encounter any rich sources of protein [10].

Evidences on dog domestication have shown that the process began in the Upper Palaeolithic period (35,000 years BP). The early process called proto-domestication could have started unconsciously around 14,000 B.C. [11]. Genomic DNA analyses have shown that the process of dog domestication most probably began in the Middle East and then rapidly spread throughout the World [11, 12]. Indian free-ranging dogs have been mentioned in numerous Indian folklore and texts throughout the ages, either as household pets or as stray dogs [5]. They have lived as scavengers in the Indian environment for many generations [13]. Domestic dogs have descended from the carnivorous Asiatic wolves *(Canis lupus chanco)* [14]. Mitochondrial DNA analysis has showed that the closest relative of all domestic dogs is the Asiatic wolf. Therefore, the Asiatic wolf is most likely the ancestor of the modern domestic dogs [14, 15]. These dogs especially those living outside of human households rarely encounter rich sources of protein. As a result these dogs have become adapted to an omnivorous diet, which could be the result of the long process of dog domestication [16].

Genomic analysis of modern canids have shown that selective pressures involving various morphological, behavioural and physiological traits were experienced by the ancestral canids. Genomic DNA analyses of domestic canids and wolves, have identified the regions of the genome which experienced directional selection during domestication of modern canids. This process included many genes involved in energy metabolism and digestion of starch which are probably connected to the change of diet of the dogs through the ages [17].

The selection has led to several rounds of duplication of the gene that coded for pancreatic amylase (Amy2B) [17]. This led to the copy number of the gene Amy2B increasing by nearly seventeen fold in dogs in comparison to the wolves [17]. The increase in Amy2B gene's copy number is associated with higher amylase activity in domestic canids compared to wolves. The copy number range of Amy2B gene varies widely in modern dogs (4-34 copies) at population level. The copy number range for wolves is 2-8 copies at the population level with 60% of the population having only 2 copies [17]. This evolutionary change has enabled the domestic dogs to adapt to more starch rich diets, comparative to the protein rich diet of their ancestor, the carnivorous wolves [18].

Indian free-ranging dogs live either solitarily or in groups on the streets [19]. They breed twice a year with each female producing only one litter per year [20]. The pups are usually weaned around 5-6 weeks of age. During the weaning process, the mother starts to refuse to suckle the pups while they continue to attempt to suckle from their mother [21]. The free-ranging pups began to eat semi-solid food around 35 days old. Around 5 weeks of age, the pups are given regurgitated food and sometimes even small animals killed by the mother. The mothers continue to suckle pups till they are around 3 months old [22, 23]. The pups, being in a stage of growth and development, have higher energy requirements than the adults. The pups have to scavenge for food during the post weaning phase of their life. These pups not only have to compete for food with their siblings and other dogs but also with their own mother. After weaning, the mother starts to refuse to share food with the pups and even competes with her pups over food [24].

The free ranging pups mainly get access to carbohydrate rich sources of food during the juvenile (3-6 months) stage of their life and rarely encounter good sources of protein (both due to scarcity of good protein food availability and also as a result of being driven away from the limited protein food available by the adult dogs). The aim of the present study was to determine if there would be any change in the behaviour of the juvenile dogs (in regards to frequencies of different behaviours) if they were provided with either a protein rich diet or a carbohydrate rich diet.

Materials and Methods

All experiments were conducted in the Khardah municipality (22^0 71'86"N, 88^0 37'81"E), North 24 Parganas district, West Bengal, India. The experimenter walked on the streets and located the juvenile dogs. Ad-libitum observations were conducted to determine movements, scavenging sites and resource availability of the dogs. All dogs which were regularly fed by people or were near meat shops and restaurants where they had better access to protein rich foods were excluded from the experiment. We also avoided the dogs that were near busy roads (as there was an increased risk of the pups dying from road accidents during the course of the experiment). The pups that had been weaned and were actively scavenging, were selected for the experiment. A total of 20 pups (11 males and 9 females) were selected and divided into 2 groups of 10 individuals each. Pups of the same litter were included in the same group. The two groups were designated as – P (protein group) and C (carbohydrate group). The pups were designated as -Do1, Do2, Do3, Do4, Do5, Do6, Do7, Do8, Do9, Do10, Do11, Do12, Do13, Do14, Do15, Do16, Do17, Do18, Do19 and Do20 (the pups were designated a number on the basis of first encounter- Do1 was the first pup encountered during the ad-libitum observation).

Table 1: Distribution of pups into the two groups -P and C

P group	C group
Do1, Do5, Do6, Do7, Do8, Do13, Do14, Do15, Do16, Do18	Do2, Do3, Do4, Do9, Do10, Do11, Do12, Do17, Do19, Do20

The litters of pups were randomly distributed into the two groups.

The pups in the P-group were provided with 200gms of raw chicken (cut into small pieces) in the morning (800-900 hrs) and in the evening (1800-1900 hrs). The pups in the C-group were provided with 200gms of cooked rice (miniket rice) in the morning (800-900 hrs) and in the evening (1800-1900 hrs).

We carried out behavioural observations on the pups in the P and C groups from the age of 4-6 months (12th April- 5th June 2015) as we were interested in the completely weaned pups, weaning occurs around 3 months old [23]. The experimenter conducted all occurrence sampling (AOS) on the two groups in two sessions, each of 2 hrs, morning session (1000-1200 hrs) and afternoon session (1500-1700 hrs) for 55 days. Thus, we had 220 hrs of AOS data. We divided all the different types of recorded behaviour into two groups-Active behaviours and Non-active behaviours. We calculated the rate (frequency per min) at which the two groups performed active and non-active behaviours separately from the AOS data. A list of the active and non-active behaviours used in the analysis has been provided in Table 2. All statistical analysis was carried out using statisti XL v. 1.8.

Table 2: Behaviours observed during all occurrence sampling

Active Behaviours	Non-active Behaviours
Walking,	Sleeping
Grooming (both self and allogrooming)	Sniffing
Begging	Raising head
Wagging tail	Lying down
Eating	Sitting
Drinking	Watching
Scavenging	Panting
Following	Yawning
Chasing	Vomiting
Playing	Stretching legs
Surrendering	Scratching
Play fighting	Shaking
Climbing	Rolling over
Tugging	Dozing
Snarling	Urinating
Chewing an inanimate object	Defecation
Digging	
Jumping	
Barking	

Ethical Note

The experiments were conducted on the pups living as stray dogs in the streets without causing any harm to them. The food used for the experiment was prepared fresh. Invasive techniques were not used in these experiments.

Results

We performed Wilcoxon Paired Sample test on the average of the frequencies of the active and non-active behaviours of the individuals of the P-group. We found a significant difference in the rate at which active and non-active behaviours were performed by the pups of the P-group (Wilcoxon Paired Sample test: $T=31.0$, $N=30$, $p=0.0001$). The non-active behaviours were performed at a much higher rate than the active behaviours (refer to figure 1).

We repeated the same statistical test on the data collected from the C-group and similarly the non-active behaviours were performed at a higher rate by the pups of the C-group (Wilcoxon Paired Sample test: T=72.500, N=30, p=0.001) (refer to figure 2).

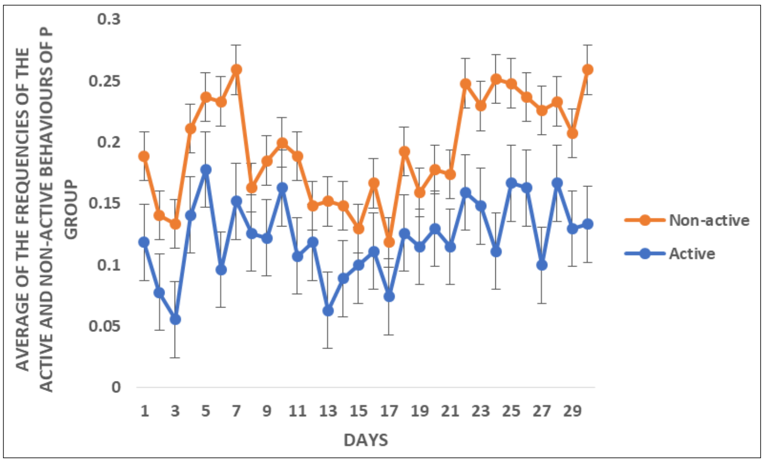

Fig 1: AOS data of average of the frequencies of the active and non-active behaviours of the pups of P-group. Non active behaviour were performed at a higher frequency *(Wilcoxon Paired Sample test: T=31.000, N=30, p=0.0001).*

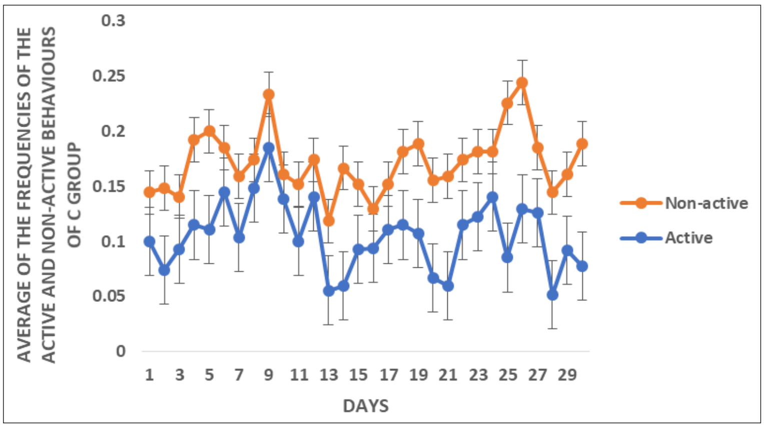

Fig 2: AOS data of average of the frequencies of the active and non-active behaviours of the pups of C-group. Non- active behaviours were performed at a higher frequency *(Wilcoxon Paired Sample test: T=72.5, N=30, p=0.001).*

The pups of both groups appear to spend majority of the time performing non active behaviours. This could be due to the pups trying to avoid unnecessary wastage of energy since there is high competition over food and majority of the hostile interactions between dogs take place at feeding grounds. We observed that the pups of the P- group performed active behaviours at a higher frequency than the pups of the C-group *(Mann-Whitney U test: U=590.000, df=30, p=0.039)*(refer to figure 3).We did not observe any significant difference in the frequencies of non-active behaviour performance between the two groups *(Mann-Whitney U test: U=509.500, df=30, p=0.390)*(refer to figure 4).

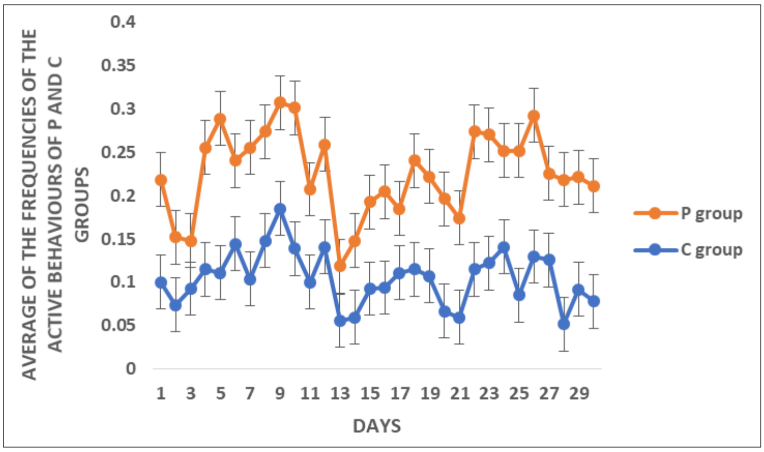

Fig 3: AOS data of the average of the frequencies of the active behaviours performed by the pups of the P and C groups. The P group pups performed active behaviours at a higher frequency *(Mann-Whitney U test: U=590.000, df=30, p=0.039).*

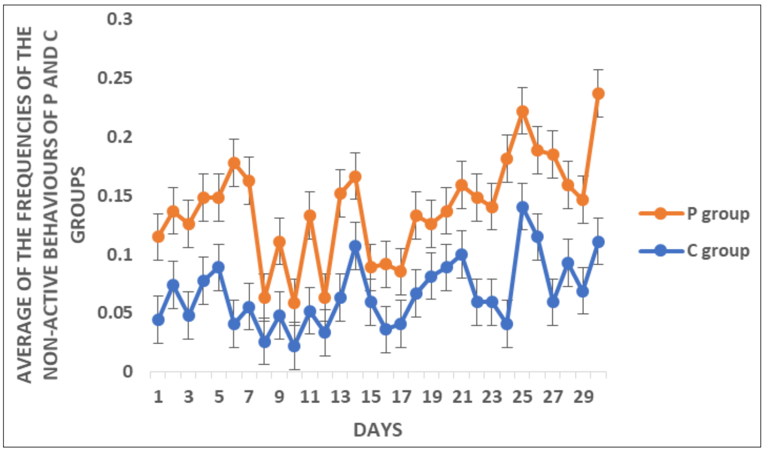

Fig 4: AOS data of the average of the frequencies of the non-active behaviours of the pups of the P and C groups. The difference found between the data recorded from both groups was found to be not significant *(Mann-Whitney U test: U=509.500, df=30, p=0.390).*

The pups of the P group were found to be more active than the pups of the C group. The dogs being descendants of a carnivorous ancestor were probably able to utilise the raw chicken (protein rich) more efficiently as energy source than the cooked rice (carbohydrate rich). This may have enabled the pups of the P group to acquire more energy from the food than the pups of the C group and so they were able to perform active behaviours at a higher frequency than the C group pups.

Discussion

The domestication of dogs was an important part of human history. It is not precisely known exactly when and why dogs were domesticated. The earliest verified dog remains (dating 12,000-11,000 years BP) was found buried in Israel together with human remains [11]. The ancestors of dogs may have initially come near human settlements to feed off the remains of the kills of the human hunter- gatherers. This probably led to the humans using the ancestral canids for hunting and guarding in exchange for food, leading to the formation of a close bond between the humans and the ancestral canids. Alternatively, the humans may have captured wolf pups and raised them. Most of the pups could have become aggressive as they grew older and thus were subsequently released into the wild. Occasionally a wolf pup was found to be friendly and remained with the humans even after it had become an adult. These canids could have been used for hunting and guarding which resulted in selection for traits that were important for these behaviours [15].

The humans gradually shifted from a nomadic hunter-gatherer to a sedentary agricultural lifestyle. With the shift in the lifestyle of the humans, there was a shift in the type of food offered to the ancestral canids. They were given a more starch rich diet in contrast to their previous protein rich diet [25]. This probably caused the ancestral dogs to undergo directional selection that targeted the gene Amy2B, increasing the gene's copy number in the dog population [18]. The Amy2B gene codes for pancreatic amylase which breaks down starch into maltose, thus enabling the canids to survive on a starch rich diet.

There are ten essential amino acids which the dogs cannot make by themselves. They obtain these essential amino acids from their protein foods [26]. The protein foods also contain the carbon chains which the dog's body uses to make glucose. The glucose is then used as a source of energy. Studies on the diet of domestic dogs have shown that when dogs are allowed to self-select they prefer to consume 39-44% of their energy requirements from proteins, 53-58% from fats and only 3% from carbohydrate [28]. Studies on the performance of working dogs have shown that a diet consisting of high fat or high protein composition with no carbohydrate resulted in better stamina and less exhaustion for the dogs. A diet consisting of higher carbohydrate resulted in higher muscle glycogen content in dogs but the glycogen was found to be used up too rapidly resulting in poor stamina and quicker exhaustion for the dogs [27]. Indian free-ranging dogs are present in an environment rich in carbohydrate but poor in protein food sources [10]. They survive by scavenging for food from garbage dumping sites or by begging humans to give them biscuits or leftover rice. The dogs face fierce competition at the feeding sites (dumps, dustbins etc) [28]. Indian free-ranging dogs, having limited access to protein foods, may have difficulty obtaining these ten essential amino acids. The pups require two times as much calories as the adults per 0.45kg of body weight [26]. The pups first start to experience semi-solid foods around 5-6 weeks of age. The mother gives the pups regurgitated food and sometimes even kills small animals for the pups. The pups are completely weaned at around 3 months old [22, 23]. These pups need to acquire nutrients from their food for not only maintaining normal body functions like the adults but also for their growth and development. At 4 months of age the pups are scavenging with their mother. During this time, the pup face competition at the feeding sites from the older dogs including their own mother and also from their siblings. This may lead to the pups being unable to get sufficient food for their normal growth and development. Our experiment shows that the pups (juvenile dogs) perform non-active behaviours at a higher frequency than active behaviours. This result could indicate that the pups try to avoid unnecessary wastage of energy. Studies done on the daily activity of Indian free-ranging dogs have shown that the dogs spend majority (52.7%) of their time either sleeping or lazing around [30]. In an environment where the pups can never be certain of their next meal, avoiding unnecessary energy wastage appears to be an efficient strategy for ensuring that they mainly utilise whatever little food they have acquired for maintaining normal physiological functions. This could probably increase their chances of surviving long enough to reach adulthood. Our experiment also show that the pups of the P -group performed active behaviours at a higher frequency than the pups of the C-group. This result could indicate that the pups were able acquire more energy from the raw chicken (protein rich) than from the cooked rice (carbohydrate rich). So it appears that the protein rich food is a better source of energy for the juvenile dogs than the carbohydrate rich food. As the studies on exercising dogs have shown that high protein diets result in the dogs having better stamina and less exhaustion from work than high carbohydrate diets. In spite of undergoing natural selection that enabled the dogs to be better adapted to more starch rich diets than their carnivorous ancestors, the dogs are still not able to utilise starch as efficiently as they are able to utilise protein as an energy source.

Conclusion

The data obtained from the experiment shows that juvenile free-ranging dogs prefer to spend most of their time performing non-activity behaviours. This enables the dogs to conserve energy and spend energy mostly on necessary activities. The protein rich diet was found to provide the pups with more energy as it enabled the pups to perform active behaviours at a higher frequency than the carbohydrate rich diet. The present study shows that protein is more efficient as an energy source than carbohydrate for Indian juvenile free-ranging dogs. So, the lack of availability of proper dietary proteins could be one of the causes for increased mortality of Indian juvenile free-ranging dogs.

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References

1. Vanak AT, Thaker M, Gompper ME. Experimental examination of behavioural interactions between free-ranging wild and domestic canids. Behav. Ecol. Sociobiol. 2009; 64:279-287.
2. Vanak A, Gompper M. Dietary niche separation between sympatric free-ranging domestic dogs and Indian foxes in Central India. J Mammal. 2009; 90:1058-1065.
3. Serpell J. The domestic dog: its evolution, behaviour and interactions with people. Second edition, Cambridge University Press, Cambridge, 2017, 342-368.
4. Kato M, Yamamato H. Survey of the stray dog population and the health education program on the prevention of dog bites and dog-acquired infections. Acta Med Okayama. 2003; 57(5):261-266.
5. Debroy B. Sarama and her children: the dog in Indian myth. First edition, Penguin, India, 2008, preface-2.
6. Ortega-Pacheco A, Rodriguez-Buenfil JC, Bolio-Gonzalez ME. A survey of dog populations in urban and rural areas of Yucatan, Mexico. Anthrozoos. 2007; 20:261-274.
7. Daniels TJ, Bekoff M. Population and social biology of free-ranging dogs, *Canis familiaris.* J Mammal. 1989; 70:754-762.
8. Boitani L. Wolf and dog competition in Italy. Acta Zool Fenn. 1983; 174:259-264.
9. Bonanni R, Valsecchi P, Natoli E. Pattern of individual Participation and cheating in conflicts between groups of free-ranging dogs. Anim. Behav. 2010; 79:957-968.
10. Bhadra A, Bhadra A. Preference for meat is not innate in dogs. J Ethol. 2014; 32:15-22.
11. Davis SJM, Valla. Evidence for domestication of dog, 12,000 years ago in the Natufian of Israel. Nature. 1978; 276:608-610.
12. Galibert F, Quignon P, Hitte C, Andre C. Towards understanding dog evolutionary and domestication history. CR Biol. 2011; 334(3):190-6.
13. Pal S. Population ecology of free-ranging urban dogs in West Bengal, India. Acta Theriol. 2001; 46:69-78.
14. Savolainen P, Zhang Y, Luo J, Lunderberg J, Leitner T. Genetic Evidence for an East Asian Origin of Domestic Dogs. Science. 2002; 298:1610-13.
15. Wills C. The Darwinian tourist: Viewing the world through evolutionary Eyes. First edition, Oxford University Press, United States, 2010, 189-207.
16. National Research Council. Nutrient requirements of dogs and cats. The National Academic Press, Washington, D.C, 2006.
17. Olivier M, Tresset A, Bastian F, Lagoutte L, Axelsson E, Arendt M *et al*. Amy2B copy number variation reveals starch diet adaptations in ancient European dogs. Royal Society Open Science. https://doi.org/10.1098/rsos.160449. 01 November, 2016.
18. Axelsson E, Ratnakumar A, Arendt M, Maqbool K, Webster M, Perloski M *et al*. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature. 2013; 495:1-5.
19. Sen Majumder S, Bhadra A, Ghosh A, Mitra S, Bhattacharjee, Chatterjee J *et al*. To be or not to social: foraging associations of free-ranging dogs in an urban ecosystem. acta ethol. 2014; 17:1-8.
20. Pal SK. Reproductive behaviour of free-ranging rural dogs in West Bengal, India. Acta Theriol. 2003; 48:271-281.
21. Malm K, Jensen P. Weaning and parent-offspring conflict in the domestic dog. Ethology. 1997; 103:653-664.
22. Pal SK. Parental care in free-ranging dogs, *Canis familiaris*, Appl. Anim. Behav. Sci. 2005; 90:31-47.
23. Pal S. Maturation and development of social behaviour during early ontogeny in free-ranging dog puppies in West Bengal, India. Appl. Anim. Behav. Sci. 2008; 111:95-107.
24. Bhadra A, Paul M, Sen Majumdar S. Selfish mothers? An empirical test of Parent-offspring conflict over extended parental care. Behavioural Processes. 2014; 103:17-22.
25. Arendt M, Cairns KM, Ballard JWO, Savolainen P, Axelsson E. Diet adaptation in dog reflects spread of prehistoric agriculture. Heredity. 2016; 117:301-306.
26. National Research Council. Your Dog's Nutritional Needs: A science based guide for pet owners. The National Academic Press, Washington, D.C, 2011.
27. Hill C. The Nutritional Requirements of Exercising Dogs. The Journal of Nutrition. 1998; 128:2686-2690.
28. Pal S, Ghosh Roy S. Dispersal behaviour of free-ranging dogs (*Canis familiaris*) in relation to age, sex, season and dispersal distance. Appl. Anim. Behav. Sci. 1998; 59:331-348.
29. Roberts M, Bermingham E, Cave N, Young W, McKenzie C, Thomas D. Macronutrient intake of dogs, self-selecting diets varying in composition offered ad-libitum. Journal of Animal Physiology and Animal Nutrition. 2018; 102:568-575.
30. Sen Majumdar S, Chatterjee A, Bhadra A. A dog's day with human-time activity budget of free-ranging dogs in India. Current Science. 2014; 106:874-878.