to minimize soil disturbance. The soil erosion map generated in the study would be helpful for land-use planners and policy makers to adopt the best site-specific best management practices to bring down the soil erosion rates within the tolerable limit.

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Protein-rich food does not affect singing behaviour and song quality in adult zebra finches, *Taeniopygia guttata*

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This study investigated whether short-term protein supplement to the seed-only diet during adulthood would impact the production and song features in male zebra finches (*Taeniopygia guttata*). In singly housed adult male zebra finches provided daily with seeds as food without or with 1 g of egg white protein supplement (equivalent to ~0.11 g egg proteins) under 12 h light : 12 h dark condition for 8 weeks, we recorded singing pattern at the beginning and end over 2 days when a female conspecific shared his cage. We found no effect of the egg protein supplement on daily song production, song bout duration and motifs per bout as well as the spectral features of the song, viz. motif duration, amplitude, pitch, goodness, mean frequency, frequency modulation, amplitude modulation or entropy. Perhaps, as sexually selected trait, song quality is not directly related to body metabolism. It is likely that seeds provided met energy requirement for vocalization (song production), and so additional egg white protein supplement did not affect the singing behaviour in adult zebra finches.

Keywords: Bird, food, protein, singing, song, zebra finch.

AMONG Passeriformes birds, male vocalization (singing) is a sexually selected trait for reproductive success, with territorial defence and mate attraction being its primary functions[1]. Male singing, as discernible by the song pattern, is species-specific[2], condition-dependent[3] and signals conspecific female about his physical condition[4]. Interestingly, and importantly, female presence can affect vocal learning and improvization of the male song[5] and a male song can have larger number of introductory notes and frequently repeated motifs when directed towards the female[6].

Among ecological factors, food has been shown to have significant effects on male singing. Pied flycatchers, *Ficedula hypoleuca*, sang more number of songs[7], and black-capped chickadees, *Poecile atricapillus*, increased dawn singing[8], when they were fed daily with 20 and 30 mealworms respectively. Similarly, Australian reed warblers (*Acrocephalus australis*) sang more on days when fed with 30 g of live blowfly maggots[9], and silvereyes (*Zosterops lateralis*) sang for a longer duration when fed

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with beef fat and sugar[10]. Further, 30% reduction in food availability early during the development impacted song learning, shown by poor copying of the tutor song in juvenile swamp sparrows (*Melospiza georgiana*)[11]. At the nutrient level, the song rate was positively related to the plasma metabolites, viz. albumin and cholesterol levels in the European starlings, *Sturnus vulgaris*[12]. Particularly in zebra finches (*Taeniopygia guttata*), a widely studied model system for bird song[13], restricted feeding decreased undirected song production (singing in the absence of female) by almost two-thirds in adult males[14]. Also, mixing of husk to provided food grains caused reduction in the rates (increased singing latency), amplitude and frequency of song, with no effect on syllable repertoire in male zebra finches[15]. Juvenile zebra finches fed on seeds mixed with seed husks in 1 : 3 ratio during the first 30 days showed an attenuated song syntax accuracy but no effect on the song amplitude, duration or repertoire size in adult birds[16]. In a similar experiment, mixing of wood chips with seeds provided during the developmental period had no effects on song learning and vocal performance in zebra finches[17]. The effect of changes in the protein content of diet during juvenile stage has been studied on the growth, metabolism and courtship rate, but not on song production, during adulthood in zebra finches[18].

Apart from the variable and inconsistent findings about food effects on song, less is known whether food quality affects 'directed' male singing (song production in the presence of female) in songbirds[17]. However, the effect of egg-supplemented enrichment of the seed-only diet during weeks 3 and 4 of the 6-week experiment has been examined on courtship behaviour in adult zebra finches[19]. Atagan and Forstmeier[19] measured the effects on courtship before, during and after the food-enrichment by monitoring for 2 consecutive days for 5 min when a female was introduced in the male's cage. The authors recorded song (individual-specific phrase repeated over 0.8 sec) and visual displays, viz. beak wiping on perch, feather fluffing, perch hopping and dance movements (twisting of head and tail). Thus, song recorded for a very brief period was only used as a supportive measure (one of the 5 parameters) to assess the courtship, and was not used as measure of the effect of food supplement reflecting on the mate choice as preferred trait by female zebra finches. We also considered 2-week protein supplement period in the study inadequate for showing an effect on a parameter directly linked with the reproductive success. Therefore, we carried out the present study to specifically examine whether protein supplement to the seed-only diet during adulthood would impact the production and/or features (motifs per bout, amplitude, amplitude modulation (AM), pitch, mean frequency, frequency modulation (FM), goodness and entropy) of directed song in zebra finches. These song features are important variables for mate choice and are sensitive to changes in conditions of the surrounding[20] including food availability[14]. In singly housed adult male zebra finches provided daily with protein supplement to food seeds over 8 weeks, we recorded singing pattern over 2-day period when a female conspecific shared his cage.

This study was conducted on adult male zebra finches (*Taeniopygia guttata*) as per approval of the Institutional Animal Ethics Committee (IAEC) of the Department of Zoology, University of Delhi, India. Birds were taken from our breeding colony and housed singly in activity cum song-recording cages (42 × 30 × 54 cm) placed inside light-tight wooden boxes (58 × 52 × 68 cm). They were maintained on 12 h light : 12 h darkness (12L : 12D; L = 100 lux at cage floor level; D = 0 lux; temperature = 22 ± 2°C) with food (*Setaria italica* seeds) and water *ad libitum*. These birds were randomly assigned to one of the 2 groups of 7 each and birds of one of these groups were given a daily dietary protein supplement of 1 g of grated hardboiled egg white for 8 weeks. A food intake of approximately 3 g per day has been reported for laboratory-bred zebra finches[21], and so 1 g protein supplement was about one-third of the daily food intake. Although not accurately measured, 1 g of egg white with more than half of the total egg proteins[22] contains an estimated amount of 0.11 g proteins. The protein content of *Setaria italica* seeds is around 10% of dry mass[23]. The choice of egg white as protein supplement was based on the following observations. Zebra finches feed predominantly on grass seeds but also include high protein insect diet[24]. In the laboratory, boiled egg is a preferred source of the protein supplement[19], although overall food intake in adult males is lower than the young developing chick[25] or breeding female[26]. Birds were maintained on the feeding regime for a period of 8 weeks. We regularly checked the food consumption, and never found leftover of the food supplement in food spillage collected every morning. The body mass was measured both at the beginning and end of the experiment to examine the effect, in any, of the dietary supplement on body condition of zebra finches.

Both at the beginning and end of the experiment, a female was introduced in each cage and singing behaviour was recorded over a 2-day period. A Behringer C-2 Studio condenser microphone fitted to each cage recorded both call and song using M-Audio profire 2626 8-channel Sound Card and Nuendo Application software from Steinberg Media Technologies GmbH, Germany. The sound was recorded at 16-bit rate and 44.1 kHz sampling frequency. First, we calculated total number of song bouts produced per 24 h for each individual, and randomly selected 30 bouts were used to calculate the duration of a song bout and the number of motifs per song bout. Then, we computed various acoustic features of 30 randomly selected song motif, viz. the duration, amplitude and amplitude modulation, pitch, frequency and frequency modulation, and Weiner entropy.

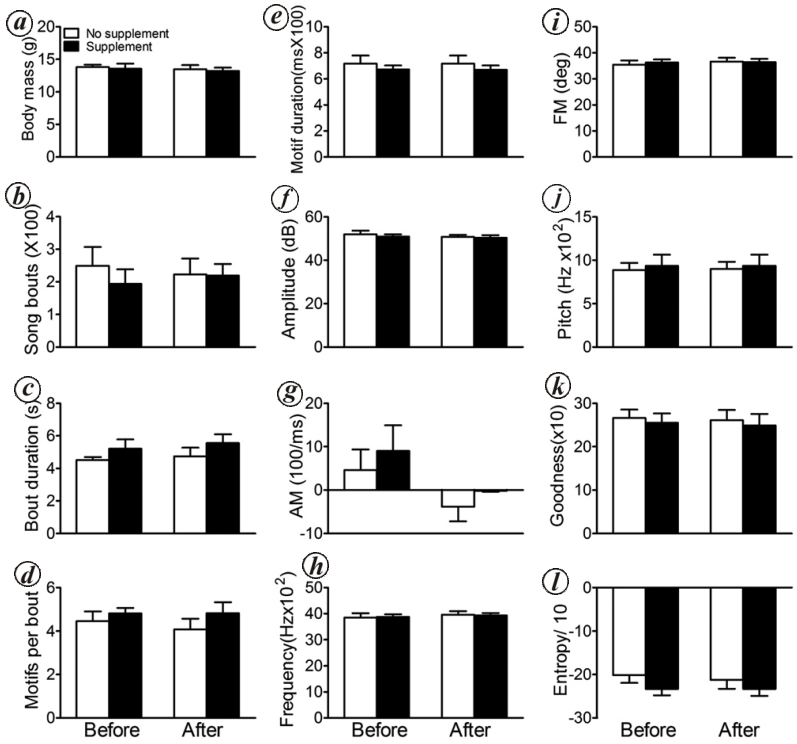

Figure 1. Mean (±SEM; $n = 7$ each) body mass (***a***) and song parameters (general features, ***a***–***c***; spectral features, ***d***–***k***) describing singing behaviour in zebra finches, *Taeniopygia guttata* before and after 8 weeks of food availability without (open bar) and with (closed bar) egg white protein supplement.

Statistical analysis was performed using IBM SPSSv20. We fitted a general linear model with repeated measures (GLM RM) to analyse differences in the song features. This considered food regime (no supplement/supplement), time (before/after) and song features (total song bout, bout duration, spectral features) together as factors (song features and time = within subject factors; food regime = between subject factors).

Figure 1 presents the results. There was no effect on body mass by the egg white protein supplement ($F_{1,12}$ = 0.21, $P = 0.66$; Figure 1 *a*). We found no effect of egg white protein supplement on the daily song production ($F_{1,12}$ =2.49, $P = 0.14$), song bout duration ($F_{1,12} = 0.02$, $P = 0.89$) and motifs per bout ($F_{1,12} = 1.78$, $P = 0.21$). Similarly, there were no differences between the two groups in spectral features of the song, viz. motif duration ($F_{1,12} = 0.25$, $P = 0.63$), amplitude ($F_{1,12} = 0.38$, $P = 0.55$), pitch ($F_{1,12} = 0.29$, $P = 0.92$), goodness ($F_{1,12} = 0.12$, $P = 0.73$), mean frequency ($F_{1,12} = 0.38$, $P = 0.55$), frequency modulation ($F_{1,12} = 0.29$, $P = 0.92$), amplitude modulation ($F_{1,12} = 0.69$, $P = 0.42$) or entropy ($F_{1,12}$ = 0.55, $P = 0.47$). Also, both the duration of experiment and its interaction with food condition (no supplement/supplement) did not show any significant effect, as above, on all the parameters analysed.

The egg-white protein supplement of about 0.11 g per day did not affect the singing behaviour, as shown by similar daily waveform (data not shown), song production and song features. These results should be viewed in the background of inconsistent findings on restricted feeding effects on song production in zebra finches[14–17]. Whereas a few studies on zebra finches showed that restricted feeding by decreasing the duration of food available[14] or by mixing husk to food grains given to adults[15] or juveniles[16] negatively impacted undirected song production in adult males[15], the other studies showed no effect of such food restrictions on adult singing[17]. Thus, our results also do not support the idea of bird song being sensitive to the energy balance during adulthood[27] in the sense that extra nutrient (protein) availability translates into effects on production and features of song in zebra finches. Interestingly, egg protein supplement has been reported to decrease the courtship rate in male zebra finches, perhaps due to detrimental effects of the high protein diet on male condition[19]. In the wild, high protein diet intake is usually during the chick-rearing period and, in general, required for enhancing the growth rates, body size, immune competence, metabolic rate and longevity[18,24]; chicks have higher protein requirement than the adult zebra finches[21]. Therefore, the availability of high protein diet in a caged situation might signal switching of hypothalamic mechanism(s) from mating efforts to parental care. This is an interesting idea that needs further study.

Our results differ from those of Atagan and Forstmeier[19] in the sense that we did not find a negative effect of egg supplement on song in adult male zebra finches. This could be due to several reasons including differences in the food supplement and measurement strategies. The previous study[19] lasted for a period of 6 weeks with only 2 weeks of protein supplement during weeks 3 and 4 of the study, and measured 0.8 s song over 5 min period on 2 consecutive days. Compared to this, we continuously provided egg supplement over 8 weeks of the experiment, and measured all song characteristics over 2-day period before and after the experiment. Further, we speculate that the negative impact of the high protein diet in previous study[19] was due to the fact that egg protein is not akin to the preferred natural source of diet protein, although it was palatable since zebra finches quickly ate all of it in the caged situation. It is also likely that as a sexually selected trait, song is not directly related to body metabolism, although frequent song bouts displaying intense singing behaviour lead to increased rates of oxygen consumption and probably account for a large proportion of direct energy demands in songbirds[26]. We would not rule out, however, that food seeds alone met energy requirement for the vocalization (song production), and so additional egg white protein supplement did not affect singing behaviour in adult zebra finches.

Conflict of interest: The authors declare that they have no competing interest.

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