Geomagnetic field absence influences adult body weight of a migratory insect, potentially through appetite regulation

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Highlights

- The GMF absence induced low phloem ingestion and body weight loss in *N. lugens*.
- The GMF absence alters *Crys* expression and orexigenic neuropeptide signaling.
- Up-regulated glucose level by the GMF absence is consistent with loss of appetite.
- The GMF could be a critical environmental cue for appetite regulation.

Abstract

The geomagnetic field (GMF) is well documented for its essential role in assisting animal orientation or navigation. Accumulating work has also shown that the GMF absence triggers stress-like responses, such as body weight loss we have found in the newly emerged adult brown planthopper, *Nilaparvata lugens*. Assuming that down-regulation of appetite is one crucial reason leading to the body weight decrease of *N. lugens* under the GMF absence, we explored magnetic responses of transcript expression of magnetoreception- and appetite-related genes, feeding behavior, and glucose level in older nymphs to a near-zero magnetic field (NZMF, i.e., GMF absence) vs. GMF. Besides the expected magnetic responses of the potential magnetosensor *cryptochromes*, the GMF absence also altered gene expression patterns of appetite-related neuropeptide genes in a way diminishing appetite. Combined investigation for feeding behavior of the 5th instar nymphs displayed a significant coincident decrease in food intake. Furthermore, a higher level of glucose, which was reported to cause food aversion, was also found in feeding defective 5th instar nymphs. These findings testify our hypothesis and highlight intriguing roles for the GMF in a migratory insect as the potential environmental regulator of appetite. This case again reminds that general magnetoreception mechanisms could be widely employed in various biological processes.

Keywords

Geomagnetic field absence; *Nilaparvata lugens*; Body weight; Appetite; Feeding behavior; Magnetoreception

1. Introduction

The geomagnetic field (GMF) is essential for orientation or navigation of many migratory animals as well as offers potential cue for the evolution of life on Earth. However, the details of the biophysical mechanisms are still an open question [1]. Three prevailing physically viable mechanisms, including radical-pair-based magnetoreception [2], magnetite-based magnetoreception [2], and electromagnetic induction detected through potential electroreceptor [3], have been suggested for terrestrial animals. Among them, cryptochrome (Cry)-mediated radical-pair-based magnetoreception have attracted the most attention, not only because it is theoretically feasible which is mainly based on quantum coherent evolution of the different spin states of radical pairs under the effects of magnetic interactions and spin-selective reactions but also due to the gaining experimental evidence from plant to animal [2,4-8].

Despite the narrow sense of magnetoreception which mainly refers to the acute behavioral responses of animals to the physiological changes in the GMF [9], increasing knowledge of the chronic magnetic responses of organisms to either physiological or nonphysiological magnetic field (including the GMF) conditions have addressed the importance of general magnetoreception in life activities across taxa [7,10-16]. More theories have been supposed especially against nonspecific magnetic responses triggered by different field intensity, such as the level mixing mechanism [17] and dipolarly coupled three-spin mechanism [18]. Among different magnetic field intensity treatments, more and more studies concern the bioeffects induced by the near-zero magnetic field (NZMF) [10,12]. On the one hand, it is urgent for human beings to prepare well for the interplanetary flights in the near future where the field is ~10,000 times weaker than that of Earth's [19], on the other hand, the NZMF can be taken as a sham control mimicking GMF canceling sham condition for the study of specific magnetoreception mechanisms [20,21]. Indeed, growing evidence from the study with NZMF has addressed the importance of GMF in maintaining organism homeostasis [15,20,22].

With three closely related species of migratory rice planthoppers, *Nilaparvata lugens*, *Laodelphax striatellus* and *Sogatella furcifera*, we showed that the GMF absence (i.e., the NZMF) could affect their physiological and behavioral processes, such as nymphal development, adult longevity, body weight, wing dimorphism, positive phototaxis, and flight behaviors, potentially through a Cry-mediated hormone signaling [13-15], which indicated the GMF's function in animal physiological homeostasis. Body weight is closely related to energy homeostasis in animals, and migratory fuelling which is characterized by gainning in body mass and food intake during migration was proved to be directed by the GMF information (intensity, direction, or both) [11,23-26]. Interestingly, recent studies showed field intensity-dependent changes in the production of reactive oxygen species (ROS) which are regarded as signaling molecules for appetite-related neurons [22,27,28]. Moreover, magnetic responses of adipokinetic hormone (AKH)/ AKH receptor (AKHR) signaling, which is reported to involve appetite regulation [29,30], was also found in adult rice planthoppers [13]. Therefore, we are curious about the potentially deleterious effect of the NZMF on the body weight of newly emerged adults, and we speculated that the GMF absence-induced body weight decrease in rice planthoppers could be due to appetite loss.

Considering both nanoscale magnetite particles [31] and potential magnetoreceptor, Crys (Cry1 and Cry2) [32], have been systematically characterized in the migratory brown planthopper, *N. lugens*. Together with the advantage of the published draft genome sequence [33], *N. lugens* is a promising insect model for the in-depth exploration of magnetoreception mechanisms. In addition to the potential magnetoreceptor Crys and multifunctional AKH-AKHR signaling, here we also explored the transcript expression of orexigenic neuropeptide F (NPF) and short NPF (sNPF) [30,34], as well as feeding behavior of older nymphs. Combining the investigation of magnetic field effects on glucose level which is suggested negatively correlated with appetite [35,36], we reported the first evidence that the GMF may work as an environmental regulator of appetite to maintain energy homeostasis in animals.

2. Material and methods

2.1. Insect stock

The migratory adults of brown planthoppers, *N. lugens* were collected from the paddy fields of Jiangsu Academy of Agricultural Science at Nanjing, Jiangsu province of China. The planthopper stocks were transferred to the test room and maintained on Taichung Native 1 (TN1) rice seedlings (15-30 days after planting) to expand the colony at temperature of 25±1□, 70-80% RH and 14:10 h light: dark cycle (Dark during 1800-0800 hours; All following assays were under the same environmental conditions). The Kimura B nutrient solution was used to provide nutrition for rice seedlings. The newly emerged macropterous adults were selected from the same generation of this expanded colony for the following experiments.

2.2. Magnetic field setups and insect exposures

Two common methods are usually utilized to mimic the absence of the GMF, either by shielding with the alloys like mu-metal or through compensation with Helmholz coils. Here, we used two DC-type Helmholtz coils system (External diameter: 1200 mm) to simulate the NZMF (i.e., the GMF absence; mimic intensity: 484 ± 29 nT) and the local GMF intensity (32° 3' 42" N, 118° 46' 40" E; mimic intensity: 50000 ± 254 nT) at same declination (−5.2 ± 1.76°) and inclination (50.2 ± 1.19°) within the effective homogeneous areas of 300 × 300 × 300 mm^3. The simulated magnetic fields were measured and adjusted with a fluxgate magnetometer (Model 191A, HONOR TOP Magnetoelectric Technology Co., Ltd., Qingdao, China) daily. To secure uniform environmental factors such as temperature, background disturbances, and other vibrations, the two coils systems were located in the same room with a distance of 6.5 m between each other (to avoid interference with each other). Follow the previous workflow and standards for the one generation exposure of rice planthoppers (from mated F0 females to newly emerged F1 adults or older nymphs before the following tests) [14]. We made one (from mated F0 females to newly emerged F1 adults) or less than one generation (from

mated F0 females to older nymphs) exposure to the NZMF vs. GMF conditions because *N. lugens* would usually begin the next wave of long-distance migration when adult offsprings emerged after at least one generation's preparation [37].

2.3. Developmental periods of nymphs and body weight of newly emerged adults

Developmental periods of nymphs were investigated following the previous work with white-backed planthopper, *S. furcifera* [14]. Considering the sex identification for the older nymphs used in the following molecular, behavioral, and biochemical assays are difficult and nonessential, we counted in both sexes for later data analyses of nymphal duration. Once the F1 adults emerged, individual *N. lugens* from the NZMF and GMF were identified to sex and weighed using precision scales (XP2U, Mettler Toledo International Inc., Columbus, OH, U.S.A.) with an accuracy of ± 0.1 μg.

2.4. Transcript expression analysis of magnetoreception-related and orexigenic neuropeptide genes

The potential magnetoreceptor *Crys* (*Cry1* and *Cry2*), and genes mediating Adipokinetic hormone (*AKH*, *AKHR*) and neuropeptide Y (NPY)-like (*NPF*, *sNPF*) signalings which involves appetite regulation were selected for transcript expression analysis by quantitative real-time polymerase chain reaction (qRT-PCR) assay. Ten individual 4th or 5th instar nymphs, which were starved for one hour were randomly mixed as one sample for each sampling time with six repeats (frozen at the same time of 1000 hours by liquid nitrogen). RNA isolation, reverse transcription, and qRT-PCR assay were conducted following previous work [13]. The reference genes in the transcript expression analyses were *RPL5* and *18S*, and all primer information for this study was listed in Table S1.

2.5. Electrical penetration graph (EPG) assay for feeding behavior of nymphs

The EPG assay was conducted to monitor the feeding behavior of the 5th instar nymphs. The test arena was set up inside a Faraday cage using a GIGA-8 DC EPG amplifier system (EPG system, Wageningen University) within the effective homogeneous area of the simulated magnetic fields. In case of magnetic field anomaly, an aluminum holder was used for supplying test channel input

terminals, and nymph individuals were wired through gold wires and conductive silver glue (diameter: length = 18.5 μm: 3 cm) on their dorsum. The 5th instar nymphs randomly sampled from different time after molting were used in the feeding behavior test. Nymphs are also starved for one hour before the test, and EPG was continuously recorded for six hours. The start time for the EPG assay was the same with daily sampling time for gene expression analysis (starve from 0900-1000 hours, and test from 1000-1600 hours which is in the middle of the objective light period). Fifteen valid replicates under the NMZF vs. GMF were respectively conducted, and four hours of records starting from the feeding beginning were analyzed with EPG Stylet + a software (Wageningen Agricultural University, 2012). Other details of the EPG assay follow our previous work with rice planthoppers [38].

2.6. Measurement of glucose content in the nymph

The glucose content of the 5th instar nymphs was determined using commercial assay kits (Glucose assay kit; Jiancheng Bioengineering Institute, Nanjing, China) according to the manual. Nymphs were starved for one hour before the test, and fifteen individual nymphs were randomly mixed as one sample for each sampling time with six repeats. The daily sampling time is the same as that for gene expression analysis.

2.7. Statistical analysis

All data were analyzed using SPSS 20 (IBM Inc., Armonk, NY, U.S.A.). In this study, all outcomes were directly separated by sampling time (developmental stage) or sex to investigate the main effect of the magnetic field. Therefore, we didn't take sampling time (developmental stage) or sex as the fixed factor in general linear models, and no post-hoc multiple comparison test for the sampling time (developmental stage) were performed. Before the analysis, data were tested for normality and homoscedasticity of variances using the Shapiro-Wilk test ($P > 0.05$) and Levene's test ($P > 0.05$), respectively. If those conditions were satisfied, a parametric one-way analysis of variance (ANOVA; $P < 0.05$) was employed, if not, a two-tailed nonparametric Mann-Whitney U test was applied to

determine differences in measured variables between experimental groups for female, male, each sampling time (developmental stage) or each specific feeding waveform. Effect sizes were estimated using partial η^2 and Cohen's d for ANOVA (small effect: partial $\eta^2 = 0.01$; medium effect: partial $\eta^2 = 0.06$; large effect: partial $\eta^2 = 0.14$) and Mann-Whitney U-test (small effect: $d = 0.2$; medium effect: $d = 0.5$; large effect: $d = 0.8$), respectively, based on the benchmarks of Cohen [39].

3. Results and discussions

3.1 Nymphal duration and the newly emerged adult body weight

To choose the instar (s) which could be potentially more sensitive to the GMF absence and thus may contribute more to the magnetic field effects on adult weight, we double-checked the nymphal development and body weight of newly emerged adult *N. lugens*. In coincide with our previous report [15], significantly prolonged duration of the 4th (+34.19%; $U_{1, 110} = 687.00$, $P < 0.001$, $d = 1.10$) and 5th (+16.96%; $U_{1, 110} = 1124.50$, $P = 0.008$, $d = 0.50$) instar and significantly decreased the body weight of newly emerged female (-14.67%; $F_{1, 47} = 24.23$, $P < 0.001$, partial $\eta^2 = 0.34$) and male (-13.17%; $F_{1, 68} = 24.14$, $P < 0.001$, partial $\eta^2 = 0.26$) adults were found in the current study (Table 1). It suggested that the GMF is indispensable to maintain the normal physiological homeostasis, especially for the nymphs at 4th and 5th instar. Therefore, we chose the 4th and 5th instar nymphs of *N. lugens* to explore the appetite regulation potentially influenced by the GMF.

Table 1. Nymphal duration and the newly emerged adult body weight of *Nilaparvata lugens*, under the near-zero magnetic field (NZMF, i.e., GMF absencce) vs. geomagnetic field (GMF).

Parameters	GMF	NZMF
Nymphal duration (days)*		
1st instar	3.28 ± 0.11 a	3.17 ± 0.13 a
2nd instar	2.87 ± 0.09 a	2.71 ± 0.10 a
3rd instar	2.98 ± 0.11 a	2.98 ± 0.09 a
4th instar	2.72 ± 0.07 b	3.65 ± 0.14 a
5th instar	3.43 ± 0.14 b	4.00 ± 0.12 a
Adult body weight (mg)**		
Female	2.025 ± 0.041 a	1.728 ± 0.044 b
Male	1.595 ± 0.029 a	1.385 ± 0.031 b

* Nymphal duration: n = 52 and 60 for each nymphal stage under NZMF and GMF, respectively.

** Adult body weight: n = 25 and 33 for female and male under NZMF, respectively. n = 24 and 37 for female and male under GMF, respectively. Newly emerged adults were measured for adult body weight.

Note: Different lowercase letters show significant differences between the GMF and NZMF tested respectively by two-tailed Mann-Whitney *U*-test for the nymphal duration at the same developmental stage, and by one-way ANOVA for the female or male adult body weight at $P < 0.05$.

3.2 Dynamic transcript expressions of magnetoreception-related *Cryptochromes* and appetite-related genes at 24h and 48h after molting into the 4th or 5th instar

We then investigated the dynamic transcript expressions of multifunctional Cry1 and Cry2. As expected, the *Drosophila-like Cry1* [8,40,41], which is the most promising magnetosensor so far, showed significantly up-regulated gene expression at 48h after molting into the 4th (+22.41%; $F_{1, 10} = 11.03$, $P = 0.008$, partial $\eta^2 = 0.52$) and 5th (+27.12%; $F_{1, 10} = 14.34$, $P = 0.004$, partial $\eta^2 = 0.59$) instar under the NZMF compared with the GMF. We also found significantly altered *Cry2* expression

pattern by elimination of the GMF at 24h after molting into the 4th (+32.32%; $F_{1, 10}$ = 22.02, P = 0.001, partial η^2 = 0.69) and 5th instar (+65.43%; $F_{1, 10}$ = 34.93, P < 0.001, partial η^2 = 0.78), and at 48h after molting into the 5th instar (-16.65%; $F_{1, 10}$ = 10.34, P = 0.009, partial η^2 = 0.51; Figure 1a). Indeed, the vertebrate-like Cry2 has also been found closely related to magnetoreception in cockroaches [8,42], while because it was classified as vestigial flavoprotein [40], and would unlikely to be the magnetoreceptor, Cry2 may work together with another chromophore (e.g., FAD in *Drosophila-like* Cry1) in magnetoreception. Moreover, given that Cry2 is a core component of the circadian clock [43,44], which has been proved to be sensitive to changes in magnetic field intensity [20,45], the altered gene expression pattern of *Cry2* here was more likely due to its role in circadian mechanisms.

Previous work in adult *S. furcifera*, the closely related species to *N. lugens*, suggested a Crys-circadian clock-AKH/AKHR signaling pathway in response to the GMF absence [13]. AKH is an insect analog of the human hormone glucagon [29]. The AKH/AKHR signaling plays crucial roles in energy mobilization [46], antioxidative stress reactions [47], anti-obesity, and appetite regulation [29]. Interestingly, AKH/AKHR signaling was also proved to involve the magnetic responses in the 5th instar nymph of *N. lugens*. The transcript expression of *AKH* was significantly up-regulated at 48h after molting into the 5th instar (+23.51%; $F_{1, 10}$ = 15.28, P = 0.003, partial η^2 = 0.60), while *AKHR*, which is a typical G protein-coupled receptor, was significantly down-regulated at both 24h (-41.93%; $F_{1, 10}$ = 31.21, P < 0.001, partial η^2 = 0.76) and 48h (-40.17%; *c*) after molting into the 5th instar. AKH in *Drosophila* is an orexigenic neuropeptide, and the AKH deficiency by knockout *AKH* or *AKHR* causes obesity with decreased food intake [29]. *AKHR* silencing was also reported to cause obesity in *N. lugens*, while AKH application produced a slim phenotype [48]. Therefore, in our case of the 5th instar nymphs, significant down-regulation of *AKHR* at 24h and 48h likely lead to hypophagia. The *AKH* and its receptor *AKHR* may work reciprocally in the 5th instar nymphs of *N. lugens* to maintain energy homeostasis stressed by the GMF absence. Two neuropeptide Y

(NPY)-like appetite regulatory factor, *NPF* and *sNPF* were also found sensitive to the GMF elimination in both 4th and 5th instar nymph. For *NPF*, consistent significant down-regulation of gene expression was found at 24h after molting into the 4th instar (-35.50%; $F_{1, 10} = 29.37$, $P < 0.001$, partial $\eta^2 = 0.75$) and at 48h after molting into the 5th instar (-16.16%; $F_{1, 10} = 11.16$, $P = 0.007$, partial $\eta^2 = 0.53$), which indicated a decreased appetite in the older nymphs. Similar significant down-regulated gene expression of sNPF was found at both 24h (-19.84%; $F_{1, 10} = 13.11$, $P = 0.005$, partial $\eta^2 = 0.57$) and 48h (-17.03%; $F_{1, 10} = 10.04$, $P = 0.010$, partial $\eta^2 = 0.50$; Figure 1b) after molting into the 5th instar.

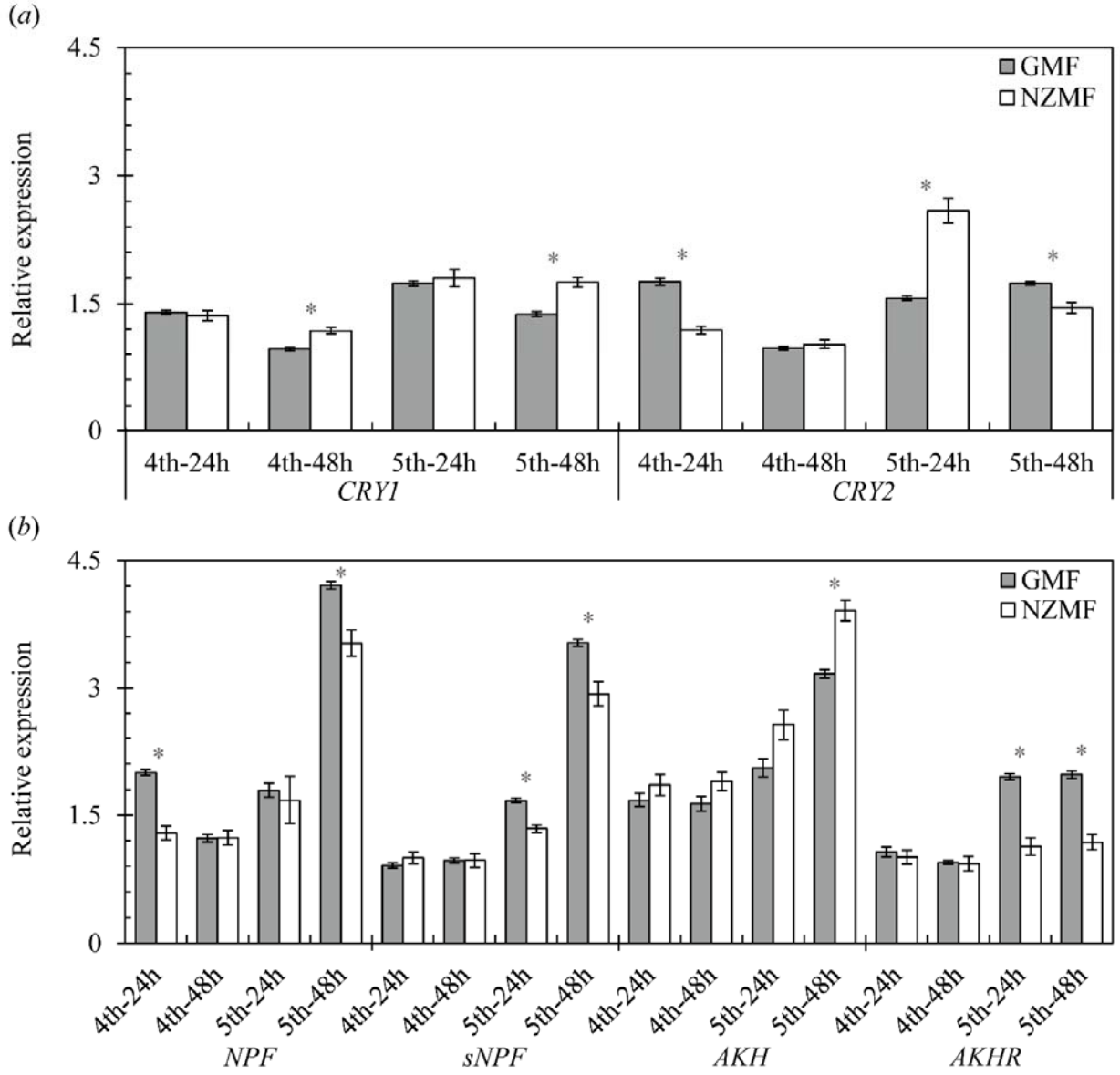

Figure 1. Temporal gene expression patterns of (a) magnetoreception-related *Cryptochromes* (*Cry1* and *Cry2*) and (b) appetite-related *NPF*, *sNPF*, *AKH and AKHR* at 24h and 48h after molting into the 4th and 5th instar of *Nilaparvata lugens*, under the near-zero magnetic field (NZMF) vs. the geomagnetic field (GMF). Ten individual nymphs were randomly mixed as one sample for each sampling time with six repeats. The columns represent averages with vertical bars indicating SE. Only significant differences between NZMF and GMF for each gene at the same sampling time are marked with asterisks tested by the one-way ANOVA at $P < 0.05$.

3.3 Analysis of feeding behavior of the 5th instar nymphs by the electrical penetration graph assay

It is reported that sNPF, unlike NPF, acts as a stimulating or suppressing factor in the more intricate species-specific way [34]. Combing the facts that both AKH/AKHR and NPY-like signaling in the 5th instar nymphs all showed magnetic responses to the NZMF vs. GMF, we measured the feeding behavior of the 5th instar nymphs to check the potential appetite down-regulation by the GMF absence. As expected, a significant decrease in the N4b feeding waveform duration (-32.02%; $F_{1, 28}$ = 4.93, P = 0.035, partial η^2 = 0.15), which indicated decreased phloem ingestion [49], suggested the loss of appetite in the 5th instar nymphs under the NZMF vs. GMF. Hence, the sNPF in *N. lugens* should be an appetite stimulator. In addition to the main nutrient intake feeding waveform N4b, duration (+48.54%; $F_{1, 28}$ = 4.37, P = 0.046, partial η^2 = 0.14) and frequency (-43.41%; $U_{1, 28}$ = 170.00, P = 0.016, d = 0.97) of feeding pathway phase P and frequency of xylem feeding waveform N5 (-66.67%; $U_{1, 28}$ = 164.00, P = 0.033, d = 0.85; Figure 2) of the 5th instar nymphs were also significantly altered in the GMF absence vs. GMF [49], which may be due to potential magnetic field effects on the salivary sheath-involved regulators [50].

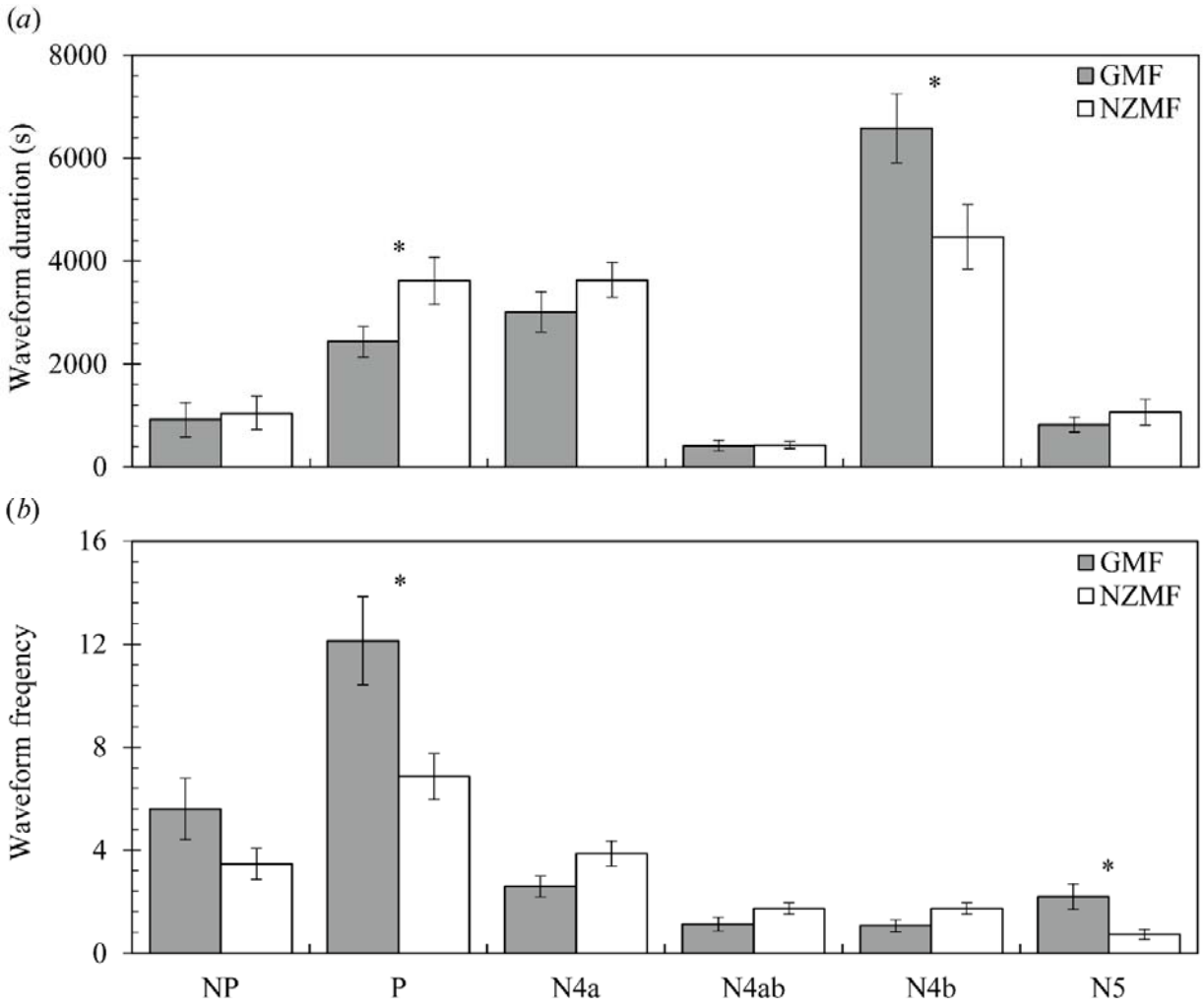

Figure 2. The geomagnetic field (GMF) absence altered both duration (a) and frequency (b) of specific feeding waveform in 5th instar nymph measured through the electrical penetration graph assay. NZMF, near-zero magnetic field; NP, non-penetration waveform; P, pathway phase, the sum of irregular mixed and transition phase prior to N4a; N4a, sieve element salivation waveform; N4ab, transition phase between N4a and N4b; N4b, phloem ingestion waveform; N5, xylem feeding waveform. n = 15 for each treatment and 4 hours of feeding behavior were recorded for each nymph. Only significant differences between the GMF and NZMF are marked with asterisk tested by one-way ANOVA for the duration or by two-tailed Mann-Whitney *U*-test for the frequency of specific feeding waves at $P < 0.05$.

3.4 Glucose content of the 5th instar nymph

It is found that larvae of *Drosophila* with increased glucose level have an aversion to feeding. Thus, glucose in fly biology was suggested as a potential negative regulator of appetite [35]. In this study, elevated glucose levels were found in 5th instar nymphs at both 24h (+16.98%; $F_{1, 10} = 9.54$, $P = 0.011$, partial $\eta^2 = 0.49$) and 48h (+20.05%; $F_{1, 10} = 9.86$, $P = 0.011$, partial $\eta^2 = 0.50$; Figure 3) after molting under the NZMF vs. GMF, which is consistent with the decreased phloem ingestion by the 5th instar nymphs in respond to the GMF absence (i.e., the NZMF). Taken together, magnetic field effects on transcript expression patterns of orexigenic neuropeptide genes, feeding behavior, and glucose level of older nymphs all pointed to the loss of appetite triggered by the GMF absence, which is accord with our speculation. Therefore, the appetite down-regulation by the NZMF vs. GMF could be the main reason for the decreased phloem ingestion in older nymphs and thus result in body weight loss in the newly emerged adults.

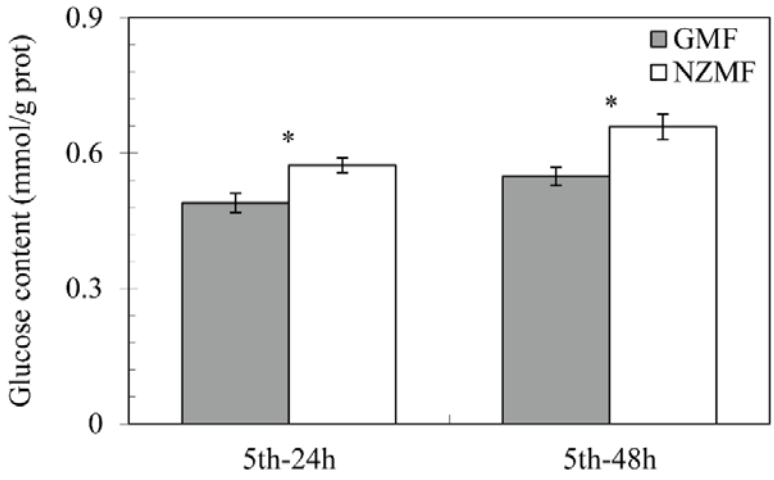

Figure 3. The geomagnetic field (GMF) absence increased the glucose content of 5th instar nymphs. NZMF, near-zero magnetic field; mmol/g prot, mmol/g protein. Fifteen individual nymphs were randomly mixed as one sample for each sampling time with six repeats. The columns represent averages with vertical bars indicating SE. Only significant differences between NZMF and GMF at the same sampling time are marked with asterisks tested by the one-way ANOVA at $P < 0.05$.

Although environmental factors like temperature, photoperiod were reported to affect animal appetite [51,52], it is still surprising that the GMF absence can result in altered feeding behavior potentially by the appetite regulation, which ultimately contributes to the adult body weight loss. However, taking a look into details, it may be easy to understand if we link the circadian clock, AKH/AKHR signaling, and NPF/sNPF signaling [53]. The potential magnetoreceptor Crys themselves play multifunctional roles in both magnetoreception and circadian clock mechanisms [54], and genetic evidence has already proved the magnetosensitivity of circadian clock to the magnetic field intensity change [20,45]. Potential interactions between their action on magnetoreception and the circadian clock could be the driving force for the chronic magnetic responses we found here, because both AKH/AKHR and NPF/sNPF signaling, and even feeding behavior are under the regulation of the circadian clock [53,55,56]. Besides, recent computational work suggested that [$FAD^{\cdot-}$–$HO_2^{\cdot}$] or [$FADH^{\cdot}$–$O_2^{\cdot-}$] can be alternative radical pairs at the origin of

magnetoreception in Crys [57], and ROS level was also proved to be sensitive to the changes in magnetic field intensity [22,27]. Moreover, it is well documented that ROS is critical in the regulation of energy metabolism and food intake due to its versatile roles in mediating the expression of vertebrate NPY (homolog of invertebrate NPF), glucose level [28] as well as AKH/AKHR pathway [47]. Therefore, another pathway which could explain the GMF-involved appetite regulation is likely to be organized by the ROS.

4. Conclusion

Migratory animals can conduct large-scale space displacement in a short time [58]. The difference in GMF intensity between the emigration and immigration areas could be environmental cues for the regulation of biological processes during their migration. Here, the changes in expression patterns of orexigenic genes, feeding behavior, and glucose content of older *N. lugens* nymphs under the NZMF vs. GMF all point to a loss of appetite, which could be responsible for the body weight loss in the newly emerged adults. Moreover, the GMF absence-induced loss of adult weight of migratory *N. lugens* potentially caused by appetite decrease implied the indispensable role of the GMF in maintaining energy homeostasis, which is critical for animal migration (e.g., fuelling behavior mediated by the GMF in several migratory birds)[11,24-26]. Taken together, we presented the first evidence in a nocturnal insect migrant that appetite-related performance can be influenced through changes in GMF intensity, suggesting GMF as a cue of the complicated system in regulating animal energy metabolism during migration.

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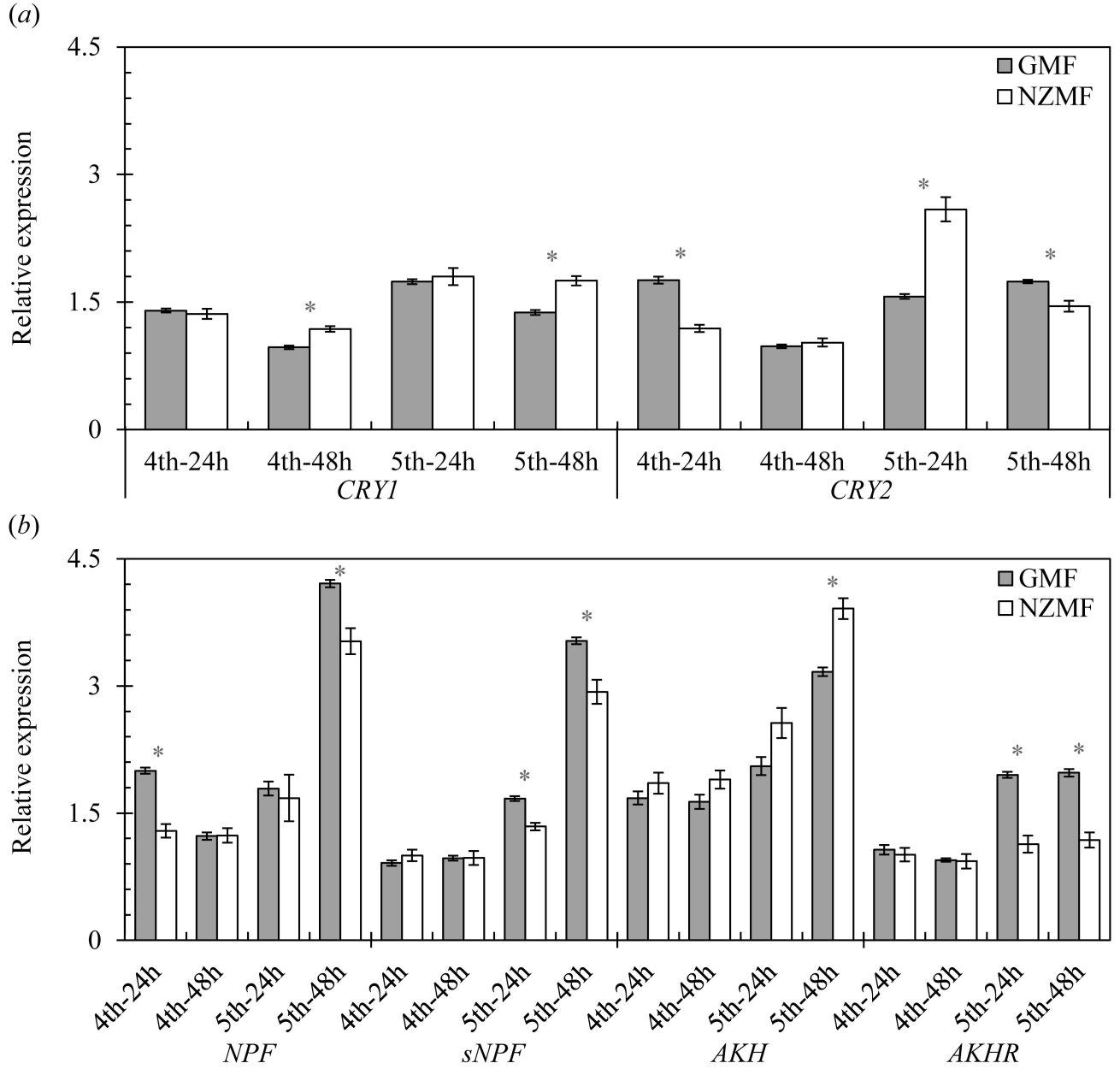
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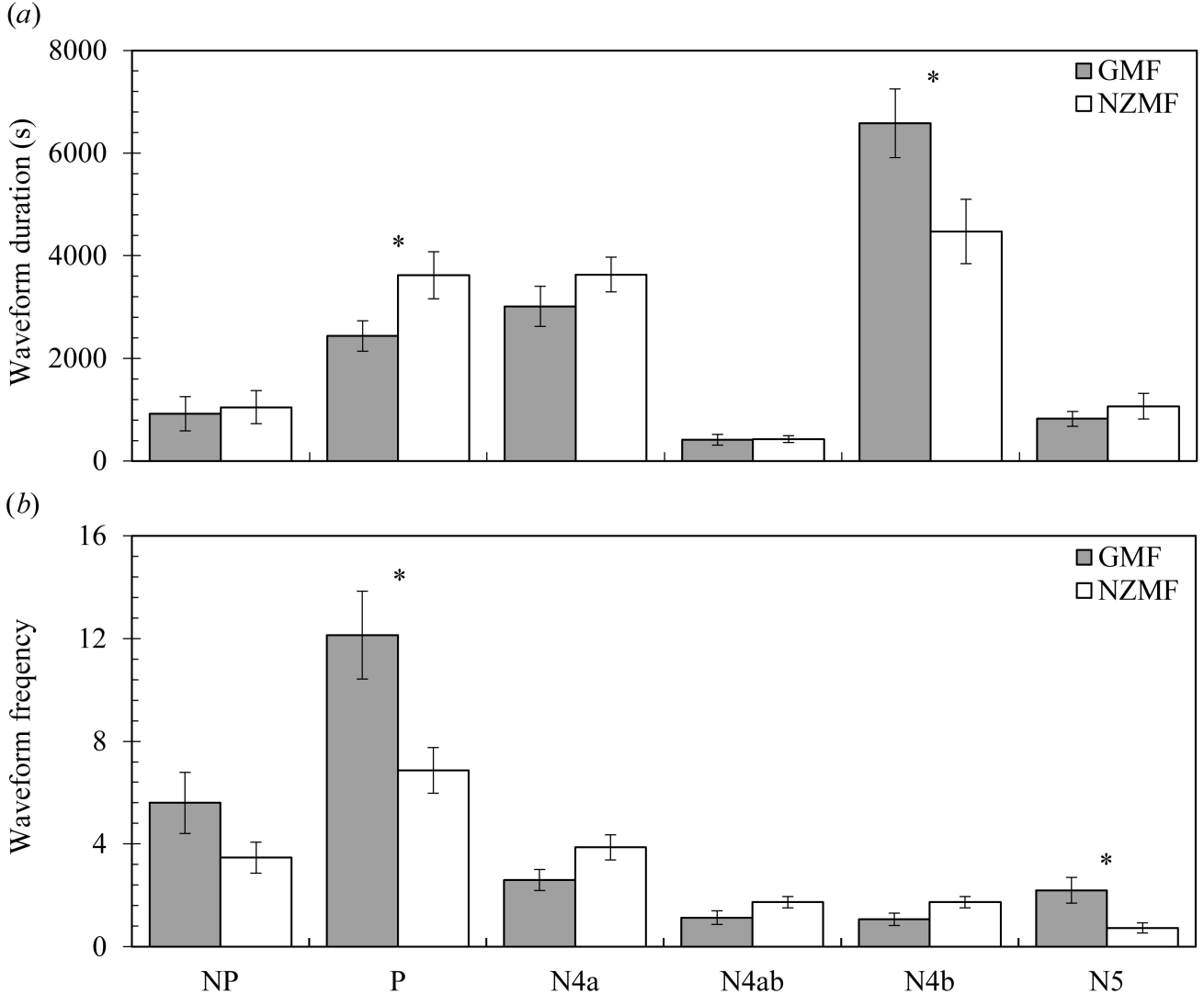
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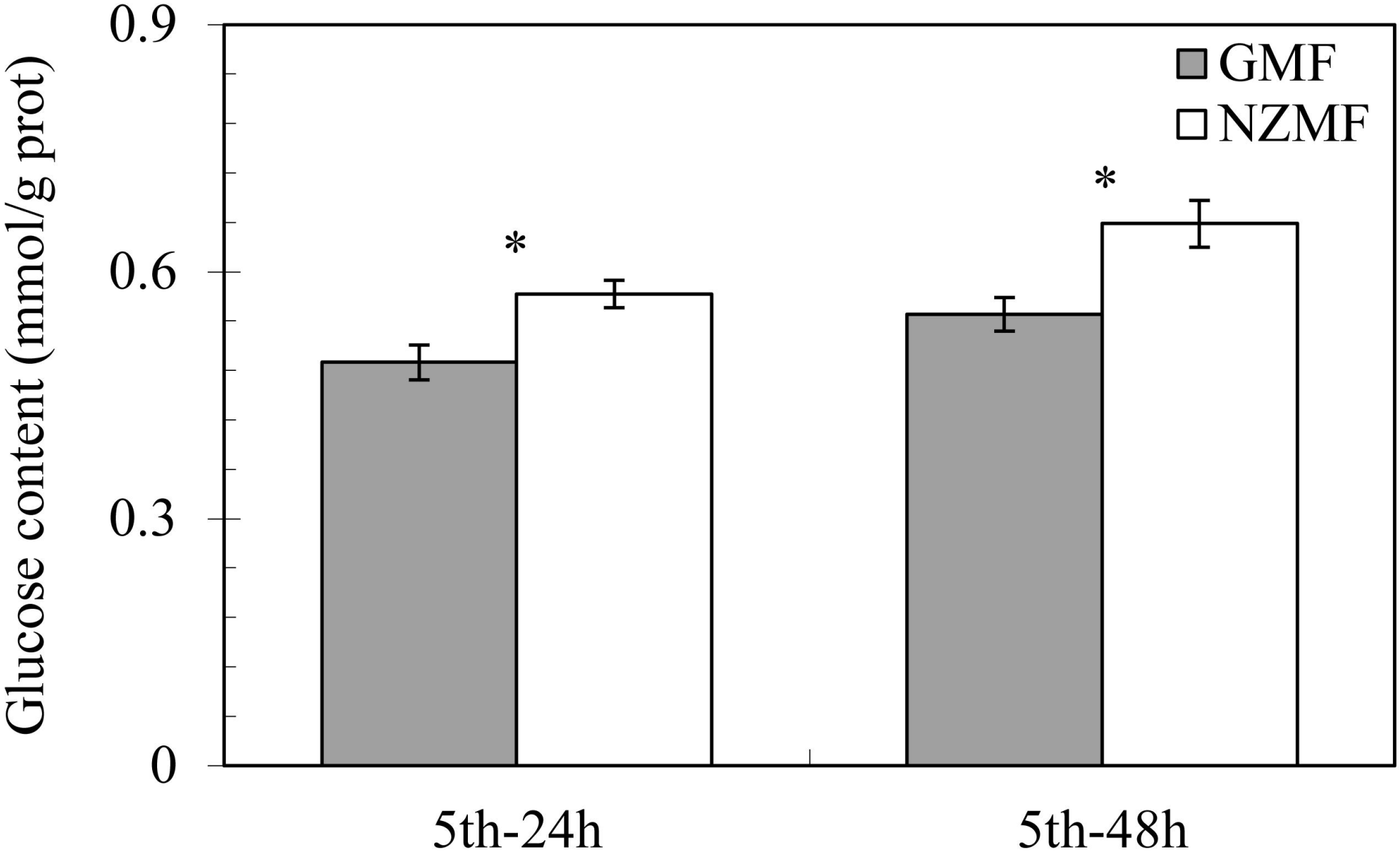
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